UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
GREAT LAKES NATIONAL PROGRAM OFFICE
77 WEST JACKSON BOULEVARD
CHICAGO, IL 60604-3590

Laura Bishop, Commissioner
Minnesota Pollution Control Agency
520 Lafayette Road N
St. Paul, MN 55155-4194

Dear Commissioner Bishop:

Thank you for your April 13, 2020 request to remove the *Eutrophication or Undesirable Algae* Beneficial Use Impairment (BUI) from the St. Louis River Area of Concern (AOC). As you know, we share Minnesota's desire to restore all the Great Lakes AOCs and to formally delist them.

Based upon a review of your submittal and the supporting data, the U.S. Environmental Protection Agency (EPA) approves Minnesota's request to remove this BUI, aka *Excessive Loading of Sediment and Nutrients,* from the St. Louis River AOC. EPA will notify the International Joint Commission (IJC) of this significant positive environmental change at this AOC.

We congratulate you and your staff as well as the many federal, state and local partners who have been instrumental in achieving this environmental improvement. Removal of this BUI will benefit not only the people who live and work in the AOC, but all residents of Minnesota and Wisconsin and the Great Lakes basin as well.

We look forward to the continuation of this important and productive relationship with your agency, the Minnesota Department of Natural Resources, and the Wisconsin Department of Natural Resources as we work together to delist this AOC in the years to come. If you have any further questions, please contact me at (312) 353-8320 or your staff may contact Leah Medley at (312) 886-1307.

Sincerely,

Chris Korleski, Director
Great Lakes National Program Office

cc: Barbara Huberty, MPCA
Matt Steiger, WNDR
Melissa Sjolund, MNDNR
Rick Gitar, Fond du Lac Band of Lake Superior Chippewa
Raj Bejankiwar, IJC

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
GREAT LAKES NATIONAL PROGRAM OFFICE
77 WEST JACKSON BOULEVARD
CHICAGO, IL 60604-3590

Stephen Galarneau, Director
Office of Great Waters – Great Lakes & Mississippi River
Wisconsin Department of Natural Resources
PO Box 7921
Madison, WI 53707-7921

Dear Mr. Galarneau,

Thank you for your April 13, 2020 request to remove the *Eutrophication or Undesirable Algae* Beneficial Use Impairment (BUI), known in the St. Louis River Area of Concern (AOC) as *Excessive Loading of Sediment and Nutrients*. As you know, we share Wisconsin's desire to restore all the Great Lakes AOCs and to formally delist them.

Based upon a review of your submittal and the supporting data, the U.S. Environmental Protection Agency (EPA) approves Wisconsin's request to remove this BUI from the St. Louis River AOC. EPA will notify the International Joint Commission (IJC) of this significant positive environmental change at this AOC.

We congratulate you and your staff as well as the many federal, state and local partners who have been instrumental in achieving this environmental improvement. Removal of this BUI will benefit not only the people who live and work in the AOC, but all residents of Minnesota and Wisconsin and the Great Lakes basin as well.

We look forward to the continuation of this important and productive relationship with your agency, the Minnesota Pollution Control Agency, and the Minnesota Department of Natural Resources as we work together to delist this AOC in the years to come. If you have any further questions, please contact me at (312) 353-8320 or your staff can contact Leah Medley at (312) 886-1307.

Sincerely,

Chris Korleski, Director
Great Lakes National Program Office

cc: Barbara Huberty, MPCA
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Raj Bejankiwar, IJC

MINNESOTA POLLUTION CONTROL AGENCY

520 Lafayette Road North | St. Paul, Minnesota 55155-4194 | 651-296-6300

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April 13, 2020

Mr. Chris Korleski
Director, Great Lakes National Program Office
United States Environmental Protection Agency
77 West Jackson Boulevard
Chicago, IL 60604-3507

RE: Approve the request to remove the Excessive Loading of Sediment and Nutrients Beneficial Use Impairment in the St. Louis River Area of Concern

Dear Mr. Korleski:

The Minnesota Pollution Control Agency and the Wisconsin Department of Natural Resources hereby request the approval of the Environmental Protection Agency's (EPA) Great Lakes National Program Office (GLNPO) staff to remove the Excessive Loading of Sediment and Nutrients Beneficial Use Impairment (BUI) in the St Louis River Area of Concern (SLRAOC).

The SLRAOC team has assessed the status of the management actions for the Excessive Loading of Sediment and Nutrients BUI as outlined in the 2013 SLRAOC Remedial Action Plan and its subsequent annual updates. All of the management actions associated with this impairment have been completed and a public review of the recommendation has been conducted. One comment was received supporting the removal recommendation and no further action was needed. A letter of support was provided by the St. Louis River Alliance, the citizens' action committee for the SLRAOC. We therefore recommend that the Excessive Loading of Sediment and Nutrients BUI be removed from the SLRAOC's impairments list. The documentation to support this recommendation is enclosed.

We value our continuing partnership with the GLNPO staff and the funding support provided to the SLRAOC through the Great Lakes Restoration Initiative. It is through your significant involvement and that of all of our federal, state and local partners that will keep us on the path to delisting the SLRAOC.

If you need further information about this request please contact either Barb Huberty at 218-302-6630 or barbara.huberty@state.mn.us or Matt Steiger at 715-395-6904 or matthew.steiger@wisconsin.gov.

Sincerely,

Laura Bishop
Commissioner

Enclosure: St. Louis River Area of Concern Beneficial Use Impairment Removal Recommendation for Excessive Loading of Sediment and Nutrients

cc: Leah Medley, SLRAOC Task Force Lead
Paul Buszka, USGS Technical Resource Lead
Matt Steiger, WDNR AOC Coordinator
Melissa Sjolund, MN DNR AOC Coordinator
Rick Gitar, Fond du Lac AOC Coordinator

St. Louis River Area of Concern

Beneficial Use Impairment Removal Recommendation for
Excessive Loading of Sediment and Nutrients

April 13, 2020

Submitted to:
U.S. EPA-Region 5
77 W. Jackson Boulevard
Chicago, IL 60604

Prepared by these implementing agencies:

NAH-GAH-CHI-WA-NONG
FOND DU LAC
RESERVATION

DEPARTMENT OF NATURAL RESOURCES

MINNESOTA POLLUTION CONTROL AGENCY

WISCONSIN DEPT. OF NATURAL RESOURCES

With major funding support from the Great Lakes Restoration Initiative.

Great Lakes RESTORATION

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ACRONYMS

- AOC – Area of Concern
- BUI – beneficial use impairment
- ca. – circa
- CBOD – carbonaceous biological oxygen demand
- chl α – chlorophyll α
- CWA – Clean Water Act
- CWMP – Coastal Wetland Monitoring Program
- DO – dissolved oxygen
- DOP – dissolved orthophosphorus
- EPT – Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies)
- FdL – Fond du Lac Band of Lake Superior Chippewa
- HSPF – Hydrologic Simulation Program—FORTRAN
- IBI – index of biotic integrity
- IEC – index of ecological condition
- MAs – management actions
- µg/L – microgram per liter
- mg/L – milligram per liter
- MNDNR – Minnesota Department of Natural Resources
- MPCA – Minnesota Pollution Control Agency
- N – nitrogen
- NRCS – Natural Resources Conservation Service
- NTU – nephelometric turbidity unit
- RAP – Remedial Action Plan
- SLRAOC – St. Louis River Area of Concern
- SLRE – St. Louis River Estuary
- TN – total nitrogen
- TIN – total inorganic nitrogen
- TMI – trimetric index
- TP – total phosphorus
- TSI – trophic state index
- TSS – total suspended solids
- UMD – University of Minnesota Duluth
- USEPA-GLNPO – U.S. Environmental Protection Agency – Great Lakes National Program Office
- USEPA-GLTED – U.S. Environmental Protection Agency – Great Lakes Toxicology and Ecology Division
- USGS – U.S. Geological Survey
- WDNR – Wisconsin Department of Natural Resources
- WLSSD – Western Lake Superior Sanitary District

COMPARISON OF UNITS USED TO EXPRESS CONCENTRATIONS:

Unit	Symbol	Also Described As	Equals
milligram per litter	mg/L	part per million (ppm)	$1/10^6$ or 0.000001
microgram per liter	µg/L	part per billion (ppb)	$1/10^9$ or 0.000000001

Conversions: 1 µg/L = 0.001 mg/L or 1 mg/L = 1000 µg/L

Within this document, mg/L will be the base unit used. Where scientists have used µg/L in their papers, the mg/L conversion will be shown in brackets to ease comparisons.

Executive Summary

Background

The United State and Canada designated 43 Areas of Concern (AOC) across the Great Lakes in 1987, including the St. Louis River Area of Concern (SLRAOC). The AOCs were designated because significant environmental damage at those locations caused specific types of Beneficial Use Impairments (BUIs). At the time of AOC designation, the International Joint Commission identified 14 BUIs in the Great Lakes Water Quality Agreement that were to be assessed at each AOC to determine their applicability. Only nine BUIs applied to the SLRAOC. Once the BUIs were identified, removal targets for each were established and management actions (MAs) to achieve the targets for each BUI were identified.

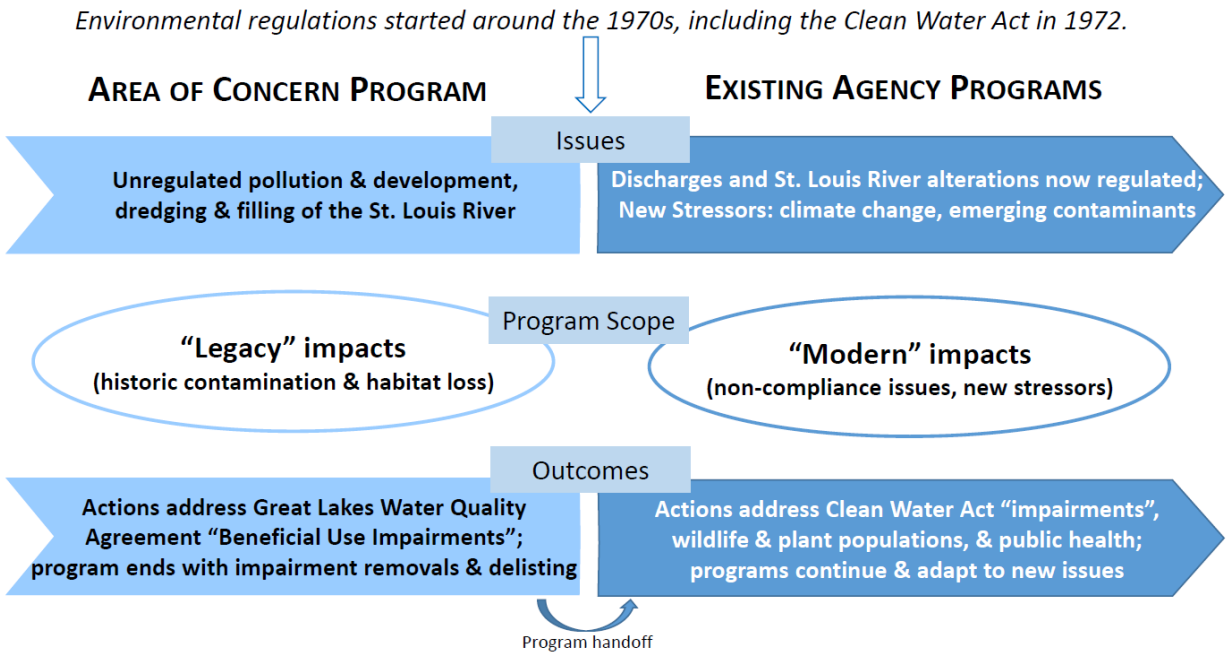
Once the MAs for a BUI are completed, a removal package is prepared for public review and, ultimately, concurrence by the U.S. Environmental Protection Agency.

This document provides the justifications supporting a removal recommendation for the Excessive Loading of Sediments and Nutrients BUI (BUI 6) for the SLRAOC, which is a modification of the "Eutrophication or Undesirable Algae" BUI from the Great Lakes Water Quality Agreement to address the SLRAOC-specific conditions. All five MAs that apply to BUI 6 are complete and the BUI 6 removal target and its four criteria are met. The removal criteria and brief conclusions pertaining to the studies applicable to each are included in this executive summary. Detailed summaries of the studies and findings for each MA are included in the main body of this document, while the study reports prepared for each management action are included in the appendices.

The voluntary AOC program was established to address "legacy" issues. These were environmental problems that caused ecosystem impairments at the time of the AOC designation and largely occurred before modern environmental regulations were in place. Legacy issues significantly impacted geographically-defined sites rather than regional-scale stressors.

For the SLRAOC, examples of legacy issues are: unregulated discharge of industrial and municipal waste, dredging and filling in the estuary, wood waste deposited in the river, and extensive logging – all of which exacerbated erosion and sedimentation problems. Since then, the Clean Water Act (CWA) and other environmental regulations are being implemented to protect the environment and human health from these types of large-scale problems.

The scope of the AOC program does not include "modern" issues that are the responsibility of many state and federal agencies under a variety of natural resources, environmental, and public health program authorities. Some examples of modern issues are: contaminants of emerging concern, water-related climate change impacts, non-compliance of point source permits, and impairments identified and regulated under the CWA. Figure ES-1 depicts the differences between the AOC and existing agency programs.



The same environmental and natural resource agencies that implemented the Area of Concern Program will address ongoing issues after the Program has ended, but under different program authorities. This will include long-term monitoring and maintenance of remediation and habitat projects, species management, and regulatory enforcement.

Figure ES 1: The Program Scope of the St. Louis River Area of Concern

As it relates to the removal of the Excessive Loading of Sediment and Nutrients BUI discussed in this report, consider climate change effects as an example of the difference between legacy and modern impacts. The SLRAOC is experiencing more frequent and intense storm events and these are affecting the intensity of seiche impacts, which are, in turn, impacting sediment and nutrient conditions in western Lake Superior and the St. Louis River Estuary (SLRE). These are modern impacts that fall under the purview of the CWA, not the SLRAOC program. The Future Actions section of this document lists a variety of future needs to be addressed by other agency programs.

It is important to note that the assessments associated with each MA are time limited. Once a MA is completed, there is not an effort to return to the endpoint of the studies to add data gathered by other agency programs since the conclusion of the study. Similarly, many implementation activities pertinent to BUI 6 are already underway by other agency programs that are outside the SLRAOC program. More recent data and activities are not reported here. Additionally, regulatory programs are ever-evolving and terminology in place when the BUI 6 studies were completed have not been substituted by newer terminology (e.g., turbidity impairments under the CWA are now total suspended sediment [TSS] impairments).

The Removal Target and Criteria Have Been Met

The removal target will have been met when:

Nutrient and sediment levels have not been shown to impair water quality and habitat, and do not restrict recreation, including fishing, boating, or body contact in the estuary and within western Lake Superior based on the following criteria:

1. *All federal, state, and local point source and nonpoint source discharge permits in the AOC are in compliance with regard to controlling sources of nutrients (particularly nitrogen and phosphorous), organic matter, and sediment;*

   CONCLUSION: As confirmed by permit compliance staff within the Wisconsin Department of Natural Resources (WDNR), all eight pollutant discharge elimination system permits within the SLRAOC area are in substantial compliance as of December 2019. Also as of December 2019, permit compliance staff from the Minnesota Pollution Control Agency (MPCA) have confirmed that there are 32 pollutant discharge elimination system permits within the SLRAOC area, of which only 21 have nitrogen, phosphorus, TSS and/or carbonaceous biological oxygen demand (CBOD) compliance conditions. Eleven permittees do not have nutrient-related requirements. Only one of the industrial permittee is noncompliant for TSS only and is following MPCA's compliance processes to address the noncompliance issues. The other 20 permittees with nutrient-related requirements are in substantial compliance with their permits.

   Additionally, the Western Lake Superior Sanitary District (WLSSD) and the City of Duluth are working to meet the conditions of a federal Consent Decree to reduce inflow and infiltration into the sanitary sewer system as a means to reduce sanitary sewer overflows.

   Both the City of Superior and the City of Duluth have also invested in stormwater management practices and outreach to reduce the impacts of non-point source, urban runoff.

2. *Total phosphorus concentrations in the Lake Superior portion of the AOC do not exceed 0.010 mg/L (upper limit of oligotrophic range);*

   CONCLUSION: Multiple data sources indicated that the Lake Superior portion of the AOC met this criterion (Table ES-1). The Lake Superior data from the 2012 and 2013 BUI study (MA 6.01) showed that total phosphorus (TP) values were slightly higher than the BUI criterion of 0.010 mg/L for Lake Superior's western arm, with an average of 12.7 µg/L [0.0127 mg/L].[1] Additional water quality parameters sampled during the study show that DO was generally near saturation and the chlorophyll α (chl α) concentrations were consistent with an oligotrophic water body. Paleolimnological study results (MA 6.03) for the Lake Superior sample location concluded that (1) water quality had improved from past periods of higher TP concentrations and (2) current prevailing concentrations of phosphorus did not exceed the TP criterion. Specifically, diatom-

inferred TP results for the Lake Superior core indicated that western Lake Superior concentrations of TP were 3 - 6 µg/L (0.003 to 0.006 mg/L). TP results for western Lake Superior were available for 1996-2015 from the U.S. Environmental Protection Agency's (USEPA's) Great Lakes Biology Monitoring Program (USEPA, Great Lakes Biology Monitoring Program, 1983 – present; Central Data Exchange). The TP results (see Appendix 11) showed that from 1996-2015 the mean western Lake Superior TP concentration was 2.6 µg/L [0.0026 mg/L] and the range was 1.0 to 8.0 µg/L [0.001 to 0.008 mg/L] and never exceeded the criterion[2].

[1]Data from this assessment was collected in nearshore conditions, which were likely biased toward St. Louis River conditions due to river water mixing with the lake at the sample sites.
[2] The USEPA's Great Lakes Biology Monitoring Program sampling point is not located within the boundary of the SLRAOC.

3. *There are no exceedances of the most protective water quality standard for either state in the western basin of Lake Superior due to excessive inputs of organic matter or algal growth attributed to loadings from wastewater overflows into the St. Louis River;*

   CONCLUSION: Data used to assess St. Louis River water quality indicate that the BUI removal criteria (MA 6.01-6.04) have been met. Additionally, these data do not indicate any excessive algal growth in or inputs of organic matter to the SLRAOC. Wastewater overflows are prohibited by Wisconsin Administrative Code Chapter NR 210.21 and are administered in Minnesota by State Statute 115.03, Minnesota Rule 7050.0210 and Minnesota Rule 7053.0205.

   Wastewater overflows, including sanitary sewer overflows, treatment facility overflows and combined sewer overflows have been drastically reduced since the time of AOC listing. Wastewater permits administered by the states have included conditions to reduce and report overflow events. In addition, as of August 2016, all facilities in Wisconsin were required to have developed and be actively implementing a Capacity, Management, Operation, and Maintenance program for operation and maintenance of sanitary sewer collection systems with goals to help address issues of inflow and infiltration which are the primary causes of overflow events. Minnesota's wastewater permitees have met similar facility management requirements. Upgrades to wastewater and collection systems in the past decade have resulted in significant reductions in overflow events. The improvements in DO, TSS and nutrients (Bellinger et al., 2016) also support this conclusion.

4. *Total phosphorus concentrations within the St. Louis River portion of AOC do not exceed an interim guide of 0.030 mg/L (upper limit of mesotrophic range) or the most restrictive water quality standards. This ensures that anthropogenic sources and activities in the St. Louis River AOC do not result in excessive productivity and nuisance conditions within the St. Louis River Estuary.*

CONCLUSION: The 5 MA's that have been completed for this BUI indicated that water quality improvements in the SLRE and Nemadji River watershed have resulted in the majority of the AOC meeting the phosphorus criterion (see Table ES-1). In addition, other water quality parameters (TSS, dissolved oxygen [DO] and chl α) indicate nutrients and sediments are not causing an impairment. Data showed a dramatic decline in TP concentrations and sediment loading in the SLRAOC since the time of listing.

Table ES-1: Summary of Water Quality Results for Management Action 6.04

Parameter	SLRE, from Fond du Lac dam to Lake Superior (Bellinger, et al., 2016)	Lake Superior[1] (Bellinger, et al., 2016)	Western Lake Superior[2] (USEPA, Great Lakes Biology Monitoring Program, 1996-2015)
TP	~60% of area below 30 µg/L [0.030 mg/L]	Average = 12.7 µg/L [0.0127 mg/L]	Average = 2.6 µg/L [0.0026 mg/L]
TSS	>85% of area below 15 mg/L	Average = 4.4 mg/L [0.0044 mg/L]	not assessed
DO	>5.5 mg/L; no hypoxia	Average = 12.2 mg/L	not assessed
chl α	>70% of area below 10 µg/L [0.010 mg/L]; oligotrophic to mesotrophic	Average = 2.7 µg/L [0.027 mg/L]; oligotrophic	not assessed

[1] The interim TP guide for Lake Superior is 0.010 mg/L. Data from this assessment were collected in nearshore conditions, which were likely biased toward SLRE conditions due to seiche mixing.
[2] The USEPA's Great Lakes Biology Monitoring Program sampling point (SU 19) is not located within the boundary of the SLRAOC

A BUI technical team of subject matter experts was established to evaluate the removal strategy and review the findings from each study and offer recommendations to address any deficiencies until the target and criteria were met (see Appendix 12 for the technical team members and their affiliations).

A public information process was conducted to obtain input from interested parties on the information provided in the removal package.

Multiple lines of evidence support a removal recommendation for this BUI. The results of the BUI 6 studies, along with support from the BUI 6 technical team, SLRAOC partners, and stakeholders have resulted in this recommendation by the SLRAOC Coordinators, leaders, and executive managers to remove the Excessive Loading of Sediments and Nutrients BUI from the SLRAOC.

Purpose

The purpose of this document is to provide the information needed to support a recommendation to remove the Excessive Loading of Sediment and Nutrients Beneficial Use Impairment (BUI) in the St. Louis River Area of Concern (SLRAOC).

St. Louis River Area of Concern Background

The 1987 US-Canada Great Lakes Water Quality Agreement designated the SLRAOC as one of 43 areas with significant environmental degradation. The SLRAOC is spatially large and geographically complex, spanning the Minnesota and Wisconsin state line and including tribal interests (see Figure 1).

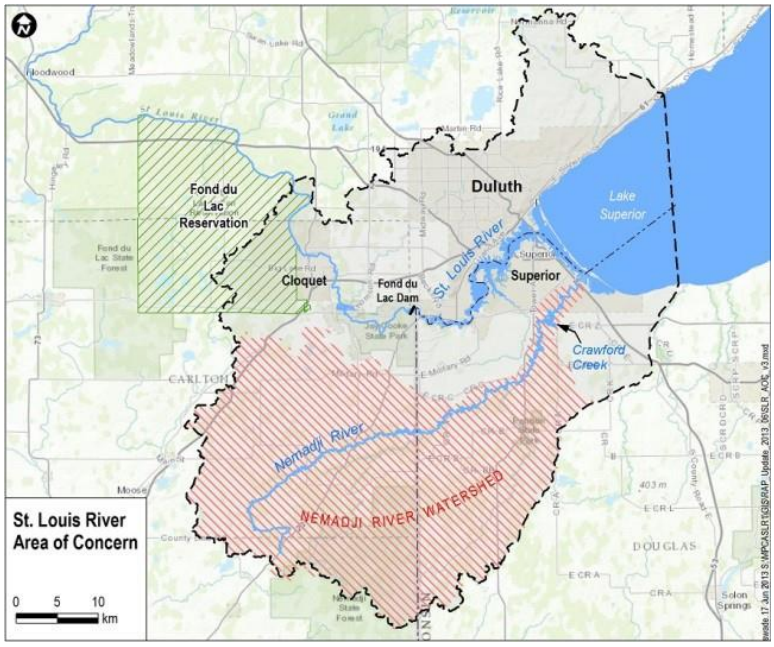


Figure 1: Extent of the St. Louis River Area of Concern

The SLRAOC is jointly managed by four implementing agencies: the Fond du Lac Band of Lake Superior Chippewa (FdL), the Minnesota Department of Natural Resources (MNDNR), the Minnesota Pollution Control Agency (MPCA), and the Wisconsin Department of Natural Resources (WDNR). MPCA and WDNR are the delegated authorities that manage official transactions with the U.S. Environmental Protection Agency - Great Lakes National Program Office (USEPA-GLNPO). Dozens of stakeholder organizations are also involved in activities related to the SLRAOC.

Efforts to reverse the BUIs are located primarily within the 12,000-acre St. Louis River Estuary (SLRE), where water from the St. Louis River and Lake Superior mix. The twin port cities of Duluth, MN and Superior, WI are located on either side of the estuary.

A Stage I Remedial Action Plan (RAP) identified these nine BUIs (MPCA and WDNR, 1992):

1. Restrictions on Fish and Wildlife Consumption
2. Degradation of Fish and Wildlife Populations
3. Fish Tumors or Other Deformities; removed in 2017
4. Degradation of Benthos
5. Restrictions on Dredging Activities
6. Eutrophication or Undesirable Algae (SLRAOC name: Excessive Loading of Sediment and Nutrients)

7. Beach Closings (SLRAOC name: Beach Closing and Body Contact Restrictions)
8. Degradation of Aesthetics; removed in 2014
9. Loss of Fish and Wildlife Habitat

The Great Lakes Water Quality Agreement "Eutrophication or Undesirable Algae" BUI was modified to become the SLRAOC's "Excessive Loading of Sediment and Nutrients" BUI 6 for two reasons. First, with the end of wholesale logging and lumber milling and the improvement of wastewater treatment in the area, the St. Louis River was no longer characterized as eutrophic. Second, undesirable algal blooms were not an identified concern. However, the delivery of excessive loads of sediment and nutrients remained as an important local concern, so BUI 6 was established to ascertain the effects of the estuary's unique turbidity, algae, and nutrient conditions.

A Stage II RAP was completed in 1995 and it was later superseded by the 2013 St. Louis River Area of Concern Implementation Framework: Roadmap to Delisting (MPCA and WDNR, 1995 and 2013, respectively). The 2013 RAP was a comprehensive listing of the BUIs, their removal targets, and the management actions (MAs) needed to achieve those targets. The 2013 RAP has been updated annually thereafter to document progress and changes to the RAP implementation plan and schedule (MPCA and WDNR, 2014-2019).

It is important to understand that the voluntary AOC program was created to address "legacy" issues or environmental problems that caused ecosystem impairments at the time of the AOC designation and largely occurred before modern environmental regulations were in place. Legacy issues significantly impacted geographically-defined sites rather than regional-scale stressors.

For the SLRAOC, examples of legacy issues are unregulated discharge of industrial and municipal waste, dredging and filling in the estuary, wood waste deposited in the river, and extensive logging – all of which exacerbated erosion and sedimentation problems. Since then, the Clean Water Act (CWA) and other environmental regulations are being implemented to protect the environment and human health from these types of large-scale problems.

The scope of the AOC program does not include "modern" issues that are the responsibility of many state and federal agencies under a variety of natural resources, environmental, and public health program authorities. Some examples of modern issues are: contaminants of emerging concern, water-related climate change impacts, non-compliance of point source permits, and impairments identified and regulated under the CWA. Figure 2 depicts the differences between the AOC and existing agency programs.

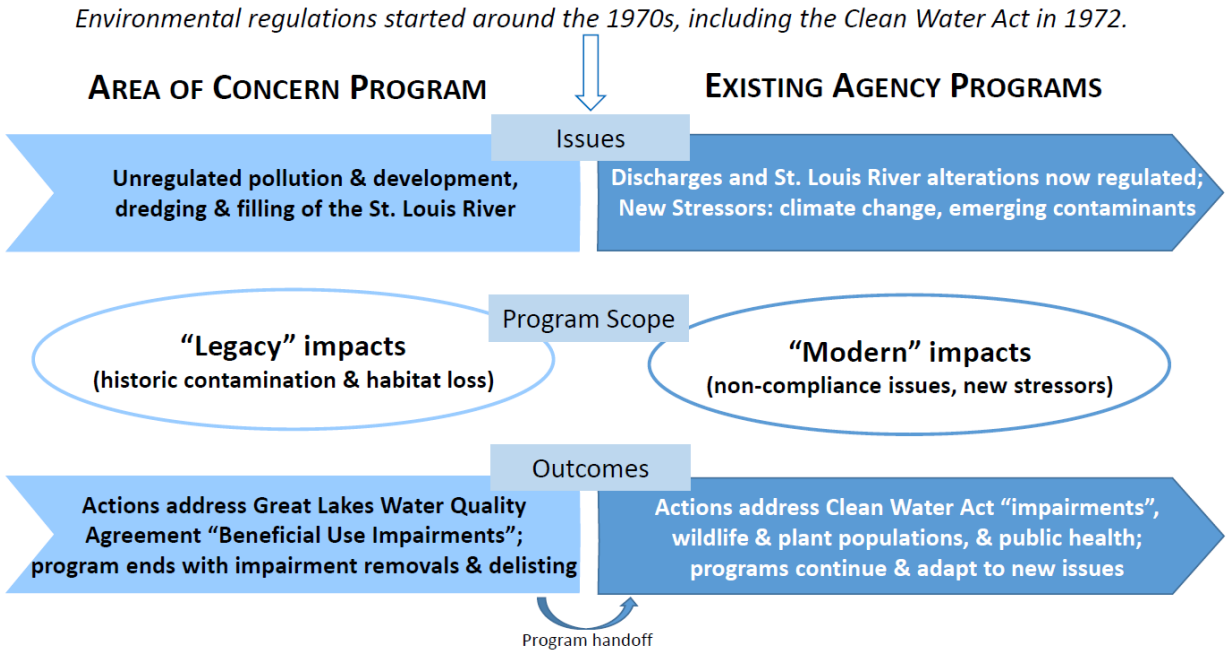


Figure 2: The Program Scope of the St. Louis River Area of Concern

As it relates to the removal of the Excessive Loading of Sediment and Nutrients BUI discussed in this report, consider climate change effects as an example of the difference between legacy and modern impacts. The SLRAOC is experiencing more frequent and intense storm events and these are affecting the intensity of seiche impacts, which are, in turn, impacting sediment and nutrient conditions in western Lake Superior and the SLRE. These are modern impacts that fall under the purview of the CWA, not the SLRAOC program. The Future Actions section of this document lists a variety of future needs to be addressed by other agency programs.

BUI Information

Rationale for Listing

The SLRAOC's RAP describes the rationale for listing this BUI as follows:

> *Prior to the improvements in wastewater treatment in the late 1970s, water quality and biological investigations characterized the St. Louis River Estuary (SLRE) as low in dissolved oxygen and high in total phosphorus and total suspended solids. At that time, the Western Lake Superior Sanitary District (WLSSD) treatment plant was built and the Superior wastewater treatment plant was upgraded. Since then, many indicators of trophic status have shown improvements. For instance, concentrations of total phosphorus have decreased and dissolved nitrogen has shown variable*

decline in St. Louis Bay. The loading of phosphorus to the estuary from point sources has been reduced substantially. At the time of AOC listing, further work was needed to ascertain the effects of nonpoint source loadings to the system and to Lake Superior. Despite the reductions in point source loadings, phosphorus concentrations in the estuary remained at levels where eutrophic conditions might be expected. Algal biomass was lower than would be expected, however, given these high phosphorus concentrations. Chlorophyll a concentrations measured in the estuary were similar to levels found in mesotrophic or oligotrophic waters. Several investigators proposed that reduced light penetration caused by turbidity and color may be a limiting factor for algal growth in the estuary. Although persistent water quality problems associated with eutrophication were not observed in the estuary, the high levels of nutrients and sediments being delivered to Lake Superior were determined to be an important concern. Therefore, the RAP used a modification of the International Joint Commission eutrophication criterion to reflect local conditions.

The St. Louis River Watershed, which drains to the St. Louis River and the SLRE near Lake Superior, has experienced more than 150 years of urban and industrial development that has altered land use, water quality, and aquatic ecosystems. Prior to the passage of the CWA, discharges from industrial and municipal sources were unregulated. Inadequately treated wastewater discharges, disposal of sawmill and paper mill waste products into the river, and runoff of forest debris in the wake of landscape-scale logging all contributed to low oxygen levels that negatively impacted aquatic life across the food web. The barren, post-logging landscape also contributed excessive loading of sediments, resulting in increased turbidity and nutrient concentrations (e.g., phosphorus, nitrogen) in the river.

The CWA spawned both state and federal laws used to control point source discharges. Because municipalities and industries can no longer discharge directly to the river without treatment to meet effluent standards, improved wastewater treatment and manufacturing processes have helped restore the water quality in the SLRE.

Removal Target

With the involvement of stakeholders, a removal target for the Excessive Loading of Sediments and Nutrients BUI was established (MPCA and WDNR 2011), stating that the removal target will be reached when:

Nutrient and sediment levels have not been shown to impair water quality and habitat, and do not restrict recreation, including fishing, boating, or body contact in the estuary and within western Lake Superior based on the following criteria:

1. *All federal, state, and local point source and nonpoint source discharge permits in the AOC are in compliance with regard to controlling sources of nutrients (particularly nitrogen and phosphorous), organic matter, and sediment; and*
2. *Total phosphorus concentrations in the Lake Superior portion of the AOC do not exceed 0.010 mg/L (upper limit of oligotrophic range); and*
3. *There are no exceedances of the most protective water quality standard for either state in the western basin of Lake Superior due to excessive inputs of organic matter*

or algal growth attributed to loadings from wastewater overflows into the St. Louis River; and,

4. *Total phosphorus concentrations within the St. Louis River portion of AOC do not exceed an interim guide of 0.030 mg/L (upper limit of mesotrophic range) or the most restrictive water quality standards. This ensures that anthropogenic sources and activities in the St. Louis River AOC do not result in excessive productivity and nuisance conditions within the St. Louis River Estuary.*

The interim guides used for the removal criteria are estimations based on existing standards. Although the St. Louis River holds some features in common with other rivers and flow-through lakes, this ecosystem is unique because of the implications of residence time, mixing, and biogeochemistry resulting from landward forcing of lake water (i.e., the result of seiche or storm surge) that mixes the lake and tributary waters. The Interim Status Indicators selected (see Table 1) are part of the BUI 6 Blueprint, (MPCA and WDNR, 2013, Appendix D).

Table 1: Water Quality Interim Status Indicators from the BUI 6 Blueprint Document

Indicator	Target	Location	Source
Water column TSS	15 mg/L	St. Louis River portion of AOC	Draft MN criteria for north river region (MPCA, May 2011)
	10 mg/L	Lake Superior portion of AOC	Draft MN criteria for class 2A waters (MPCA, May 2011)
Water column TP	30 µg/L (1)	St. Louis River portion of AOC	Final Delisting Target: Note the discrepancy between current MN and WI TP criteria that might also be used for the SLR AOC - MN draft TP criterion for the north river region is 55 µg/L (MPCA, 2011); WI TP criterion for St. Louis River is 100 µg/L (WDNR, November 2010; N.R. 102.06(3)(a))
	10 µg/L (2)	Lake Superior portion of AOC	Final Delisting Target: Note WI, but not MN, has a TP criteria that should be considered of 5 µg/L (WI TP standard for Lake Superior of 5 µg/L includes open and nearshore waters- WDNR, November 2010; N.R. 102.06(5))
Chlorophyll *a*	10 µg/L	St. Louis River portion of AOC	Draft MN criteria for north river region (MPCA, November 2010)
	1.3 µg/L	Lake Superior portion of AOC	Number derived from Annex 4 of the Great Lakes Water Quality Agreement target TP loading of 3400 metric tons per year (IJC 1983); corresponding TP is 5 µg/L.
	3 µg/L	MN Class 2A	7050.0222 Specific Water Quality Stds for Class 2; Aquatic Life and Recreation
Dissolved Oxygen	7 mg/L	MN Class 2A	Daily minimum and compliance with the standard 50% of the days at which the flow of receiving water is equal to the $7Q_{10}$
Un-ionized Ammonia (NH_3)			Criteria are many and varied, depending on agency and methodology. Therefore, it is not appropriate at this time to list existing Wisconsin and Minnesota standards as an interim status indicator without further review and historical data analysis.

In addition, the following measurable indicators are applicable to discharge permits and wastewater overflows.

Indicator	Measurement Basis	Target
Federal, state, and local permitted dischargers, including MS4s in the AOC	Determined through review by WDNR and MPCA	All permittees in compliance with regard to controlling sources of nutrients (particularly nitrogen and phosphorus), organic matter, and sediment
Municipal wastewater collection systems and WWTP permittees within the AOC	Determined through review by WDNR and MPCA	All permittees in compliance with permit conditions with regard to controlling sewage overflows

The SLRAOC RAP interprets this to mean that the removal of the Excessive Loading of Sediment and Nutrients BUI will be justified when:

1. *All federal, state, and local point source and nonpoint source discharge permits in the AOC are in compliance with regard to controlling sources of nutrients (particularly nitrogen and phosphorus), organic matter, and sediment.*
2. *Assessment of current water quality data for the Lake Superior and the SLRE portions of the SLRAOC indicate that water quality meets the water quality goals established by the strategy described below.*
3. *Watershed management objectives for the Nemadji River watershed that are in the Nemadji Basin Plan (NRCS, 1998) are adopted and progress towards implementing the objectives is being made.*

The RAP goes on to explain that:

Total phosphorus data alone will not provide the level of confidence needed to show that nutrient and sediment concentrations do not impair water quality and habitat and do not restrict recreation, including fishing, boating, or body contact in the estuary. Therefore, to protect and restore the condition of the SLRAOC related to the listing of this BUI, a thorough review of historical data and a statistical analysis of the current water quality condition based on the recommended seven status indicators listed below are necessary. These analyses will allow the BUI Technical Team to assess the trends and current condition of the SLRE in relation to BUI removal. The seven status indicators include:

- *Chemical – total phosphorus, un-ionized ammonia, dissolved oxygen*
- *Biological – chlorophyll a*
- *Physical – total suspended solids (TSS) and turbidity or other loading metric based on tons of sediment*
- *Watershed – progress toward meeting management objectives to reduce runoff rates and sediment delivery in the Nemadji River watershed*

The RAP further acknowledges that:

This work is not intended to set or replace State water quality standards, but to develop BUI removal objectives agreeable to both States and FdL that are consistent with the intent of the BUI removal target. The BUI removal objective water quality goals are to: protect the riverine and estuarine portions of the AOC from a eutrophic classification, to protect the Lake Superior portion of the AOC from a mesotrophic

classification, and to achieve desired levels of sediment and nutrient loading to Lake Superior. SLRAOC managers and the BUI Technical Team decided that additional water quality goals were not necessary for BUI removal. Sufficient information is available to justify BUI removal using the parameters in the BUI removal target.

Removal Strategy

Five management actions were established in the RAP to support the removal of the Excessive Loading of Sediments and Nutrients BUI and all have been completed (see Table 2).

Table 2: Completed Management Actions for BUI 6

Mgmt. Action	Name	Description
6.01	*Perform Area-Wide Water Quality Sampling and Analyses*	*Identify data needs, develop sampling design based on Bellinger et al. (2012) and evaluate results.*
6.02	*Perform Expanded Historical Data Analysis*	*Conduct a thorough review of current and historical data and a statistical analysis of the six water quality indicators (total phosphorus, un-ionized ammonia, dissolved oxygen, chlorophyll a, TSS and turbidity) and evaluate long-term trends in water quality.*
6.03	*Paleolimnological Investigation*	*Perform a paleolimnological investigation of the St. Louis River Estuary to reconstruct the algal and geochemical history and develop models to characterize trends in natural and anthropogenic drivers in water quality.*
6.04	*Develop Water Quality Goals (Compilation of 6.01, 6.02, and 6.03)*	*Assess results of 6.01, 6.02, and 6.03 and determine appropriate water quality goals for the reference condition of biological, chemical and physical indicators of water quality.*
6.05	*Assessment and Implementation Planning in the Nemadji River Basin*	*Assess sediment impairments through biological, water quality, and sediment monitoring, and HSPF modelling of historic sediment loads. Support implementation of the Nemadji Basin project recommendations to reduce sedimentation through stakeholder and landowner planning efforts.*

The strategy outlined in the RAP for each of the management actions is described below.

Strategy for MA 6.01– Perform Area-Wide Water Quality Sampling and Analyses

Perform area-wide water quality analyses in the SLRE based on the 2012 monitoring protocols in Bellinger et al. The objective of this project is to work with SLRAOC program staff and other groups responsible for monitoring and assessing conditions in the SLRE to identify data needs, develop a sampling design to meet those needs, and evaluate the relevancy of the results. Analysis of the water quality indicators will be used to estimate conditions within geographic zones and/or estuary-wide. Results will be used to report whether the SLRE is trending toward or has reached the reference condition or range of conditions considered reasonable for the estuary. Understanding changes in

water quality and associated biological conditions that meet BUI removal objectives is the focus of this work and it will include the six chemical water quality status indicators to:

a. *Provide a summary of the six chemical water quality indicators for a period of two to three years and*
b. *Assess and verify the relevance of all six status chemical indicators within the SLRE or by geographic zone, if necessary, to determine if the estuary is impaired for these parameters based on agreed-upon reference conditions and accounting for any unique conditions.*

Strategy for MA 6.02 – Perform Expanded Historical Data Analysis

Perform an expanded historical data set analysis based on methodologies used in Hoffman (2011) to evaluate long-term trends in water quality as it relates to the six chemical status indicators. Determine the appropriate water quality goals for the reference condition of any or all of the status indicators appropriate for the SLRE and western portion of Lake Superior that will meet approval by Minnesota and Wisconsin as appropriate for the SLRAOC.

Strategy for MA 6.03 – Paleolimnological Investigation

Perform a paleolimnological investigation of the SLRE to reconstruct the algal and geochemical history for approximately the last 300 years (management action 6.03). Diatom-based (i.e., microfossil algae) models will be applied to identify historical temporal and spatial variations in biological (i.e., chlorophyll, algal load), chemical (i.e., phosphorus, ammonia) and physical (i.e., TSS, turbidity) water quality indicators. Combined with the results of the monitoring data and trend analyses described in the strategies for 6.01 and 6.02, the paleolimnological data will provide quantitative and qualitative reconstructions of the important physical, chemical and biological trends that have resulted from natural and anthropogenic drivers.

Strategy for MA 6.04 – Develop Water Quality Goals (Compilation of 6.01, 6.02, and 6.03)

Determine the appropriate water quality goals for the reference condition of any or all of the status indicators appropriate for the SLRE and western portion of Lake Superior that will meet approval by Minnesota and Wisconsin as appropriate for the SLRAOC.

Strategy for MA 6.05 – Assessment & Implementation Planning in the Nemadji River Basin

Document progress toward meeting watershed management objectives from the Nemadji Basin Plan (NRCS, 1998) as an indicator of sediment loading to the SLRAOC. The Nemadji plan established watershed objectives to reduce runoff rates and sediment delivery from the Nemadji River watershed into SLRAOC.

Once the work for the five management actions is complete, the RAP directs an assessment of the status of the SLRE in relation to BUI removal:

1. *For the water quality indicators:*
    a. *If the assessments show the current conditions are sustained and the water quality has improved to where it meets the water quality goals, then removal targets are met.*
    b. *If the assessments show the current conditions are not sustained and water quality is not meeting the water quality goals, then removal targets are not met. Determine possible sources and develop an action plan to address the source(s). Then, re-evaluate annually until it can be shown that water quality meets applicable water quality goals for two consecutive years.*

2. *For the watershed indicator:*
    a. *If watershed management objectives for the Nemadji watershed are met or progress over time to meet the objectives can be demonstrated, this information will help support removal of the sediment loading aspect of this BUI.*

It is important to note that the assessments associated with each MA are time limited. Once a MA is completed, there is not an effort to return to the endpoint of the studies to add data gathered by other agency programs since the conclusion of the study. Similarly, some implementation activities pertinent to BUI 6 are already underway by other agency program that are outside the SLRAOC program. More recent data and activities are not reported here. Additionally, regulatory programs are ever-evolving and terminology in place at the time BUI 6 studies were completed have not been substituted by newer terminology (e.g., turbidity impairments under the CWA are now TSS impairments).

Management Actions Methods, Findings, and Conclusions

6.01 Area-Wide Water Quality Sampling and Analyses

Historical and current water quality conditions for a variety of parameters were evaluated to compare concentration estimates with BUI removal criteria established by SLRAOC stakeholders. Current water quality condition was assessed both seasonally and spatially using data collected in 2012 and 2013 (MA 6.01). For the historical component, 60 years of water quality data (1953 – 2013) from two fixed stations was used to determine how nutrient and sediment concentrations and loads changed in the SLRE (MA 6.02). These MA's were combined into one scientific paper*, Water quality in the St. Louis River Area of Concern, Lake Superior: Historical and current conditions and delisting implications* (see Appendix 1). This work was completed by the U.S. Environmental Protection Agency – Great Lakes Toxicology and Ecology Division (EPA-GLTED) under the direction of Dr. Joel Hoffman and Dr. Brent Bellinger (Bellinger, et al., 2016) and has been summarized below (see Appendix 1 for the scientific paper).

6.01 Methods

Long-term water quality trends in the SLRE were assessed at both the Highway 23 Bridge (i.e., upper estuary) from 1953 to 2013 and the Interstate 535/US Highway 53 John A. Blatnik Bridge (i.e., lower estuary) from 1973 to 2013 (see Figure 3). Data were available for dissolved oxygen (DO), total phosphorus (TP), total nitrogen (TN), dissolved nitrate/nitrite-N, ammonium/ammonia-N, and TSS. Chlorophyll α (chl α) was not available as a historical measurement. This summary focused on trends in both concentration and loadings for TSS and TP, in particular, as well as trends in DO concentration. For TSS and TP, a conservative mixing model was used to estimate the concentration in the river, absent a lake effect. The study was intended to better understand how water quality has changed from the industrial era to the present day and whether the levels today meet BUI removal objectives.

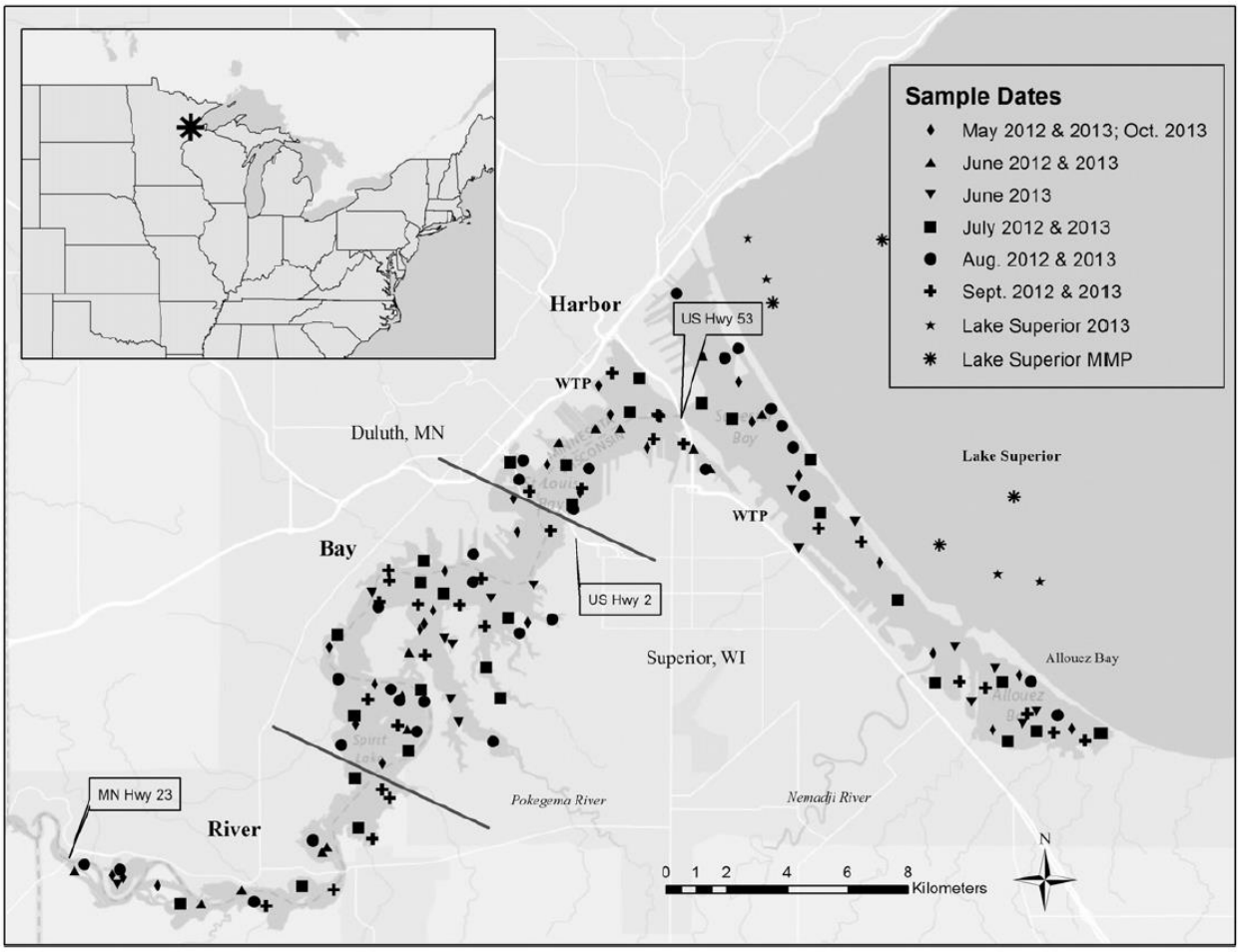


Figure 3 Sampling Locations for Management Action 6.01

Current water quality conditions for the estuary were assessed from 2012-2013 for TP, TSS, DO, and chl α to estimate the proportion of the estuary's surface area below the BUI removal criteria concentrations. A random, spatially balanced sampling design was developed to provide unbiased, area-weighted water quality concentration estimates for DO, TP, and TSS across the SLRAOC (see Figure 3). The design was then used to determine the areal extent of the SLRAOC that either met or was in exceedance of a specific water quality criteria. The sampling event locations were identified and subsequently assigned to three zones with distinct hydrologic and geochemical character: River (i.e., upper estuary), Bay (i.e., central estuary or St. Louis Bay, and the Harbor (i.e., lower estuary or Superior Bay).

6.01 Findings – Historic Water Quality Trends (1953-2013)

Sediment and nutrient loads, as represented by TSS and TP, respectively, declined between 1953 and 2013. See Figure 4, where:

- panels A and C: temporal trends in monthly TP and TSS concentrations
- panels B and D: annual TP and TSS loads
- monitoring stations: upper estuary (closed circles) and lower estuary (open circles)
- dashed lines = BUI removal criteria of 0.030 mg/L TP and 15 mg/L TSS.

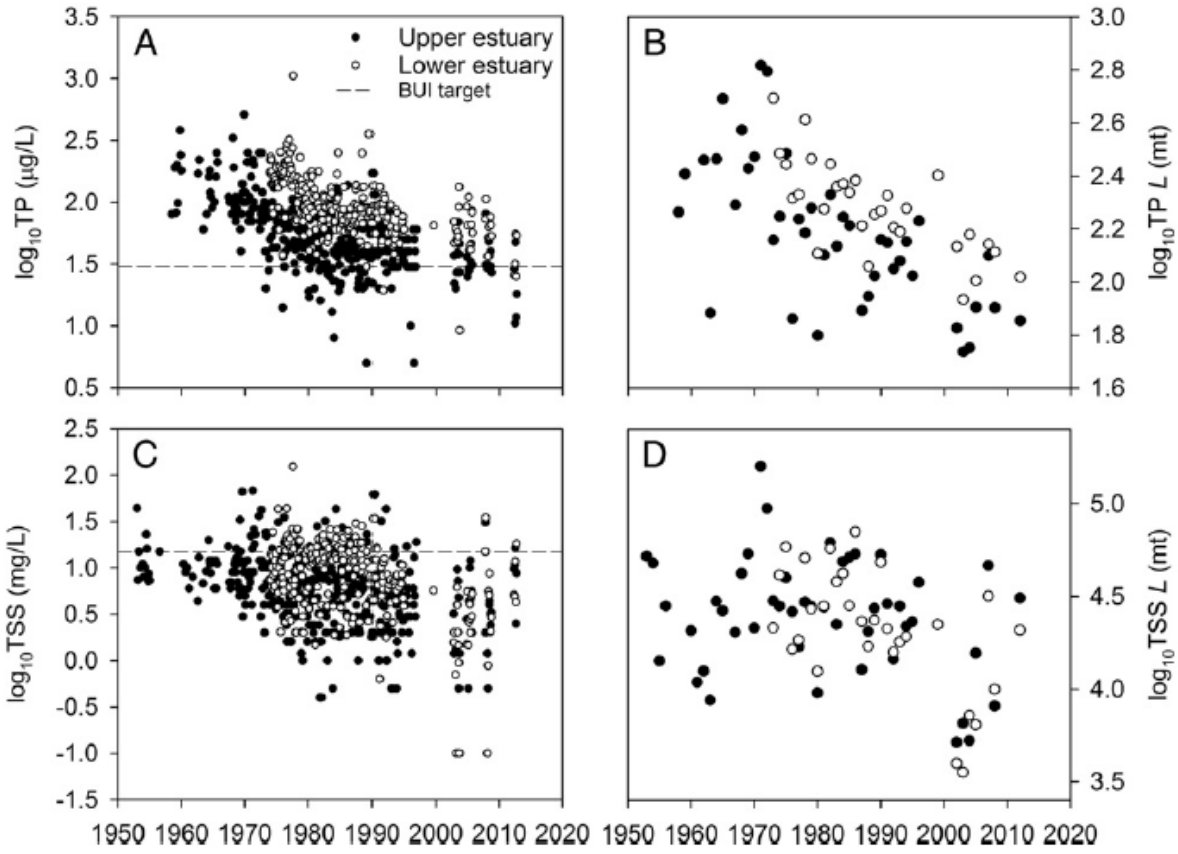


Figure 4: Total Phosphorus and Total Suspended Solids Results from Management Action 6.02

Annual mean TP concentrations and loads to Lake Superior declined significantly over time; the change in concentration was faster at the lower estuary station than the upper estuary station. Since 2000, TP concentrations at the upper estuary stations have generally ranged between 20 and 50 µg/L [0.020 and 0.050 mg/L]. Though concentrations have declined, monthly and annual average TP concentrations frequently exceeded the BUI removal criterion of 30 µg/L [0.030 mg/L] over the period of record; however, the majority of concentrations greater than the criterion precede 1990. The ratio between the mean annual load and river discharge (known as the mean mass per unit discharge), revealed a decline over time, indicating that the decline in TP load was the result of changes in TP concentration rather than discharge. The decline in TP concentration can be attributed to a combination of factors, including reduced TP inputs to the system, improved retention of TP within the watershed, and reduced resuspension of legacy organic matter inputs. Through time, TP concentrations in the lower estuary were higher than in the upper estuary, implying there were internal TP sources (e.g., resuspension of sediment) or tributary additions. From 2002 to 2012, the estimated mean annual TP load was 76 tons at the upper estuary station and 133 tons at the lower estuary station, for an average annual net addition of 57 tons.

Annual mean TSS concentrations significantly declined over time at both stations; as with TP concentrations, the decline was faster at the lower estuary station than the upper estuary station.

Average annual TSS concentrations were above the interim status indicator of 15 mg/L (see Table 1) three times prior to 1978. After 2000, relatively low TSS concentrations (≤5 mg/L) were measured at both stations except for two instances (2007, 2012) in which elevated TSS concentrations (31.0 and 16.3 mg/L, respectively) coincided with large discharge events (354 and 120 m^3/s, respectively).

Annual TSS loads to the SLRE declined over time at both stations, until the 2012 flood. TSS loads to Lake Superior also declined faster at the lower estuary station than the upper estuary station. Notably, at the beginning of the time-series, the estuary between stations was a source of TSS, compared to its current neutral status or that of a TSS sink, which suggested a substantial shift in TSS dynamics within the estuary. As with TP loads, the ratio of the mean annual load and discharge (i.e., the volume-weighted mean TSS) declined over time, indicating that the change in TSS load was due to change in TSS concentration rather than discharge.

A long-standing concern for water quality in the SLRAOC has been low DO (see Appendix 1). Historically, this was strongly influenced by the discharge or dumping of materials with high biological oxygen demand, such as wood waste and sewage. At both monitoring stations, the last recording of summer hypoxia (<2 mg/L DO) was 1964; DO values <5 mg/L were infrequent after 1975. The DO standard (as a daily minimum) in Minnesota and Wisconsin is 5 mg/L for class 2B (warmwater) streams. The period for which hypoxia was present somewhere in the river was likely longer than the time series suggest because the available longitudinal DO concentration data indicated that the lowest DO concentrations in the river were typically located between the upper and lower estuary stations (i.e., between river km 20 and 35). Nevertheless, low DO concentrations have not been observed in the thalweg (i.e., the deepest part of the river channel) since the mid-1970s. At the upper estuary station, late-summer (July–September) DO concentrations increased from 1953 to ca. 1990, after which the concentrations leveled-off and possibly declined slightly (generally, between 7 and 9 mg/L). Data from the lower estuary followed a similar pattern. Since 2000, monthly summer concentrations were always above 5.5 mg/L at both stations.

6.01 Findings – Current Water Quality Conditions (2012-2013)

In both 2012 and 2013, about 60% of SLRE area between Fond du Lac dam and Lake Superior was below the BUI removal criterion for TP of 30 µg/L [0.030 mg/L]; thus, 40% of SLRE area exceeded the TP removal criterion. The spatial distribution of TP is shown in Figure 5 for 2012 and in Figure 6 for 2013. The highest TP exceedances were seen in “hotspots” that had unique characteristics compared to normal SLRE conditions (i.e., primarily near wastewater treatment facility outfalls or in clay-influenced bays). System-wide TP concentrations ranged from 4.7 µg/L [0.0047 mg/L] to 195.4 µg/L [0.1954 mg/L] with a median concentration across years of 28.7 µg/L [0.0287 mg/L]. The weighted mean TP concentration for 2012 (30.9 µg/L [0.0309 mg/L]) and 2013 (30.7 µg/L [0.0307 mg/L]) were not significantly different from the BUI criterion (30 µg/L [0.030 mg/L]). The TP hotspots identified in the Wisconsin clay-influenced bays justified the study included as part of MA 6.04.

The WLSSD hotspot was likely an anomaly, potentially associated with sanitary sewer overflows during the 2012 flood. This conclusion is supported by WLSSD’s publically available records, indicating a later change to load-based TP limits, its permit compliance record, and its operational excellence awards.

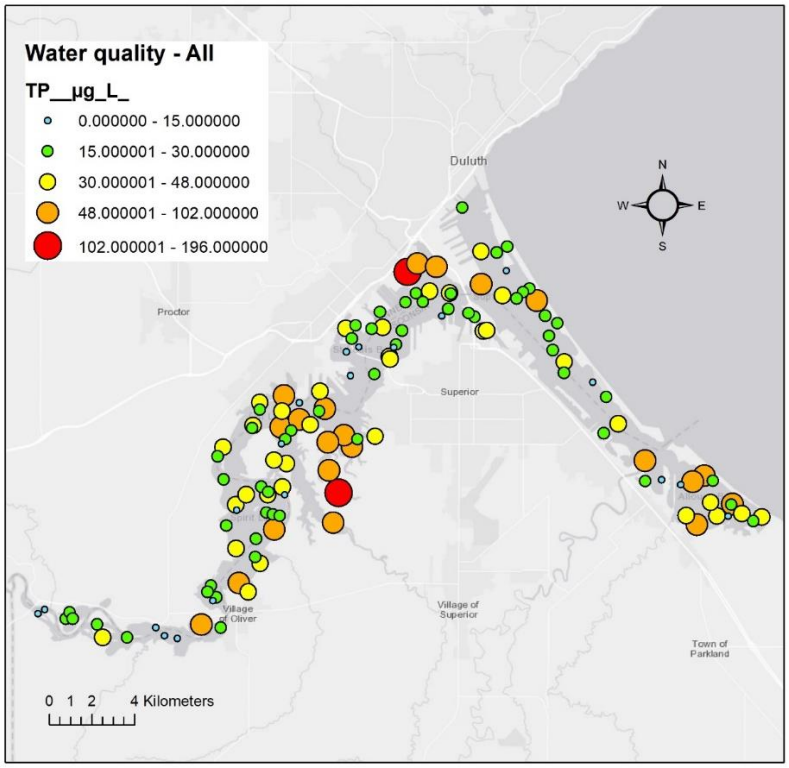


Figure 5: 2012 Total Phosphorus Results from Management Action 6.01

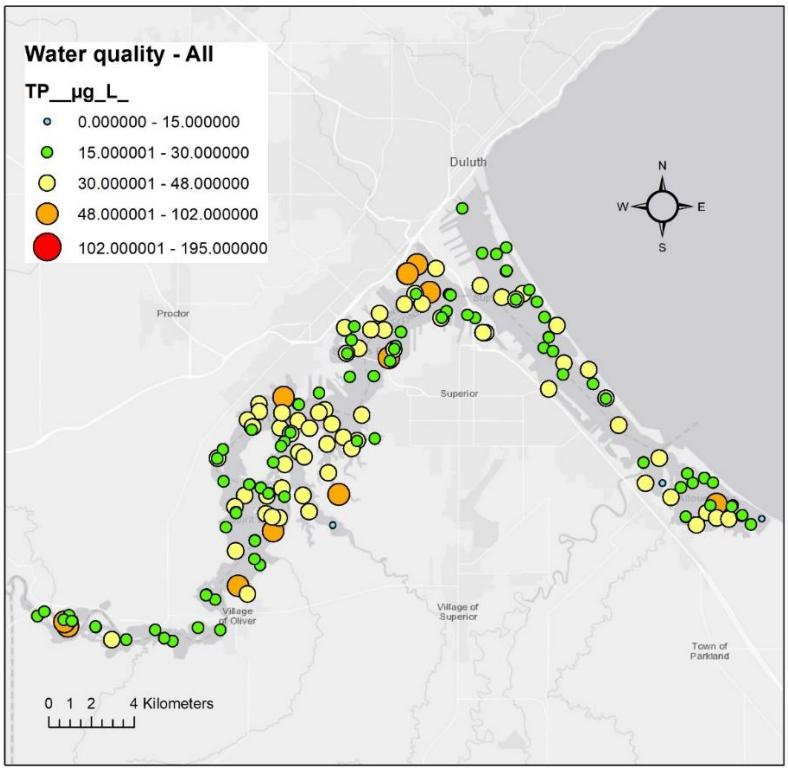


Figure 6: 2013 Total Phosphorus Results from Management Action 6.01

At least 85% of the area of the SLRE between Fond du Lac dam and Lake Superior had TSS concentrations below the 15 mg/L interim status indicator in both years. TSS concentrations varied from 2.3 mg/L to 71.4 mg/L, with a median concentration of 8.6 mg/L. The weighted mean TSS concentrations for 2012 (12.0 mg/L) and 2013 (9.9 mg/L) were significantly below the BUI criterion of 15 mg/L.

Chlorophyll α concentration data were not available in the time-series monitoring to assess trophic status. For both years, over 70% of the area of the SLRE between Fond du Lac dam and Lake Superior had chl α concentrations below the interim status indicator of 10 µg/L [0.010 mg/L], as listed in Table 1. Chlorophyll α concentrations ranged from 0.6 µg/L [0.0006 mg/L] (October 2013) to 49.9 µg/L [0.0499 mg/L] (September 2012). The weighted mean chl α concentrations in 2012 and in 2013 were significantly below the BUI criterion.

For the Lake Superior portion of the SLRAOC, TP concentrations were greatest in June and averaged 12.7 µg/L [0.0127 mg/L]. Average TSS concentration was 4.4 mg/L, ranging from 0.4 to 15.1 mg/L. Dissolved oxygen was always at or near 100% saturation; concentration for the season averaged 12.2 mg/L. Chlorophyll α concentration was greatest in June (4.5 mg/L) and averaged 2.7 mg/L.

It should be noted that the Lake Superior sampling locations for this study were just outside of the estuary and were influenced by river water mixing with the lake, which likely contributed to higher results than would have been seen in the open water areas of Lake Superior (Figure 3). Additional data from the USEPA's Great Lakes Biology Monitoring Program and the paleolimnology study (MA 6.03) were used as additional lines of evidence to justify that Lake Superior BUI removal criteria have been met.

6.01 Conclusions – Area-Wide Water Quality Sampling and Analyses

Since the 1950s, there has been a dramatic decline in TP concentrations in the SLRAOC, with concentrations generally ranging from 80-380 µg/L [0.080 – 0.380 mg/L] in the 1950s to 20-50 µg/L [0.020 – 0.050 mg/L] in the 2000s. In 2012 and 2013, about 60% of SLRE area between Fond du Lac dam and Lake Superior was below the BUI removal criterion of 30 µg/L [0.030 mg/L]. Similarly, there has been a dramatic decline in TSS, with concentrations generally ranging from 7-20 mg/L in the 1950s to 1-10 mg/L in the 2000s. At least 85% of the area of the SLRE between Fond du Lac dam and Lake Superior had TSS concentrations below 15 mg/L, the interim status indicator, in 2012 and 2013. Along with these changes, DO concentrations improved and no indications of hypoxia (<2 mg/L) have been observed in the SLRE thalweg since 1964. The current chl α concentrations observed in the SLRE are generally indicative of an oligotrophic to mesotrophic waterbody, ranging from <1 µg/L [0.001 mg/L] to nearly 50 µg/L [0.050 mg/L]. In 2012 and 2013, over 70% of the area of the SLRE between Fond du Lac dam and Lake Superior had chl α concentrations below the interim status indicator of 10 µg/L [0.010 mg/L].

In the Lake Superior portion of the SLRAOC, the DO was generally near 100% saturation and the chl α concentrations were consistent with an oligotrophic water body. Total phosphorus values measured near the estuary entry points (average of 12.7 µg/L [0.0127 mg/L]) were generally higher than typical values measured in offshore waters in the western arm of Lake Superior (generally <5 µg/L [<0.005 mg/L]). Nearshore environments of Lake Superior are expected to be more productive (and closer to

the upper limits of the oligotrophic range) than offshore waters, due to riverine and other nearshore inputs. Most of the SLRE area and Lake Superior were below the status indicators for each parameter (See Table 3).

Table 3: Summary of Water Quality Results for Management Action 6.04

Parameter	SLRE, from Fond du Lac dam to Lake Superior (Bellinger, et al., 2016)	Lake Superior[1] (Bellinger, et al., 2016)	Western Lake Superior[2] (USEPA,'Great Lakes Biology Monitoring Program, 1996-2015)
TP	~60% of area below 30 µg/L [0.030 mg/L]	Average = 12.7 µg/L [0.0127 mg/L]	Average = 2.6 µg/L [0.0026 mg/L]
TSS	>85% of area below 15 mg/L	Average = 4.4 mg/L [0.0044 mg/L]	not assessed
DO	>5.5 mg/L; no hypoxia	Average = 12.2 mg/L	not assessed
chl α	>70% of area below 10 µg/L [0.010 mg/L]; oligotrophic to mesotrophic	Average = 2.7 µg/L [0.027 mg/L]; oligotrophic	not assessed

[1] The interim TP guide for Lake Superior is 0.010 mg/L. Data from this assessment were collected in nearshore conditions, which were likely biased toward SLRE conditions due to seiche mixing.
[2] The USEPA's Great Lakes Biology Monitoring Program sampling point (SU 19) is not located within the boundary of the SLRAOC.

6.02 Perform Expanded Historical Data Analysis

Management Action 6.02 was to conduct a thorough review of current and historical data and conduct a statistical analysis of the six water quality indicators (TP, un-ionized ammonia, DO, chl α, TSS and turbidity) and evaluate long-term trends in water quality. To establish long term trends in the portion of the SLRE below the Fond du Lac dam, staff from USEPA-GLTED analyzed data sets from four stations in the lower St. Louis River that were monitored by the MPCA from the early 1950's (for some sites) until 2008 when their Milestone Monitoring Program was discontinued. The collected data were provided by MPCA to USEPA-GLTED and subsequently included in the public STORET database. The milestone stations utilized were the MN Hwy 23 Bridge, the Oliver Bridge, the former Arrowhead Bridge/U.S. Hwy 2 Bong Bridge, and the U.S. Hwy 53 Blatnik Bridge. The length of time for the data series at each location varied.

Dissolved inorganic nitrogen, TP, and TSS data were available from October 1974 and May 1975 from the four locations. Historic data were not available for chl α, soluble reactive phosphorus, or TN for these stations. To characterize current conditions, data collected by USEPA-GLTED researchers were analyzed. Data from April-September 2002-2007 at numerous locations were available, though only one station was sampled regularly within the same year.

Data analyzed for MA 6.02 was merged with the 1953-2013 data evaluated as part of the 6.01 Area-Wide Water Quality Sampling and Analyses effort and published in Bellinger, et al., 2016 (see Appendix 1). Findings and conclusions for the merged data sets were described in MA 6.01, above.

6.03 Paleolimnological Investigation

6.03 Methods and Findings

The historical magnitude and extent of sediment and nutrient impacts had not been well understood for the years preceding water quality improvements due to environmental regulations and systematic long-term monitoring of water quality (pre- 1953-1973, depending on location). Therefore, a paleolimnology study of the SLRE was initiated to close the knowledge gap. To help understand this history, seven cores were taken from SLRE sites believed to have undisturbed sediments and continuous depositional environments (see the red dots on Figure 7). These cores provided good representation of the conditions present in the SLRE; they represent western Lake Superior, the St. Louis River thalweg, and the nearshore portions of the SLRE.

Figure 7: Paleolimnological Core Locations for Management Action 6.03

The cores were evaluated for retrospective analyses by staff from the Natural Resources Research Institute, of the University of Minnesota-Duluth. The primary goal, especially related to the excessive loading of sediment and nutrients BUI, was to determine pre-industrial water quality conditions and to track, through time, the anthropogenic impacts and the extent of loading reductions. In order to do this, sediments in the core samples were dated using isotopic analyses and fossil remains (i.e., diatoms, pigments, pollen, and phytoliths) were identified in concordance with other stratigraphic indicators (i.e., organic and inorganic materials, contaminants, and sedimentation rates) to reconstruct the history of

the system from 1850 to the present. That work (Reavie, et al., 2016; Alexson et al. 2018) was summarized here and contained in Appendix 2.

Diatoms in relation to water quality

Diatom assemblages were assessed from sediment intervals and these assemblages were used to infer trophic conditions using a regional diatom-based model for Great Lakes coastlines. Interpretations were based on diatom-based models that contained known species responses to water quality, which were applied to fossil assemblages. The diatom records indicated varying ecological histories and trajectories depending on the location within the SLRE. Deeper core locations (e.g., near the federal navigation channel, Lake Superior) indicated water quality improvement from past periods of higher total phosphorus concentrations and algal productivity, and that current, prevailing concentrations of phosphorus, based on inferred total phosphorus concentrations from core samples, did not exceed the SLRAOC BUI removal phosphorus criterion of 0.030 mg/L. However, the near-coastal (e.g., North Bay, Pokegama Bay, Allouez Bay) reconstructions revealed a recent increase in inferred phosphorus. At these locations, the inferred phosphorus levels based on the diatom species model would have been in exceedance of the BUI removal criterion. It is noteworthy that the earliest dated concentrations (~1850) were also inferred to be above the criterion, reflecting the productivity of these systems at that time.

It should be noted that a later study (Alexson, et al., 2018) determined that there is possible uncertainty in the inferred total phosphorus concentration or diatom-inferred TP data. That study showed a close, but not exact, relationship in the TP and diatom-inferred TP concentration trends. Additionally, the diatom-inferred TP concentrations in that study were found to be lower than those observed by Bellinger, et al., 2016 at the most comparable sampling location, but also provided possible reasons for those differences.

One core was taken in Lake Superior within the AOC boundary. The inferred total phosphorus concentrations from the Lake Superior core showed concentrations of TP that ranged from 3 - 6 µg/L [0.003 to 0.006 mg/L]; these were less than the Lake Superior BUI criterion of 0.010 mg/L.

Geochemistry in relation to water quality and nutrient loading

Algal pigment concentrations in the sediment profiles concurred with diatom-based inferences. Main channel cores did not indicate recent increases in algal abundance, however the increasing presence of cyanobacterial pigments in two bays (North Bay, Billings Park) indicated increases in potentially undesirable algae; an indicator of increasing nutrients in those locations.

Historical sediment accumulation rates (organic and inorganic) indicated that recent sediment loads to the estuary remained higher than loads estimated around 1850. However, three sites (Lake Superior, Allouez Bay and Billings Park) exhibited reduced sediment loads since the peak period of development in the mid-20th century. This finding aligned with the results of other sediment load studies in the Nemadji River watershed.

In addition to water quality information, cores were analyzed for heavy metals and organic contaminants indicative of human activity and industrialization. This work was intended to better understand general trends and to see if the science behind the analysis could provide a line of evidence

that supports overall water quality improvement through time. Mercury was included as a marker of human activities such as mining, burning of fossil fuels, and untreated sewage disposal. Sediment mercury concentrations peaked in the mid-1900s, but more recently declined to near pre-impact concentrations, indicating recent decreases in some combination of direct atmospheric deposition, watershed runoff, and point source domestic and industrial discharges. There were distinct mid-1900s peaks in cadmium, zinc, lead, tin, antimony and magnesium, likely resulting from watershed disruptions that exposed materials to erosion and runoff and/or industrial discharges. With improved regulation of these activities, a concurrent reduction in metals was seen. Sedimentary organic contaminants analyzed from the single core from the harbor had concentrations below the detection levels.

6.03 Conclusions – Paleolimnological Investigation

Overall, paleolimnological results from Lake Superior and the main stem of the St. Louis River indicated improvements in nutrient loads or a discontinuation in the enrichment trends that were observed through the 1970s. Since the onset of environmental regulations, there have been clear improvements in TP concentrations in the water column, as inferred from paleo-diatom analyses from three mid-channel cores and one core from western Lake Superior, largely due to wastewater treatment and stormwater management improvements that have occurred in the SLRAOC over the past ~40 years.

Increasing nutrient loads were seen in the three nearshore/bay cores. However, in terms of nearshore phosphorus, the study generated evidence that pre-industrial impact concentrations of phosphorus likely exceeded the BUI removal criterion of 30 mg/L for TP by approximately 10 – 15 µg/L [0.010-0.015 mg/L] for TP. Also, nearshore changes in water quality may have been the result of phenomena outside the rationale for listing this BUI, such as climate change, increasing precipitation, phosphorus recycling, and perhaps other indirect mechanisms. A more detailed paleolimnology investigation, including speciation of phosphorus and development of nutrient (i.e., carbon, nitrogen, and phosphorus) budgets for the system would be needed to determine the factors influencing the nearshore areas.

These data indicated that BUI removal objectives were being met in over fifty percent of the SLRE. The clay-influenced Wisconsin bays were an area where the removal objectives were not being met and was another reason why the clay-influenced bays study was added as a BUI 6 activity (see Section 6.04). It is noteworthy that the earliest dated estuary phosphorus concentrations (~1850) were inferred to be above the BUI criterion, reflecting the productivity of these systems before industrial influence and putting the BUI removal criteria into context with the natural productivity of the nearshore areas.

The inferred phosphorus concentrations from the Lake Superior core did not exceed the removal criteria for TP in water of 0.010 mg/L.

The overall improvement seen is one line of evidence to support BUI 6 removal, given the rationale for listing.

6.04 Develop Water Quality Goals (Compilation of 6.01, 6.02, and 6.03)

The purpose of MA 6.04 was to assess the findings of MAs 6.01, 6.02, and 6.03 and determine appropriate water quality goals for the reference condition of biological, chemical, and physical

indicators of water quality in the SLRAOC and to use the MA findings to determine if the SLRAOC met these goals. Since the numeric BUI criteria were recommended based on interim values, the BUI Technical Team was tasked with evaluating those criteria. After reviewing results of four assessments performed under AOC management actions, the BUI Technical Team agreed that the indicators included in the BUI removal target were an appropriate goal to justify BUI removal and additional water quality goals were not needed for BUI removal evaluation. The upper limit of mesotrophic range (0.030 mg/L) was identified as being appropriate for riverine and estuarine portions, while the upper limit of oligotrophic range (0.010 mg/L) was deemed appropriate for the Lake Superior portion of the SLRAOC.

Although these three MAs showed that sediment and nutrient conditions were improving in the SLRE, the improvements were not uniformly distributed throughout the SLRE. In particular, clay-influenced nearshore bays in Wisconsin had higher nutrient levels than the rest of the SLRAOC; however, eutrophication that might be expected under those conditions was absent. Additionally, these same bays had higher sediment loads than the rest of the SLRAOC. No comprehensive dataset existed to determine if these higher nutrient and sediment conditions were having a negative impact on aquatic life. As a result, a study was added to MA 6.04 to assess the clay-influenced bays in the Wisconsin portion of the SLRAOC (Roesler, 2018; Appendix 3) and provide data that could be used to determine if any additional AOC action was needed and whether site-specific water quality goals for these bays would be appropriate.

Background: Saint Louis River Estuary Clay-Influenced Bay Assessment

A BUI removal criterion of 0.030 mg/L for TP was established for the SLRAOC. This criterion was established to ensure that anthropogenic sources and activities in the SLRAOC were not resulting in excessive productivity and nuisance conditions within the SLRE. Diatom-inferred TP concentrations from sediment core analyses (Reavie, et al., 2016) indicated that TP concentrations in some SLRE bays exceeded the BUI removal criterion, but they had been at or above this criterion prior to development in this watershed.

Three bays on the Wisconsin side of the SLRE were selected for monitoring and assessment: Allouez Bay, Pokegama Bay, and Kimball's Bay (Figure 8). These sites were selected because very limited pre-existing water quality data was available for these bays and because they are the major clay-influenced bays within the SLRE. Watersheds for these bays contain clay-rich soils that are highly erodible and prone to high rates of surface runoff.

Figure 8: Clay-Influenced Bays Sampled for Management Action 6.04

The monitoring at these three bays was intended to:

- Document the current water quality and biotic conditions in these SLRE clay-influenced bays.
- Determine if current nutrient and suspended solids concentrations are negatively affecting aquatic life.
- Provide data that could be used to determine if site specific water quality goals are warranted.

Methods

The three bays were monitored twice per month during May – October of 2017 for water quality, algae, sediment chemistry, and benthic invertebrates. Tributary streams for the bays were monitored for water quality. Pre-existing water quality and biotic information was reviewed and summarized (Roesler, 2018). A companion project to assess fish communities in the bays was also conducted in 2017 (Nelson, 2019).

Findings: Clay-Influenced Bay Characteristics and Water Quality

The three bays had some unique characteristics relative to nearby main channel waters that influenced their water quality during the 2017 sampling period, as described below.

- Allouez Bay is the largest (1,011 acres) and shallowest and it is subject to frequent wind-induced mixing. The mouth of the bay is adjacent to the Superior entrance to Lake Superior and seiche-induced backflows of Lake Superior water influence the bay.

- Allouez Bay was mostly well-mixed, with intermittent thermal and DO stratification. There were indications that seiche-induced inputs of cooler Lake Superior water flowed along the bay bottom at times. Mean TP, TSS, and chl α concentrations were 85 µg/l [0.085 mg/L], 21 mg/L, and 7.1 µg/l [0.071 mg/L], respectively. TP and TSS concentrations were highest in May and

October when more runoff and suspended sediment were entering the bay. Chl α concentrations were highest in June and August when runoff and suspended sediment loads were lower and water clarity was higher than the other months.

- Pokegama Bay (441 acres) has the largest watershed area and so its water quality is heavily influenced by Pokegama River inflow. The bay is also affected by wetlands that fringe its narrow upstream end.

- Pokegama Bay also was mostly well-mixed, with intermittent thermal and DO stratification. Lower DO concentrations occurred more frequently at the surface in the upstream end of the bay, likely due to decomposing organic matter and overall high respiration rates in the fringe wetlands. There were likely occasional releases of sediment phosphorus from wind mixing of intermittently anoxic bottom waters in deeper areas of the bay, in addition to runoff-driven pulses of phosphorus from the fringe wetlands. Such intermittent inputs of phosphorus, and nitrogen (mostly as ammonium-N), are a characteristic of shallow lakes, ponds, and embayments. Mean TP, TSS, and chl α concentrations were 121 µg/L [0.121 mg/L], 32 mg/L, and 6.2 µg/L [0.062 mg/L], respectively. TP and TSS concentrations were highest in May and October at the two more downstream monitoring stations when more watershed runoff was entering the bay. TP and TSS concentrations were more variable at the most upstream monitoring station which is most strongly influenced by variability in Pokegama River inflows. Just as occurred in Allouez Bay, chl α concentrations were highest in July and August when watershed runoff was low and water clarity was higher than the other months.

- Kimball's Bay (101 acres) is the smallest of the three bays. Steep sloped, wooded banks line the bay's perimeter. The narrowness of the bay and the high wooded banks, along with its greater mean depth (Table 1) tend to minimize the frequency and extent of wind-induced mixing relative to other bays, although it is still a shallow system. The single water quality monitoring site in the bay is close to the bay mouth and strongly influenced by seiche-induced mixing of main channel and Lake Superior water.

- Kimball's Bay was more frequently stratified (i.e., temperature, DO, and other parameters) than other bays, despite the seiche influence. Phosphorus release from sediment during periods of bottom water anoxia was evident and prolonged during July and August. Inflow from the small tributary stream appeared to be mostly flowing along the bottom of the bay and producing higher turbidity (implying higher TSS) near the bottom. Mean TP, TSS and chl α concentrations were 63 µg/l [0.063 mg/L], 5 mg/L, and 7.6 µg/L [0.076 mg/L], respectively. TP concentrations were somewhat higher in May and October and increased from mid-June to early September, presumably due to sediment phosphorus release. As for the other bays, TSS levels were higher in May and October and chl α levels were higher in July through early September when water clarity was higher.

For the three bays, mean TP concentrations were 2-4 times higher than those found in the rest of the SLRE (Bellinger, et al., 2016). Mean chl α concentrations were lower than those found in the rest of the SLRE. Mean TSS concentrations were lower at the Kimball's Bay site and higher at the Allouez and Pokegama Bay sites compared to the rest of the SLRE (see Table 4).

Table 4. Summary of Mean Total Phosphorus, Total Suspended Solids and Chlorophyll α Concentrations in 2017

	Size (acres)	Mean Depth (ft)	Mean TP (µg/L)	Mean TSS (µg/L)	Mean Chl a (µg/L)
Allouez Bay	1,011	6	**85 [0.085 mg/L]**	**21 [0.021 mg/L]**	7.1 [0.0071 mg/L]
Pokegama Bay	441	5	**121 [0.121 mg/L]**	**32 [0.032 mg/L]**	6.2 [0.0062 mg/L]
Kimball's Bay	101	12	**63 [0.063 mg/L]**	5 [0.005 mg/L]	7.6 [0.0076 mg/L]
Estuary Mean	NA	NA	31 [0.31 mg/L]	11 [0.011 mg/L]	9.4 [0.0094 mg/L]

(Bold #s indicates values higher than the estuary mean)

Water quality monitoring was also conducted in the tributary streams that enter these bays. Stream TP and TSS concentration means ranged from 106-224 µg/L [0.106-0.224 mg/L] and 28-106 mg/L, respectively. Watershed non-point sources of phosphorus include pasture and hayfield runoff (including the influence of manure spreading), barnyards, and septic systems. Streambank and bluff erosion along streams is not believed to be a large phosphorus source (Bahnick, 1977), but is believed to be the largest source of TSS. Additional tributary information is contained in the full report in Appendix 3.

Findings: Bay Chlorophyll α Relationship to Other Trophic State Indices

Chl α concentrations in the three bays were much lower than would be predicted based on TP concentrations using either the Carlson Trophic State Index (TSI; Carlson, 1977, used to measure biological productivity) or the MN and WI statistical modeling of relationships between TP, Secchi depth, and chl α for inland lake assessments (MPCA, 2016 and WDNR, 2020). Chl α concentrations were only 3 -18% of what is typically found at the TP concentrations predicted by the Carlson, 1977 equations. Water clarities (i.e., Secchi depths) were also lower (i.e., poorer) than typical for comparable chl α concentrations.

Total algal cell densities were highest in all bays in July, August, and September. Pokegama Bay had the highest total cell density on July 10th (10,343 cells/ml). All algal phyla occurred in higher densities during those three months. Total suspended solids concentrations and turbidity were lower during these months, which increased light availability for algal growth (see further discussions below). Water temperatures were higher during these months which can also promote algal growth.

Poor light availability due to suspended sediment and dissolved organic carbon (as opposed to nutrients like phosphorus and nitrogen) likely limits algal growth in the bays, as also happens in shallow, turbid lakes. The brown "tea" color of SLRE waters, from dissolved organic matter draining from wetlands, also contributes to lower light availability for algal growth, as well as its high variability. Lack of typical TSI

parameter relationships complicates water quality goal setting since it makes it difficult to predict responses to water quality improvements.

Findings: Bay Sediment Characteristics

Mean clay content of sediment in all three bays (40 – 46%) was significantly higher than that found in the remainder of the central and lower SLRE, where clay content averaged about 14.7% (NOAA DIVER 2018); this was not surprising given the clay-rich soils in the watersheds of the bays. Clay content of sediment (% clay) was moderately well correlated with phosphorus concentration ($R^2 = 0.75$) and iron concentration ($R^2 = 0.76$); this was also not surprising since iron readily attaches to the extensive bonding surfaces of clay particles and phosphorus attaches to the iron.

Findings: Clay-Influenced Bay Biological Indicators

Multiple biological indicators were assessed to provide a better understanding of how water quality conditions effect the habitat and overall biological health of the bays. Four biological areas were examined and described below: benthic macroinvertebrates, aquatic macrophytes, wetlands, and the fishery. The results for each community, separately and collectively, provided further lines of evidence that an impairment does not exist. The bays were shown to sustain adequate biological health despite TP conditions that exceeded the BUI removal criterion.

Benthic Macroinvertebrates

The trimetric index (TMI) (Angradi, et al., 2016), an index of benthic invertebrate community quality, was developed specifically for the SLRE. Due to their unique clay conditions, Allouez and Pokegama Bays were excluded from the development of the TMI and the accompanying ephemerid density index. This complicated the interpretation of the benthic data conditions reported, however the TMI was the most useful benthic invertebrate indices available for these bays and provided a basis of comparison to the rest of the SLRE.

The median TMI value was poor for Allouez Bay, fair for Pokegama Bay, and poor for Kimball's Bay. The quality of the benthic invertebrate community in all three bays was below average in comparison to the rest of the SLRE. The physical characteristics of sediment with high clay content (and corresponding high-water content) likely provided poor habitat for some benthic invertebrates in these bays. Periods of anoxia at two sites in Kimball's Bay probably also contributed below average benthos.

The median ephemerid (mayflies) density index value (Angradi et al. 2016) was good for Allouez Bay, excellent for Pokegama Bay, and poor for Kimball's Bay, with Allouez and Pokegama Bays above average in comparison to the rest of the SLRE.

Aquatic Macrophytes

Aquatic macrophyte surveys from 2004-2015 were summarized and statistically analyzed (Danz, et al., 2017) to develop the Coefficient of Conservatism (C*) as an index of tolerance to disturbance (see Table 5). Mean C* values for Allouez and Pokegama Bays were similar and somewhat higher than the mean for all SLRE surveys, while Kimball's Bay was substantially poorer, likely due to physical conditions and less littoral zone area in the bay compared to the other Bays and SLRE.

Table 5. 2017 Aquatic Macrophyte Survey Data for Management Action 6.04

	Allouez Bay	Kimball's Bay	Pokegama Bay	All SLRE surveys
Number of species	155	74	148	NC**
Species per plot	8.8	5.0	5.8	NC**
Mean C* value	5.6	3.6	5.4	5.06

C* = coefficient of conservatism, an index of tolerance to disturbance. **NC = not comparable because a number of species and species per plot are influenced by the size of area surveyed and survey methods, and so the data do not offer a simple means of comparison.

Wetlands

Recent wetland monitoring data (2011-2017) was available for all three bays from the Great Lakes Coastal Wetland Monitoring Program (Brady, 2018).

Wetland nutrient, turbidity, and chl α concentrations were generally similar to those found at open water sampling sites in 2017, although Kimball's Bay TP concentrations were higher than in open water.

Daytime DO concentrations in wetlands were low (<3 mg/L) for 5-25% of the measurements, with Kimball's Bay having the most low oxygen periods.
Wetland macroinvertebrate IBI's were taken from the Coastal Wetland Monitoring Program (CWMP) data for Allouez and Pokegama Bays for 2011 and 2012. Most Allouez Bay sites showed moderate impacts, while Pokegama Bay showed moderate impacts to most pristine.

Wetland fish IBI ratings for 2011, 2013, 2015, and 2016 for Allouez Bay ranged from moderate impacts to mild degradation. The rating for the 2017 fish study (Nelson, 2018) was generally similar; Pokegama Bay showed mild impacts and Kimball's Bay showed moderate degradation.

Wetland bird and frog survey results (2012-2013) were also available for Allouez and Pokegama Bays (Tozer 2014) and for one or more years during 2014 -2017 for all three bays (Brady, 2018). A summary of the wetland bird and frog survey assessments were compiled in Table 6 and Table 7.

Table 6: Summary of Bird Survey Data for Management Action 6.04

Year	Allouez Bay	Pokegama Bay	Kimball's Bay	L. Superior Coastal Wetlands
2012	Fair IBI	Fair IBI	NA	Fair for 14 sites
2013	NA	NA	NA	Fair for 14 sites
2014	High quality IEC	NA	NA	NA
2015	NA	NA	NA	NA
2016	High quality IEC	Mildly impacted	Degraded	NA
2017	High quality IEC	NA	NA	NA

Table 7: Summary of Frog Survey Data for Management Action 6.04

Year	Allouez Bay	Pokegama Bay	Kimball's Bay	L. Superior Coastal Wetlands
2012	Good	Very Good	NA	Excellent for 13 sites
2013	Good	Very Good	NA	NA
2014	Excellent	NA	NA	NA
2015	NA	NA	NA	NA
2016	Excellent	Moderately Impacted	Moderately Impacted	NA
2017	Excellent	NA	NA	NA

Fishery

Bay fisheries were monitored during 2017 using gill nets and shoreline electrofishing and compared to 2017 estuary wide gill netting data from MNDNR (Nelson, 2019). Results are summarized in Table 8.

Table 8. Comparison of 2017 St. Louis River Gill Net Data for Management Action 6.04

Gill Net Data	Allouez Bay	Kimballs Bay	Pokegama Bay	21 MN SLRE gill net sites
Total number of species	12	6	9	19
Median number of species/net lift	9	3	9	8
Mean fish/net lift	39.9	3.6	19.3	27.5
Mean kg fish/net lift	21.9	1.3	8.3	13.0
Gill Net plus Electrofishing Data				
Total number of species	22	15	21	not applicable
Number of native species	18	14	16	not applicable
Number of non-native species	4	1	5	not applicable
Number of intolerant species	4	4	3	not applicable

Allouez and Pokegama Bays gill net data was generally similar to data collected by the MNDNR during 2017 from 21 SLRE gill net sites for number of species/net lift, mean fish/net lift, and mean kg of fish/net lift. Data from Kimball's Bay indicated a poorer fish community than the MNDNR data averaged from 21 sites within the SLRE.

The conclusion from the fishery survey report stated: "Despite turbid conditions that may lead to the perception of poor water quality or habitat, locally popular sport fish species like walleye, northern pike, black crappie, and yellow perch were well represented in both Allouez and Pokegama Bays. Other species of interest to anglers and state fisheries management agencies were also found in these bays including lake sturgeon, muskellunge, bluegill, and channel catfish. While increased turbidity in Allouez and Pokegama Bays may influence the presence or abundance of specific species, it has not diminished the fishery value or eliminated desirable gamefish species from these areas." (Nelson, 2019)

Biological Indicators Summary

Although nutrient and sediment loads were higher in the clay-influenced bays than in the other areas of the SLRE, this study showed that biotic health was not limited as a result, as seen in the summary of available biological indicators for the three bays in Table 9.

Table 9: Summary of Biological Indicators for Management Action 6.04

BIOLOGICAL COMMUNITY	INDICATOR	ALLOUEZ BAY	KIMBALLS BAY	POKEGAMA BAY
Benthic invertebrates	Trimetric index[1]	median = poor (poorer than average for SLRE)	median = poor (poorer than average for SLRE)	median = fair (poorer than average for SLRE)
Ephemerid mayflies	Ephemerid density index[1]	median = good (better than average for SLRE)	median = poor (poorer than average for SLRE)	median = excellent (better than average for SLRE)
Aquatic macrophytes	Species richness[2]	155	74	148
Aquatic macrophytes	Species richness per plot[2]	8.8	5.0	5.8
Aquatic macrophytes	Mean C value[2]	5.6; species that tolerate moderate disturbance; better than SLRE mean value of 5.06	3.6; generalist species that are tolerant of disturbance; poorer than SLRE mean value of 5.06	5.4; species that tolerate moderate disturbance; better than SLRE mean value of 5.06
Bay fish	multiple[6,7]; no applicable IBI available	Number fish/gill net lift = 145% of 21 site SLRE mean; kg fish/gill net lift = 168% of 21 site SLRE mean; number species /gill net lift = 112% of 21 site SLRE median; % native species = 92%; number of intolerant species = 4; "...popular sport fish species...are well represented in Allouez ...Bay."	Number fish/gill net lift = 13% of 21 site SLRE mean; kg fish/gill net lift = 10% of 21 site SLRE mean; number species /gill net lift = 38% of 21 site SLRE median; % native species = 99%; number of intolerant species = 4	Number fish/gill net lift = 70% of 21 site SLRE mean; kg fish/gill net lift = 63% of 21 site SLRE mean; number species/gill net lift = 112% of 21 site SLRE median; % native species = 79%; number of intolerant species = 3; "...popular sport fish species ... are well represented in ... Pokegama Bay."

Table 9 (continued): Summary of Biological Indicators for Management Action 6.04

BIOLOGICAL COMMUNITY	INDICATOR	ALLOUEZ BAY	KIMBALLS BAY	POKEGAMA BAY
Wetland Macroinvertebrates	Wetland macroinvertebrate IBI[4]	2011, 2012 = moderately impacted; not enough non-clay influenced SLRE surveys to allow comparison.	IBI not available	2011, 2012 median = mildly impacted; not enough non-clay influenced SLRE surveys to allow comparison.
Wetland Vegetation	Wetland vegetation IBI[4]	2011-2017 median = moderately impacted = median for non-clay influenced SLRE surveys	2014, 2016 = moderately degraded, which is poorer than the median for non-clay influenced SLRE surveys (moderately impacted).	2011, 2012, 2016 median = moderately impacted = median for non-clay influenced SLRE surveys
Wetland Fish	Wetland fish IBI[4]	2011-2017 median = moderately impaired to moderately degraded, which is slightly poorer than the median for non-clay influenced SLRE surveys (moderately impaired).	2014 = moderately degraded, which is poorer than the median for non-clay influenced SLRE surveys (moderately impaired).	2012 = mildly impacted, which is better than the median for non-clay influenced SLRE surveys (moderately impaired).
Wetland Birds	Bird IBI[3]	31.8; fair - just below median value of 33.3 found for 14 Lake Superior coastal wetlands, mostly outside of SLRE	no data	34.0; fair - just above median value of 33.3 found for 14 Lake Superior coastal wetlands, mostly outside of SLRE
Wetland Birds	Bird IEC[4]	2014 2016, 2017 median = high quality, which is better than the median for non-clay influenced SLRE surveys (moderately impacted)	2016 = degraded, which is poorer than the median for non-clay influenced SLRE surveys (moderately impacted)	2016 = mildly impacted, which is better than the median for non-clay influenced SLRE surveys (moderately impacted)
Wetland Frogs	Frog IBI[3]	60.0; good - below median value of 86.5 found for 13 Lake Superior coastal wetlands, mostly outside of SLRE	no data	70.3; very good - below median value of 86.5 found for 13 Lake Superior coastal wetlands, mostly outside of SLRE
Wetland Frogs	Frog IEC[4]	2014 2016, 2017 median = reference condition, which is better than the median for non-clay influenced SLRE surveys (mildly impacted)	2016 = moderately degraded, which is poorer than the median for non-clay influenced SLRE surveys (mildly impacted)	2016 = moderately impacted, which is poorer than the median for non-clay influenced SLRE surveys (mildly impacted)

[1]Angradi, TR, Bartsch, WM, Trebitz, AS, Brady, VJ, Launspach, JJ. 2016. A depth-adjusted ambient distribution approach for setting numeric removal targets for a Great Lakes Area of Concern beneficial use impairment: degraded benthos. J Great Lakes Res.

[2]data from Danz, et al. 2017 (get full reference)

[3]Tozer, D. 2014. LSRI nearshore monitoring project: 2012-2013 bird and frog indices of biotic integrity. EPA assistance no. GL00E00500-0.

[4]Uzarski, DG, et al. 2017. Standardized measures of coastal wetland condition: implementation at a Laurentian Great Lakes basin-wide scale. Wetlands (37:15).

[6]Nelson, A. 2018. St. Louis River Bays – Douglas County; 2017 fish community survey. Wisconsin Dept. of Natural Resources, Superior, WI. Unpublished report.

[7]Pinkerton, J. 2018. Personal communication. Minnesota Dept. of Natural Resources fisheries specialist, Duluth, MN.

Conclusions: Clay-Influenced Bays

Considering the findings of the SLRE Clay-Influenced Bay Assessment, members of the BUI Technical Team reached agreement that establishing site specific water quality goals for the Wisconsin bays would not be necessary. Many standard indicators were not tailored to these unique estuary and clay-influenced conditions and best professional judgement was needed to properly interpret these results. Although this study was a comprehensive look at the bays, tributary streams and biota, it was limited to one season of water quality data and only limited conclusions could be made. However, the biological condition in the bays was dependent on water quality and a better long term indicator of bay health was shown to be the condition of aquatic life. Despite some differences seen amongst the bays and between the bays and the remainder of the SLRE, the study did not indicate that the biota in these environments were impaired by higher levels of sediment and nutrients. In fact, the study found that some of these areas contained unique high quality habitats and species assemblages.

Monitoring of aquatic life in the SLRE will continue because aquatic life is one beneficial use addressed under MPCA's and WDNR's 303(d) programs. One goal of these 3030(d) programs is to reverse identified impairments and protect beneficial uses according to the requirements of the CWA.

6.04 Overall Conclusions: Develop Water Quality Goals (Compilation of 6.01, 6.02, & 6.03)

The findings of MAs 6.01, 6.02, 6.03 and the SLRE Clay-Influenced Bay Assessment support BUI removal.

The comprehensive approach used to assess the current status against BUI criteria included studies that detailed historical, current, and site-specific water quality and biologic indicators. The BUI Technical Team was part of the review and discussion of each of the studies. Due to the magnitude of unique conditions and habitats found in the AOC, specific water quality goals were not established in addition to the BUI removal target. BUI criteria for the SLRE (0.030 mg/L) and Lake Superior (0.010 mg/L) remained an appropriate measure of nutrient improvements for the SLRAOC.

Estuary Conditions

The BUI Technical Team and AOC Coordinators concluded that, given that a large percentage of the area in the SLRE is composed of clay-influenced bays that have unique combinations of water quality

indicators that are due to natural background conditions, the 60% of the SLRE that met the BUI TP criterion of 0.030 mg/L phosphorus during the study period fulfilled the criteria for BUI removal. Additional water quality indicators were used to support this conclusion, including improving trends in TSS, DO, and chl α (see Table 10). The clay-influenced bay study supported the hypothesis that the SLRE ecosystem was reasonably well adapted to current sediment, nutrient, and other biophysical conditions, and no AOC impairment caused by excessive sediment and nutrients remained.

Lake Superior Conditions

The BUI Technical Team and AOC Coordinators concluded that information from MA 6.01, 6.03 and 6.04 suggested average Lake Superior water quality was not exceeding the BUI criterion of 0.010 mg/L TP; therefore, the BUI removal criterion was met.

Multiple data sources were used to evaluate the Lake Superior-specific BUI criterion. The inferred phosphorus conditions from the paleolimnological core (MA 6.03) showed that Lake Superior conditions had not exceeded the BUI criterion. The core location is within the AOC boundary and showed concentrations of TP ranging from 3 - 6 µg/L [0.003 to 0.006 mg/L].

MA 6.01 also gathered data from Lake Superior sample locations, but the average values were slightly above the criterion (0.0127 mg/L). This is attributed to data from this assessment being collected in nearshore conditions, which were likely biased toward St. Louis River conditions due to river water mixing with the lake at the sample sites. DO and chl α data were consistent with oligotrophic waters.

Additional data for the western Lake Superior sampling point (SU 19), which is part of the USEPA's Great Lakes Biology Monitoring Program (1983- present; https://www.epa.gov/great-lakes-monitoring/great-lakes-biology-monitoring-program), were reviewed to supplement the findings in MA's 6.01 and 6.03. This sampling point was not located within the boundary of the SLRAOC, but still provided a longer-term record of the nutrient conditions in the western portion of Lake Superior compared to the data collected in MA 6.01. Select data available from USEPA's Great Lakes Environmental Database via the Central Data Exchange (https://cdx.epa.gov/) was used for this comparison. Data from 1996-2015 showed the mean western Lake Superior TP concentration was 2.6 µg/L [0.0026 mg/L] and the range was 1.0 to 8.0 µg/L [0.001 to 0.008 mg/L] (Table 10, Figure 9). Data selected for BUI comparison represented upper water column samples including epilimnion (top 10 m) or spring integrated sample designations. Hypolimnetic or deep-water samples were excluded from the BUI comparison.

Table 10: Summary of Water Quality Results for Management Action 6.04

Parameter	SLRE, from Fond du Lac dam to Lake Superior (Bellinger, et al., 2016)	Lake Superior[1] (Bellinger, et al., 2016)	Western Lake Superior[2] (USEPA, Great Lakes Biology Monitoring Program 1996-2015)
TP	~60% of area below 30 µg/L [0.030 mg/L]	Average = 12.7 µg/L [0.0127 mg/L]	Average = 2.6 µg/L [0.0026 mg/L]
TSS	>85% of area below 15 mg/L	Average = 4.4 mg/L [0.0044 mg/L]	not assessed
DO	>5.5 mg/L; no hypoxia	Average = 12.2 mg/L	not assessed
chl α	>70% of area below 10 µg/L [0.010 mg/L]; oligotrophic to mesotrophic	Average = 2.7 µg/L [0.027 mg/L]; oligotrophic	not assessed

[1] The interim TP guide for Lake Superior is 0.010 mg/L. Data from this assessment were collected in nearshore conditions, which were likely biased toward SLRE conditions due to seiche mixing.

[2] The USEPA's Great Lakes Biology Monitoring Program sampling point (SU 19) is not located within the boundary of the SLRAOC.

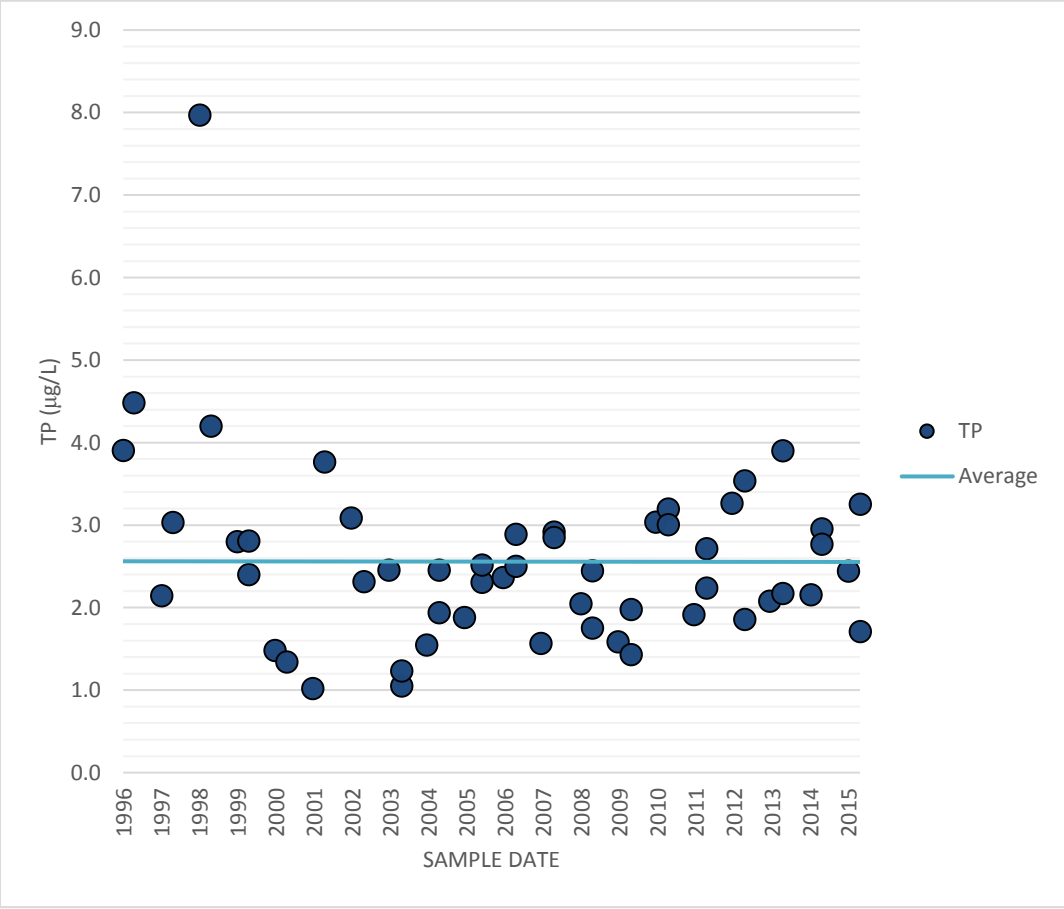


Figure 9: 1996-2015 Lake Superior Upper Water Column Total Phosphorus at SU 19 Great Lakes Biology Monitoring Program

6.05 Nemadji River Basin Studies

The Nemadji River Basin comprises a large portion of the SLRAOC (see Figure 10).

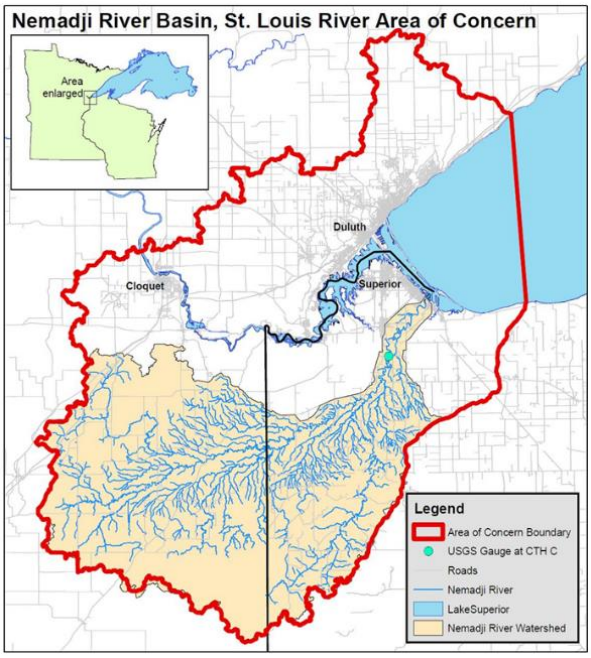


Figure 10: Map of the Nemadji River Basin within the St. Louis River Area Concern

In geologic terms, the Nemadji River Basin is relatively young. The Nemadji River and its tributary streams are still changing to reach slope equilibrium after elevations changed when the Laurentide ice sheet retreated approximately 10,000 years ago. As the river and stream channels adjust, steep valley walls, sloughing clay banks, and high sediment loading to the SLRE and Lake Superior result. Historic logging and agricultural practices have exacerbated the erosion problem in some areas. By removing old growth forest cover and draining wetlands, stormwater runoff to the channel and peak flows are increased. The Nemadji River Basin Project Report (NRCS, 1998) outlines many recommended actions and best management practices to help reduce the impacts of land use on peak flows and sediment loading in the Nemadji River Basin.

As a follow-up to the Natural Resources Conservation Service (NRCS) Report, and to show progress towards implementing the report's objectives has been made, several studies were conducted in the Nemadji River Basin to assess sediment impairments and evaluate the <40% open lands watershed objective that was previously a BUI removal objective. As part of MA 6.05, WDNR (through the Great Lakes Protection Fund) and Carlton County, MN funded a GIS based open lands assessment in the Nemadji River Watershed. A comparison of 2002 data to the 2014 analysis showed that the <40% open land objectives in the Nemadji River Basin Plan had not been met (Appendix 4; Community GIS, 2016).

However, there were several issues with requiring this objective to be met for BUI removal and the <40% open lands objective was removed from the BUI removal strategy in 2014 and MA 6.05 was adapted to assess the biological condition of the Nemadji River and to determine if excessive sediment is an impairment.

The open lands assessment identified small hydrological units within the Nemadji River watershed exceeding 40% open land status by digitizing agricultural and urban land and timber stands ≤15 years in age. The 280,787-acre Nemadji watershed with its 171 sub-watersheds were delineated for this study, of which 26.9% had more than 40% open land.

While land use trends have an impact on peak flows and erosion in the basin, there are several caveats to using the <40% open lands objective in the Nemadji Basin in the RAP. The "open land" classification includes urban, agriculture, grasslands, hay fields, shrublands, and young forest; but each of these land cover types influences peak flows differently (Verry 1976, Verry et al. 1983, Verry 1986). Verry's work found that at moderate percentages (40 -60%) open lands, snowmelt peak flows are desynchronized and thus reduced. Because of this desynchronization and the differences in water uptake among different types of open lands, there is a lack of consensus among resource managers about what the appropriate percent open lands target should be. Also, because "slow the flow" efforts are not limited to reforestation (but also include wetland restoration, ditch plugging, elimination of unused roads, field borders, filter strips, etc.), using the percent of open lands in the basin as the target metric does not accurately assess physical results of efforts that have been implemented to reduce sediment in the Nemadji River. In fact, initial assessments of fish and macroinvertebrates at several sites on the Nemadji in Wisconsin do not indicate there is an impairment due to sediment (Roesler, 2014).

The adapted MA 6.05, described in the following sections, justifies BUI removal based on historical sediment load modeling and biological conditions, and implementation of the Nemadji Basin Plan through stakeholder and landowner planning workshops in the Nemadji River Basin. The planning component included communication of the results of the open lands assessment to stakeholders and landowners in the Nemadji Basin.

SLRAOC managers adopted the strategy to evaluate the Nemadji River through: sediment monitoring and HSPF modelling of historic sediment loads, biological and water quality assessments, and planning efforts to better understand the following conditions.

1. Sediment loading:
    a. During pre-settlement, peak agriculture, and current conditions using an existing HSPF model.
        i. See Appendix 5: Current and Historical Sediment Loading in the Nemadji River Basin (Butcher, 2016)
    b. Comparing current sediment loading to 1970's sediment loads as reported in the Nemadji River Basin Project Report (NRCS, 1998).
        i. See Appendix 6: Sediment Characteristics of Northwestern Wisconsin's Nemadji River, 1973-2016 (Fitzpatrick, 2020)

2. The health of natural biological communities through an assessment of fish, macroinvertebrate, and water quality samples.
    a. See Appendix 7: Lower Nemadji River-Douglas County Fish Community Survey (Nelson, 2015)
    b. See Appendix 8: Nemadji River and Tributaries Water Quality Assessment (Roesler, 2014)
    c. See Appendix 9: Lower Nemadji River Water Quality and Macroinvertebrate Community Assessment (Roesler, 2015)
3. How making progress towards meeting watershed management objectives identified in the Nemadji River Basin Project Report (NRCS 1998) is advanced by completing an implementation planning effort aimed at educating citizens and local government officials in the Nemadji River Basin and identifying landowners to implement best management practices on their property.
    a. See Appendix 10: Nemadji River Watershed Implementation Planning Report (Ostern, 2017)

Each of the listed projects is summarized below and the full reports are contained in the appendices noted above.

Current and Historic Sediment Loading in the Nemadji River Basin

Methods and Findings

As part of their obligation to identify impaired waters and make Total Maximum Daily Load determinations, MPCA developed Hydrologic Simulation Program—FORTRAN (HSPF) models for all eight of the basins in Minnesota. One of the basin models encompasses both the Minnesota and Wisconsin portions of the Nemadji River watershed. These models were developed to better understand water quality and predict how it could change under different land management practices.

Tetra Tech used MPCA's existing HSPF basin model to evaluate and document changes in sediment loading caused by the conversion of land use from old growth evergreen forest to agriculture and new growth forests. The basin-wide HSPF model was calibrated and modified to provide watershed-level detail for the Nemadji River watershed portion of the basin. The model represented sediment loading from both upland and channel sources and provided a credible match to observed suspended sediment concentrations and loads at multiple monitoring points. Due to its relatively coarse spatial scale, the model was not an ideal simulation tool to specifically address loading from bluff slumping, believed to be the major source of sediment loading in the Nemadji. Nonetheless, the Nemadji model provided a useful framework to investigate potential changes in sediment loading over time.

Analyses with the HSPF model were used to compare current conditions to the probable sediment loading patterns under pre-settlement conditions (prior to harvesting of the mature white pine forests that previously covered most of the watershed) and under peak agriculture conditions. A date around 1930 was selected for peak agriculture primarily due to the availability of the Bordner Survey maps that provide a detailed representation of land use and land cover in Wisconsin during the Great Depression. (In fact, peak agriculture in the basin may have occurred somewhat later, during the 1940s and 1950s,

but comparable land use surveys are not available for that time period.) Pre-settlement conditions were based on survey notes from the original Land Office grants in the watershed in the 1850s.

Initial settlement of the watershed was followed by harvest of the old-growth evergreen forest, followed by drainage and conversion to agricultural uses. Massive fires further altered the watershed and its sediment generating characteristics at the beginning of the 20th century. The early period of forest harvest included massive disruption to the natural stream network as channels were straightened and de-snagged to promote floating of logs to mills in Superior, WI, including use of splash dams that were used to build up flow and then dynamited to so logs could move downstream. Insufficient records existed to simulate the likely massive impacts on sediment loading that occurred from these events.

By the 1930s almost all of the old growth forest had been cut and areas that were previously in mature white pine had either been replaced by subsistence agriculture (primarily small grains) or reverted to second-growth deciduous aspen forest. These conditions promoted increased sediment loading from the uplands and also increased peak flows in the streams, which likely exacerbated erosion from stream banks and bluffs. Another important change was the drainage of wetlands, which were estimated to have declined from 38% of the watershed during the pre-settlement era to 13% of the watershed ca. 1930. This caused a loss in the wetland functions of mitigating peak erosive flows in streams and trapping sediment eroded from the uplands. Since the 1930s, wetlands have increased to approximately 17% of the watershed, but most of the recovery has been in herbaceous wetlands rather than the pre-settlement dominance of forested wetlands.

A major unknown in the analyses was how channel geometry may have changed from pre-settlement conditions. No data were available for the pre-settlement period, but anecdotal evidence suggests that stream channels may have been more stable, with greater roughness (due to large wood debris), greater sinuosity (and thus smaller slope), and less entrenchment of stream channels.
It must be recognized, however, that the Nemadji watershed is geologically young, with unstable clay soils. The Nemadji River is a highly erosive system influenced by ongoing slope adjustments to post-glaciated conditions, especially the changing base levels in Lake Superior resulting from glacial recession.

Application of the HSPF model suggests that upland sediment loads in the Nemadji watershed increased more than threefold from pre-settlement conditions to ca. 1930, but have since recovered to the point that current upland loads are less than twice pre-settlement loads (see Table 3.3 in Appendix 5). The major sediment source in the Nemadji is from bank and bluff contributions (estimated at about 75% of the total load). The bank and bluff contributions do not change much under model simulations that assume channel geometry pre-settlement is similar to current conditions. However, reasonable assumptions about pre-settlement channels with greater sinuosity and lower gradient prior to logging suggest this component, while still significant, may be about 27% less than under current conditions.

In summary, clear-cut logging during the late 19th century increased sediment loads over three-fold from the pre-settlement era. The end of landscape-scale logging decreased sediment loading. Additional progress in reducing loads has been made since the peak agriculture period, but upland loads were still

estimated to be nearly double those that would have occurred under pre-settlement white pine and forested wetland cover.

The total sediment load is primarily derived from channel erosion and bluff slumping where the river intersects the valley walls, a natural characteristic of the watershed. That problem is exacerbated by changes in land use in the watershed that increase peak flows. Therefore, reducing erosive flows in the Nemadji and its tributaries (e.g., through wetland restoration) can help ameliorate, but not eliminate, sediment loads from these sources.

Processes such as evolving head cuts, ongoing expansion of the drainage network, and responses to changing base levels in Lake Superior that affect channel bank and bluff erosion may not be readily amenable to management interventions.

It should be noted that the modeling conducted for this analysis is limited in its predictive power because the HSPF model is constructed at a relatively coarse scale (approximately HUC12 sub-basins) and quantitative data on contributions from bluff and channel bank sources is lacking. Nonetheless, the model provides a credible basin-scale indication of the changes that have likely occurred over time. Use of a finer-scale model informed by detailed stream surveys would help in identifying local hotspots of sediment loading where management intervention might be beneficial. More sophisticated channel evolution models, informed by detailed channel measurements, would also help to better constrain model predictions.

Sediment Characteristics of Northwestern Wisconsin's Nemadji River, 1973-2016

Methods and Findings

Over the last 45 years, a variety of sediment samples were collected and analyzed periodically using different field and analytical techniques by the USGS, the WDNR, and the MPCA at the USGS stream gage on the Nemadji River near South Superior (USGS identification number 04024430). Most of the samples were of suspended sediment concentration. In 1973-86, the USGS collected samples for suspended-sediment concentration analysis and a limited number of bedload samples, including two in 1978. Starting in 2006 and continuing through the present, the WDNR and MPCA have been collecting TSS data.

Three objectives were identified for this study:

1. Develop a calibration curve between suspended sediment-concentration (SSC) and total suspended solids (TSS) data.
2. Compare SSC-based sediment rating curves from the 1973-86 with adjusted TSS-based curves from 2006-15 and determine if there has been a change in suspended sediment discharge.
3. Describe 2015-16 total sediment discharges, comparing USGS and WDNR data, which were determined directly by collecting suspended sediment, bedload and bed material samples and measuring suspended sediment discharge and bedload discharge and indirectly by calculating total sediment discharge using the modified Einstein procedure.

Study methods included:

- Gathering published historical and ongoing sediment concentration, water discharge, and sediment discharge data collected by the USGS, WDNR, and MPCA at the USGS stream gage on the Nemadji River.
- Collecting comparative measurements of suspended sediment, bedload, and bed material in 2015-16.
- Calculating instantaneous total sediment discharges for 2015-16 samples by summing the measured suspended sediment discharge and bedload discharge.
- Calculating the estimated total sediment discharge using the modified Einstein procedure.
- Comparing sediment concentration-water discharge rating curves using analysis of covariance.

Hydrologic conditions were variable over the two periods of historical suspended sediment data collection. Mean annual flows during 2006-15 were about 84% less than during 1973-86. In contrast, two extreme floods in 2011 and 2012 were over 2.5 times larger than any peak flow in the 1973-86 period.

The 2009-16 annual total sediment discharges ranged from a low of 18,000 tons/year in 2015 to almost 180,000 in 2012. Bedload discharges ranged from 20 percent of the total sediment discharge during low mean annual flow years to only 5 to 6% during high flow years. A sediment rating curve for suspended sediment concentration and water discharge for 2006-15 had a similar slope but a lower intercept than its 1973-86 counterpart. Although not statistically significant, the negative offset resulted in a potential reduction of about 15% of the annual suspended sediment discharge for an example data set of annual discharges from 2009-16. Altogether, these various data sets collected over different time periods and using different methods helped to describe present and past sediment characteristics as well as provide a calibration tool for future sediment data collection.

The hydrologic context with what is seeming to have more year-to-year variability will likely become more important than the overall value of annual loading at face value. The 10-fold increase in the size of sediment discharges during extreme floods compared to more average flood condition suggests that restoration done at the mouth of the Nemadji River needs to be resilient to large floods and sporadic, highly variable sediment deposition, even though overall the amount of suspended sediment per unit of water discharge may have been reduced.

6.05 Sediment Loading Assessment Conclusions

Results from both sediment loading assessments document a recovery from higher sediment loading in the past. These results support the efforts of the Nemadji River Basin Plan (NRCS, 1998) and the “slow the flow” initiative as watershed management programs continue making progress toward sediment load reduction and meeting plan objectives.

Lower Nemadji River-Douglas County Fish Community Survey
Methods and Findings

This work was completed to assess the fish community present in the Lower Nemadji River watershed (Nelson, 2015). Electrofishing sampling was conducted at six wadable and non-wadable stations. This method was chosen because it eliminated bias from net locations, mesh sizes or openings on nets or traps, or fish behavior and allowed for standardized Index of Biotic Integrity sampling. All captured fish were identified by species. Gamefish and panfish species were measured to the nearest tenth of an inch and larger individuals were weighed. All other non-gamefish species were counted. All fish captured in the survey were released back to the river, except for voucher species used to confirm species identification.

At the time of the study, the Nemadji River supported a diverse, primarily native, fish assemblage; 24 different fish species were documented in the 6 stations assessed in 2015. Minnows were the most abundant and widely distributed fish species and were represented mainly by common and emerald shiners. Silver redhorse, shorthead redhorse, rock bass, smallmouth bass and walleyes were also widely distributed throughout the Nemadji River, but didn't occur in the higher abundance seen in the minnow species. Muskellunge, largemouth bass, yellow perch, and channel catfish were also present, but in smaller numbers.

The fish communities from each station were scored and rated using the Lake Superior warmwater IBI rating to determine if the site is degraded and to what extent. Despite not being able to incorporate weight data, the non-wadable stations that were assessed scored between 56.25 and 75 points and were minimally rated from "Fair" to "Good". The IBI score for the wadable stations were rated as "Excellent". Despite relatively poor instream and riparian habitat in the Lower Nemadji River and some difficulty sampling fish, the fish communities documented reflected good water quality. In some instances, the lower scores for the IBI metrics reflected lower fish diversity in the Lake Superior basin rather than environmental degradation.

Nemadji River and Tributaries Water Quality Assessment
Methods and Findings

The Nemadji River and five of its tributaries (Crawford Creek, Black River, Balsam Creek, Clear Creek, and Mud Creek) were monitored for fish and macroinvertebrate communities, water chemistry, and stream habitat from 2008-2010 to assess water quality conditions and to determine if these streams should be placed on Wisconsin's 303d list of impaired waters (Roesler, 2014).

Fish communities were assessed by electroshocking and calculating IBI ratings. Macroinvertebrate communities were assessed by collecting kick samples from riffles. Water samples were collected and field parameters were measured following standard WDNR protocols. Stream habitat was assessed based on fish community-temperature relationships.

Fish community IBIs on the Nemadji River, Black River, Balsam Creek, and Mud Creek were rated excellent and the IBI for Clear Creek was rated good. The fish community IBI for Crawford Creek was rated as fair. Macroinvertebrate IBI ratings were excellent or good at all sites except Crawford Creek, which was rated as fair. Hilsenhoff biotic index ratings (mostly influenced by organic matter loading and the resultant dissolved oxygen concentrations) ranged from good to excellent. Streams ranged from cool-cold headwaters to warm mainstems.

Sampling frequency and duration for water chemistry varied by site; no water samples were collected from the Black River. Median concentrations of TP and TN were low to moderate at the two Nemadji River sites, with more than one nutrient sample analysis. These sites had low concentrations of ammonia and nitrate plus nitrite. All sites had fairly high TSS concentrations, fairly high turbidity, and fairly low transparency. Daytime DO concentrations were generally good. Median conductivities ranged from 195 – 520 µmhos/cm and pH median values ranged from 7.5 to 8.0.

Common stream concerns in this area include:

- High peak flows resulting from rapid runoff from clay soils.
- Low base flows resulting from limited groundwater discharge.
- Stream bed scouring and bank erosion resulting from high peak flows.
- High bed loads of sand and silt, reducing the substrate quality for fish and macroinvertebrates.
- High TSS and turbidity, and low transparency resulting from erosion of clay soils.

The Nemadji River was added to Wisconsin's 303d list in 2010 based on the state's narrative standard due to its high sediment load (Wisconsin does not have a standard for turbidity or TSS). The Nemadji River was placed on Minnesota's 303d list in 2004 due to exceedances of Minnesota's turbidity standard. The two states are working together to develop a comprehensive turbidity Total Maximum Daily Load for the entire watershed. Crawford Creek was placed on Wisconsin's 303d list in 1998 due to chronic aquatic toxicity. The data collected from this project did not support 303d listing of any of the other streams monitored.

Lower Nemadji River Water Quality and Macroinvertebrate Community Assessment Methods and Findings

Past water quality monitoring in the lower 8.8 miles of the Nemadji River was affected by Lake Superior's seiche causing partial backflow in the lower river. Previously, the most downstream water quality data was collected at the County Rd C crossing, 11.9 miles above the river mouth. Furthermore, deep water and lack of coarse substrate discouraged macroinvertebrate sampling, with the most downstream macroinvertebrate sample previously collected at County Rd W, 31.2 miles above the river mouth. With higher percentages of urban and agricultural land use in the lower portion of the watershed, inflow from Crawford Creek and discharges from point source outfalls could have been expected to contribute to poorer water quality and macroinvertebrate communities in the lower portion of the watershed, which is only 3.7% of the total watershed area. Therefore, monitoring of water

quality and macroinvertebrate sampling were done in 2015 to evaluate lower river conditions (Roesler, 2015).

Water quality monitoring was conducted monthly at three sites from May to October on the second Wednesday of each month to provide a systematic, random distribution of samples. A Kemmerer sampler was used to collect water samples near the river center, where the river continued to move downstream, in an attempt to avoid the seiche effects of observed backflows moving upstream near the stream banks. Water quality samples were collected and field parameters were measured following standard DNR protocols.

Macroinvertebrate communities were assessed by collecting kick samples at six sampling sites. Due to the lack of riffles and scarcity of coarse substrate (gravel/cobble), all but one sample was collected from woody debris draped with leaf packs and other vegetative debris. One sample was collected from cobble substrate to allow a comparison of a nearby sample collected from woody debris/leaf snags. Samples were preserved in 85% ethanol before the macroinvertebrates were counted and identified to the lowest possible taxa. Biotic indices and other statistics were generated.

Water quality results were as follows:

- DO concentrations exceeded the 5 mg/L water quality standard for fish and aquatic life.
- Conductivity ranged from 93 to 275 µmhos/cm; lowest conductivity occurred when flows were higher.
- Transparency ranged from 3 to 65 cm; lowest transparencies occurred during highest flows. Soil erosion was greatest during high flows.
- TP concentrations ranged from 33 to 501 µg/L [0.033 to 0.501 mg/L]; they were highest when flows were highest. Median TP concentrations (49–56.3 µg/l [0.049-0.0563 mg/L]) were below Wisconsin's stream water quality standard of 75 µg/L [0.075 mg/L]. Relatively higher TP concentrations corresponded with relatively higher TSS and turbidity concentrations. (This is comparable to the findings in Roesler 2015 where larger mean daily flows corresponded with larger concentrations of TP, TSS, turbidity and E. coli.
- Dissolved orthophosphorus (DOP) concentrations ranged from <1.7–13 µ/L [<0.0017-0.013 mg/L]. The percent of TP as DOP ranged from 2.2 – 25%, with a tendency for DOP to comprise a smaller percentage of TP when flows were higher and more particulate bound TP was present.
- Total Kjeldahl nitrogen concentrations ranged from 0.56 to 1.62 mg/L; highest concentrations occurred when flows were higher.
- Ammonium-nitrogen and nitrate plus nitrite-nitrogen concentrations were very low (they ranged from <0.0150 – 0.0303 mg/L and <0.0190 – 0.0868 mg/L, respectively).
- TSS concentrations and turbidity ranged from 5.8 – 393 mg/L and turbidity ranged from 7.1 – 729 nephelometric turbidity units (ntu's). Both parameters were much higher during high flows. Median turbidities ranged from 24.9 to 26.9 ntu's. Although all three sites are in Wisconsin, the values are very close to Minnesota's 25 ntu standard.

Other factors that may be impacting water quality:

- During seiche events, the water back-flowing up the lower reach of the Nemadji River is derived mostly from the SLRE, with additional contributions from Lake Superior. In general, backflow of SLRE water is expected to contribute to lower TP, TSS, and DO concentrations, higher nitrate-nitrogen concentrations, and conductivity and temperature increases.
- Water quality conditions are dominated by upstream inputs. Runoff from the lower Nemadji River sub-watershed is expected to increase concentrations or loads of TP less than 3%. Increased concentrations or loads of TN and TSS are also likely to be small.
- Crawford Creek's watershed is about half the area of the Lower Nemadji River sub-watershed and about 1.8% of the total Nemadji River watershed. The creek is contaminated with creosote and PAH's from a former wood preserving facility, contributing a slight increase in downstream Nemadji River conductivities.
- Three point sources have discharges to the lower Nemadji River that may be impacting its water quality.
    - The Superior combined sewer treatment plant discharges intermittently following heavy rainfalls, when Nemadji River flows are usually high, and so considerable dilution capacity is usually available. However, discharges can, at times, have high concentrations of BOD5 (2-60 mg/L), *E. coli* (100-250,000cfu/100ml), ammonia (0.2-5.36 mg/L), TP (40-793 µg/L [0.040-0.793 mg/L]), and TSS (9-189 mg/L).
    - Enbridge Energy had a much larger than usual pipeline pressure test in 2015 that resulted in water discharges during most of October, slightly increasing TP in the river. At that time, average concentrations of BOD5, ammonia, and TSS were unlikely to produce measurable impacts in the Nemadji River. Conductivity of the discharges was not reported, so that was a possible contributor to higher conductivities in the river.
    - The Burlington Northern Sante Fe Railway Company discharge is comprised primarily of runoff from the taconite storage pile plus a small amount of treated maintenance water; both are treated in a retention/settling pond. With the exception of chloride, this point source appears unlikely to produce measurable impacts to the Nemadji River.

There may be other potential influences on temperature and DO. The Nemadji River widens, deepens, and slows between County Rd C and U.S. Highway 2/53. Solar radiation inputs may also be a contributor to the increases. DO decreases may be due to reduced oxygen solubility that is a function of temperature increases and sediment oxygen demand might be higher in the lower river if temporary deposition of organic solids is occurring due to reduced stream velocities.

Macroinvertebrate sampling did not occur at multiple sites as planned due to low discharge rates and inadequate current velocities that did not meet Wisconsin's protocols for applying WDNR macroinvertebrate biotic indices for streams or rivers. Furthermore, the periodic backflows prevented any accumulation of leaf packs or other vegetative debris on a suitable sampling substrate. Despite this, very healthy macroinvertebrate communities were found at all six sites. All samples had high macroinvertebrate IBIs rated as excellent. Hilsenhoff biotic index values ranged from good to excellent,

indicating oxygen availability is consistently good and little organic pollution is present (Table 11). Species richness ranged from 19 to 41. Percent EPT individuals (Ephemeroptera-mayflies, Plecoptera-stoneflies, Trichoptera-caddisflies) was high (40-75%), and percent Chironomidae individuals was low (2-21%), which both also suggested good water quality.

Table 11. 2015 Lower Nemadji River Macroinvertebrate Sample Results for Management Action 6.05

Site	SWIMS station #	Date	Macroinvertebrate Index of Biotic Integrity (MIBI)	MIBI Condition Category	Hilsenhoff Biotic Index (HBI)	HBI Condition Category
Nemadji R. 15 m DS Dedham Rd.	10044435	11/02/2015	8.75	excellent	3.99	Very good
Nemadji R. 25 m US Finn Rd.	163233	10/22/2015	9.04	excellent	4.96	Good
Nemadji R. 10 m DS Finn Rd.	163233	10/22/2015	9.32	excellent	2.78	Excellent
Nemadji R. 135 m DS STH 35	163048	11/02/2015	8.69	excellent	3.85	Very good
Nemadji R. 60 m US CTH C	163003	10/22/2015	11.62	excellent	3.73	Very good
Nemadji R. 3 mi. DS CTH C	10044397	10/22/2015	11.34	excellent	3.61	Very good

Two samples were collected at one station from different substrates for comparison. The downstream sample was collected from leaf packs snagged on woody debris, while the upstream sample was collected from cobble. The cobble had fairly heavy coatings of periphyton and silt. The sample from cobble had a similar macrophyte IBI, a poorer Hilsenhoff biotic index, higher species richness, a lower percent EPT, and a higher percent Chironomids. The coatings of periphyton and entrapped silt on the cobble substrate were probably a major reason for these differences.

Overall, the high quality of the macroinvertebrate community found in the lower Nemadji River is consistent with past findings for the Nemadji River, despite higher levels of turbidity and sediment loads.

6.05 Water Quality and Biotic Assessment Conclusions

Results from the three assessments document that the biota in the Nemadji River do not indicate an impaired condition in relation to BUI status. These results show that many sites in the Nemadji River Basin contain high quality species assemblages despite the wide variety of sediment conditions present.

Nemadji River Watershed Implementation Planning Methods, Findings, and Conclusions

The purpose of this project was to conduct Nemadji River implementation planning activities as an element of the BUI removal strategy. The work was completed in two phases. During the first phase, a Nemadji River Implementation Plan (Plan) was developed that included the following activities:

- Developed a Nemadji Watershed Implementation Strategy
- Compiled a landowner database
- Compiled natural resource information for the watershed
- Compiled watershed maps
- Developed a newsletter and mail to resident landowners (approximately 1600 residents)
- Conducted a watershed informational workshop
- Coordinated with the Carlton County Soil and Water Conservation District

Implementation of the Plan began during the second phase, during which these activities were conducted by Douglas County, WI:

- Convened and coordinated a Wisconsin stakeholder group
- Developed informational workshops to provide information on water quality issues.
- Identified a minimum of 3-5 landowners who agreed to explore cost-share opportunities to implement best management practices on their property.
- Maintained communication with the Carlton County Soil and Water Conservation District and other groups involved with Nemadji Watershed research to identify ways to continue to collaborate on outreach activities.
- Developed supporting documents that included the Implementation Plan, a map of parcels for landowners that scheduled site visits, stakeholder committee contacts and meeting agendas, an open house flyer, a workshop invitation, a landowner site visit form, a newsletter, and photos.

At the close of this grant project, primary considerations for next steps were recommended, as follows:

- Identifying needs for project design assistance, cost share and other support for implementing best management practices for reducing runoff and erosion
- Developing strategies for continuing funding and outreach efforts in the watershed
- Expanding watershed partnerships to include groups such as (for example) Northern Institute of Applied Climate Science, West Wisconsin Land Trust, Wisconsin Towns Association, Wisconsin Farmers Union, Ruffed Grouse and American Woodcock Society. This will form the foundation for a coalition with the capacity to further develop and implement watershed protection, restoration and participation into the future and beyond any one grant-funded project.

As a result of this work, Douglas County, Wisconsin increased the local capacity for addressing watershed issues in the Nemadji River through the engagement of landowners, community leaders, and local decision-makers. Educational workshops have increased stakeholder knowledge of water resource problems and provided information on best management practices to reduce runoff and facilitate the implementation of projects that will improve watershed health. These accomplishments documented important progress in the effort to promote and implement the Nemadji River Basin Plan (NRCS, 1998) objectives and fulfilled the intent of the Nemadji River Watershed BUI removal strategy.

6.05 Overall Conclusions

The comprehensive assessments and planning effort included in MA 6.05 document Nemadji River Basin water quality, sediment loading, and biological conditions. Results do not indicate that an impairment exists in relation to the SLRAOC BUI removal. Watershed level management and implementation of best management practices identified by MA 6.05 will continue outside of the AOC program.

Future Actions

Sediment and nutrient management in relation to water quality and habitat is an ongoing effort needed on a watershed scale. Following the completion of the management actions for BUI 6, a variety of future actions outside of the AOC program still exist, including: planning, monitoring, and research needs. Additionally, there are a number of programs that are already implementing actions related to modern issues. The following descriptions portray a sampling of the ongoing programs and additional needs, but it is not intended to be a fully inclusive list.

Planning and Program Implementation

The MPCA has completed a Watershed Restoration and Protection Strategy for the upper portion of the Nemadji River Watershed located in Minnesota (MPCA, 2017). This includes a compilation of slump inventories, which show locations that may be contributing to erosion-based P.
Implementation planning for the Minnesota portion of the Nemadji River Watershed is being led by the Carlton County Soil and Water Conservation District following Minnesota's One Watershed One Plan process (https://carltonswcd.org/nemadji-1w1p). The plan is expected to be ready in late 2020.

The cities of Duluth and Superior are implementing Municipal Separate Storm Sewer System Permit programs to manage stormwater in their communities. Implementation of these ongoing programs helps manage runoff and its resultant erosion.

The MNDNR, MPCA, and WDNR websites that contain SLRAOC information will be maintained as information repositories from which stakeholders will be able to obtain information generated to complete this BUI. Although it contains some SLRAOC project information, the St. Louis River Stories and Science website (www.stlouisriverestuary.org) goes beyond the goals of the SLRAOC. It is currently being maintained by the University of Wisconsin-Extension staff and its continuance will depend on

future communication needs identified by the broader SLRE community and the ability to obtain continued funding.

Water Quality and Biological Monitoring

The MPCA currently completes high-resolution stream monitoring at major tributaries to the SLRE. This tributary monitoring approach is in place and will continue in the future. TSS, TP, dissolved orthophosphate, nitrogen and total Kjeldahl nitrogen are sampled 35 times per year and are paired with USGS flow data. This allows the MPCA to determine concentrations and loadings specific to the main tributaries to the estuary on an annual basis. The St. Louis River sampling location is at Scanlon, MN and the Nemadji River sampling site is near South Superior, WI (see Figure 11). The St. Louis River sampling location has consistently low levels of TP and TSS. In general, the Nemadji River carries higher sediment and phosphorus loads to the estuary. This monitoring effort will continue into the future and drives the modeling used to develop Watershed Restoration and Protection Strategies for these two watersheds. MPCA is prepared to ensure that activities are managed so that water quality standards are met at the outlets of these major watersheds.

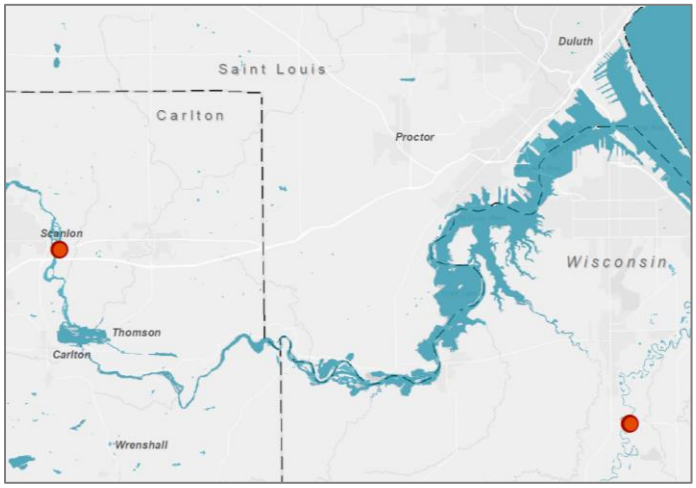


Figure 11: Location of USGS Gaging Stations on the St. Louis and Nemadji Rivers

Moving forward, MPCA and WDNR monitoring staff are conversing to determine what approach and frequency of surface water monitoring in the estuary is appropriate under existing state monitoring programs to determine ambient conditions for aquatic recreation and aquatic life uses.

As these conversations ensue, evaluating whether to use constituent-specific probes or continuous constituent-based surrogate statistic models of nutrient and sediment concentrations at strategic stream locations should be considered as a way to improve concentration monitoring and load estimates for the St. Louis River and the Nemadji River sites. These techniques can help identify real time water quality patterns between sampling events to indicate nutrient fluxes and, in places, the possibility of best management practice-related improvements. The USGS, MPCA and WDNR data from these sites may be appropriate for surrogate model computation. Additionally, historical water quality

data collected by state and federal agencies from the SLRE should be assessed. This could include comparing USGS's Scanlon station nutrient and sediment concentration and load data to MPCA's comparable data, taking into account storm-event flows and utilizing continuous nutrient or sediment surrogate models to improve concentration monitoring, detection of concentration peaks between samples, and load estimates. Such a comparison could also help validate future efforts and identify potential differences in nutrient loads based on sampling methods and how hydrologic conditions are represented in the two data sets.

MPCA and WDNR also have ongoing programs to monitor surface waters and identify impairments under Section 303(d) of the Clean Water Act. Additionally, each agency administers permit programs to address impairments if found in the future. Three tributaries to the St. Louis River are considered impaired for total phosphorus: Bear Creek, Bluff Creek and the Pokegama River; however, these tributaries are located in the clay plain and assessing these waters based on statewide water quality standards may not be appropriate. At this time, there are no 303(d) nutrient impairments in the St. Louis River within Minnesota's portion of the SLRAOC. The Nemadji River is listed as impaired for turbidity in both Minnesota and Wisconsin and is being managed jointly under Total Maximum Daily Load rules.

The Lake Superior National Estuarine Research Reserve routinely monitors water quality under its System-Wide Monitoring Program, which began in 2013. This program perpetuates the long-term data series collected by MPCA under the Milestone Monitoring Program. The Reserve collects and analyzes TSS and nutrients (i.e., TP, TN, dissolved nitrate-N, ammonium/ammonia-N), as well as chl α and DO at both upper river (Oliver Bridge) and lower river (Blatnik Bridge) sites. The sampling locations and collection methodology allow for direct comparison of results to historic MPCA data. Current and future (i.e., post-2013) data can thus be added to the historic sediment and nutrient annual load estimates (using methods of Bellinger et al., 2016) to evaluate long-term water quality trends post BUI 6 removal. This congruence will allow for critical assessment of sediment and nutrient dynamics as the SLRE exits an historic period strongly affected by unregulated discharges and poor land use practices to an era of recovery. The Reserve will continue monitoring water quality to assess impacts from current and future stressors such as precipitation regimes, flood events, and warming temperatures. Additionally, the continuation of chl α and DO monitoring will help assess how future changes impact SLRE's productivity.

There are many other monitoring programs that may also continue to generate SLRE data in the future, such as:

- USEPA's Biology Monitoring Program
- USEPA GLTED's mission-oriented research, including the Cooperative Science and Monitoring Initiative program, as well as remedy and restoration effectiveness monitoring for the AOC program
- CWMP's coastal wetland monitoring

Continuation of USGS cooperative stream flow monitoring is recommended to provide flow context for individual samples collected by state and federal agencies. Hydrologic data from those programs should be mentioned if they are part of future research and watershed improvements.
There is a need to determine how to integrate all these monitoring efforts to develop a collaborative and comprehensive SLRE monitoring program by assessing current monitoring efforts, identifying future monitoring needs and funding sources, and creating a structure to collaboratively administer a comprehensive monitoring program for the SLRE.

Research

Sediment and nutrient cycling and predictors of harmful algal blooms in the SLRE are poorly understood. In particular, there is a need to understand whether recent water temperature changes and shifts to cyanobacterial populations are a factor in TP increases in the SLRE and how those factors are related to sediment cycling. Based on similar observations in degrading systems in western Lake Erie and southern Lake of the Woods (Ontario and Minnesota, respectively), the nearshore eutrophication observed in the SLRE may be due to factors such as periodic recycling of stored sedimentary phosphorus (regulated by the extent and duration of oxygen depletion during warm months coupled with intermittent wind mixing events). These conditions may be further aggravated by climate change related to increased winds and stormwater runoff, more frequent and larger storms, stronger thermal stratification in the ice-free season, or other indirect mechanisms, such as water clarity, light penetration, and nutrient availability. As described above, comprehensive, long-term water quality monitoring with periodic data evaluation and public reporting is needed, including a more detailed paleolimnology investigation of the nearshore environment coupled with a speciation of phosphorous study, development of a nutrient budget, long-term chl α data collection, and a comprehensive food web study. This knowledge will help develop an understanding of factors that may be contributing to nearshore eutrophication in the SLRE, identify vulnerabilities, and provide anticipatory and cost-effective management of the SLRE.

More frequent and intense storms and flood events cause peak flows that generate outliers in data sets that skew background data. An evaluation of peak flows over time is needed to identify how TP and TSS correlate with high flows and at what point higher loads cause nutrient resuspension. These analyses, supported with additional long-term streamflow data and watershed-specific precipitation data, are needed throughout the estuary, including in the clay-influenced bays. Potentially, analyses of nutrient and sediment sources and loads from the smaller, clay-influenced tributaries discharging to the SLRE may require automated streamflow and storm-based water quality sampling to represent rapidly developing conditions leading to nutrient and sediment mobility into the clay-influenced bays. An assessment of the 2012 flood is also needed to determine its effect on post-2013 conditions. Further, a cumulative frequency analysis for both base flow and peak flow regimes is needed to determine the effects of each.

UMD is conducting multiple research efforts related to nutrients and sediments, including evaluating the effect of nutrient and water clarity changes on algal productivity and erosion risk in the Nemadji River Watershed.

Pursuit of the actions described above will be the responsibility of individual organizations, or collaborations of organizations, acting under authorities outside of the AOC program that will exist after BUI 6 has been removed.

BUI Assessment Conclusions

With the completion of the five MA's and their review and interpretation by the BUI Technical Team, the BUI Target has been reached for each of the BUI criteria, as summarized below (see Table 12).

Table 12: Summary of Water Quality Results for Management Action 6.04

Parameter	SLRE, from Fond du Lac dam to Lake Superior (Bellinger, et al., 2016)	Lake Superior[1] (Bellinger, et al., 2016)	Western Lake Superior[2] (USEPA, Great Lakes Biology Monitoring Program 1996-2015)
TP	~60% of area below 30 µg/L [0.030 mg/L]	Average = 12.7 µg/L [0.0127 mg/L]	Average = 2.6 µg/L [0.0026 mg/L]
TSS	>85% of area below 15 mg/L	Average = 4.4 mg/L [0.0044 mg/L]	not assessed
DO	>5.5 mg/L; no hypoxia	Average = 12.2 mg/L	not assessed
chl α	>70% of area below 10 µg/L [0.010 mg/L]; oligotrophic to mesotrophic	Average = 2.7 µg/L [0.027 mg/L]; oligotrophic	not assessed

[1] *The interim TP guide for Lake Superior is 0.010 mg/L. Data from this assessment were collected in nearshore conditions, which were likely biased toward SLRE conditions due to seiche mixing.*

[2] *The USEPA's Great Lakes Biology Monitoring Program sampling point (SU 19) is not located within the boundary of the SLRAOC*

The Removal Target and Criteria Have Been Met

Nutrient and sediment levels have not been shown to impair water quality and habitat, and do not restrict recreation, including fishing, boating, or body contact in the estuary and within western Lake Superior based on the following criteria:

1. *All federal, state, and local point source and nonpoint source discharge permits in the AOC are in compliance with regard to controlling sources of nutrients (particularly nitrogen and phosphorous), organic matter, and sediment;*

   CONCLUSION: As confirmed by permit compliance staff within the WDNR, all eight pollutant discharge elimination system permits within the SLRAOC area are in substantial compliance as of December 2019. Also as of December 2019, permit compliance staff from the MPCA have confirmed that there are 32 pollutant discharge elimination system permits within the SLRAOC area, of which only 21 have nitrogen, phosphorus, TSS and/or CBOD compliance conditions, Eleven permittees do not have nutrient-related requirements. Only one of the industrial permittee with nutrient-related requirements is noncompliant for TSS only and is following MPCA's compliance

processes to address the noncompliance issues. The other 20 permittees with nutrient-related requirements are in substantial compliance with their permits.

Additionally, WLSSD and the City of Duluth are working to meet the conditions of a federal Consent Decree to reduce inflow and infiltration into the sanitary sewer system as a means to reduce sanitary sewer overflows.

Both the City of Superior and the City of Duluth have also invested in stormwater management practices and outreach to reduce the impacts of non-point source, urban runoff.

2. *Total phosphorus concentrations in the Lake Superior portion of the AOC do not exceed 0.010 mg/L (upper limit of oligotrophic range);*

CONCLUSION: Multiple data sources indicated that the Lake Superior portion of the AOC met this criterion (Table ES-1). The Lake Superior data from the 2012 and 2013 BUI study (MA 6.01) showed that TP values were slightly higher than the BUI criterion of 0.010 mg/L for Lake Superior's western arm, with an average of 12.7 µg/L [0.0127 mg/L].[1] Additional water quality parameters sampled during the study show that DO was generally near saturation and the chl α concentrations were consistent with an oligotrophic water body. Paleolimnological study results (MA 6.03) for the Lake Superior sample location concluded that (1) water quality had improvemed from past periods of higher TP concentrations and (2) current prevailing concentrations of phosphorus did not exceed the TP criterion. Specifically, diatom-inferred TP results for the Lake Superior core indicated that western Lake Superior concentrations of TP were 3 - 6 µg/L (0.003 to 0.006 mg/L). TP results for western Lake Superior were available for 1996-2015 from the USEPA's Great Lakes Biology Monitoring Program (USEPA, Great Lakes Biology Monitoring Program, 1983-present; Central Data Exchange). The TP results (see Appendix 11) showed that from 1996-2015 the mean western Lake Superior TP concentration was 2.6 µg/L [0.0026 mg/L] and the range was 1.0 to 8.0 µg/L [0.001 to 0.008 mg/L] and never exceeded the criterion[2].

[1] Data from this assessment was collected in nearshore conditions, which were likely biased toward St. Louis River conditions due to river water mixing with the lake at the sample sites.
[2] The USEPA's Great Lakes Biology Monitoring Program sampling point is not located within the boundary of the SLRAOC.

3. *There are no exceedances of the most protective water quality standard for either state in the western basin of Lake Superior due to excessive inputs of organic matter or algal growth attributed to loadings from wastewater overflows into the St. Louis River;*

CONCLUSION: Data used to assess St. Louis River water quality indicate that the BUI removal criteria (MA 6.01-6.04) have been met. Additionally, these data do not indicate any excessive algal growth in or inputs of organic matter to the SLRAOC. Wastewater

overflows are prohibited by Wisconsin Administrative Code Chapter NR 210.21 and are administered in Minnesota by State Statute 115.03, Minnesota Rule 7050.0210 and Minnesota Rule 7053.0205.
Wastewater overflows, including sanitary sewer overflows, treatment facility overflows and combined sewer overflows have been drastically reduced since the time of AOC listing. Wastewater permits administered by the states have included conditions to reduce and report overflow events. In addition, as of August 2016, all facilities in Wisconsin were required to have developed and be actively implementing a Capacity, Management, Operation, and Maintenance program for operation and maintenance of sanitary sewer collection systems with goals to help address issues of inflow and infiltration which are the primary causes of overflow events. Minnesota's wastewater permitees have met similar facility management requirements. Upgrades to wastewater and collection systems in the past decade have resulted in significant reductions in overflow events. The improvement in DO, TSS and nutrients (Bellinger, et al,, 2016) also support this conclusion.

4. *Total phosphorus concentrations within the St. Louis River portion of AOC do not exceed an interim guide of 0.030 mg/L (upper limit of mesotrophic range) or the most restrictive water quality standards. This ensures that anthropogenic sources and activities in the St. Louis River AOC do not result in excessive productivity and nuisance conditions within the St. Louis River Estuary.*

   CONCLUSION: The 5 MA's that have been completed for this BUI indicated that water quality improvements in the SLRE and Nemadji River watershed have resulted in the majority of the AOC meeting the phosphorus criterion. In addition, other water quality parameters (TSS, DO and chl α) indicate nutrients and sediments are not causing an impairment. Data showed a dramatic decline in TP concentrations and sediment loading in the SLRAOC since the time of listing.

Public Involvement Process

Many types of public involvement activities are conducted as part of the SLRAOC program. Some are specific to projects and BUIs and others are related to the SLRAOC program more broadly and they are too numerous to be mentioned here. Three specific activities fall in the public involvement realm for this BUI:

1. The activities associated with the BUI 6 technical team (see Appendix 12 for the members and their affiliations). The technical team members assisted the SLRAOC Coordinators with activities associated with reaching the RAP's BUI 6 removal target, including: making recommendations on data collection and analyses, reviewing the findings, and providing input on the removal package.
2. The process to obtain public input on the BUI removal package. A thirty-day public comment period about the BUI 6 removal recommendation was held from February 24, 2020 through March 24, 2020. The draft removal document was placed on MPCA's SLRAOC web site and, over the course of the public comment period, there were 110 unique visitors. A public meeting was

scheduled for March 19, 2020, but had to be cancelled due to the need for social distancing due to the coronavirus pandemic. A cancellation notice was sent to the approximately 400 recipients on the SLRAOC's master contacts list and the notice was also shared by the St. Louis River Alliance on their Twitter and Facebook accounts. As an alternative to the meeting, the posters and presentation prepared for the meeting were posted on the MPCA SLRAOC web site and their availability was promoted in the cancellation notice. Only one comment was received during the comment period (see Appendix 12). Since it was in support of the removal recommendation, no changes to the removal document were needed.

3. Additional outreach. A presentation about the BUI 6 removal recommendation was made at the St. Louis River Summit on March 3, 2020 and to the Harbor Technical Advisory Committee on March 4, 2020. About 270 people attended the Summit and 35 people attended the Committee meeting.
4. SLRA Letter of Support. The St. Louis River Alliance is the designated citizens' action committee for the SLRAOC. Information about the BUI 6 removal recommendation was made available to the members of the Alliance's External Affairs/Issues Committee. This information included the BUI 6 removal document and the posters and presentation prepared for the public meeting. As a result of their review, a letter of support for the removal of BUI 6 was submitted on behalf of the St. Louis River Alliance (see Appendix 12).

Removal Recommendation

The results of the BUI 6 studies show multiple lines of evidence that, taken together, demonstrate improved conditions warranting a removal recommendation for BUI 6. Such a recommendation is supported by the BUI 6 technical team; the SLRAOC partners; and the SLRAOC Coordinators, leaders, and executive managers who collectively request that the Excessive Loading of Sediments and Nutrients BUI be removed from the SLRAOC. Feedback received as a result of the public involvement efforts also supports this removal request.

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Appendix 1

Water quality in the St. Louis River Area of Concern, Lake Superior: Historical and current conditions and delisting implications (Pertaining to management actions 6.01 and 6.02.)

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Water quality in the St. Louis River Area of Concern, Lake Superior: Historical and current conditions and delisting implications

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ABSTRACT

Water quality in the St. Louis River Area of Concern (AOC) was assessed at two stations over a 60 year period (1953–2013) and system-wide for 2012–2013 to determine if the AOC beneficial use impairment (BUI) of "Excessive loading of sediment and nutrients" should be considered for removal. Based on the time-series analysis, concentration and loading of total suspended solids and total phosphorus to Lake Superior from the St. Louis River have decreased over time, and episodic hypoxia in the mainstem of the estuary was eliminated after 1975. Detection of temporal patterns in nitrogen concentration and loading, particularly in the lower estuary, were complicated by Lake Superior nitrogen inputs and changes in wastewater treatment practices. For the system-wide assessment, sample locations were based on a probabilistic survey design. In 2012 and 2013, there was significant monthly (May–October) variability in water quality constituents. Based on area-weighted estimates, 60–85% of the estuary surface area was below BUI criterion for total phosphorus, total suspended solids, and chlorophyll *a*. Water quality in the western arm of Lake Superior in 2013 was indicative of oligotrophic conditions, satisfying delisting requirements. The long-term improvements in water quality followed improvements in watershed land-use practices and treatment of wastewater. The stratified system-wide survey provided unbiased estimates of spatial and temporal condition and identified some outlier sites. The data from this study supports the BUI removal process for the St. Louis River AOC.

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Introduction

The St. Louis River Estuary (SLRE), located at the western end of Lake Superior, is the largest estuary of the Great Lakes (50 km^2; Fig. 1). The SLRE is bordered by the port cities of Duluth, MN, and Superior, WI. The Duluth–Superior area developed rapidly during the late 1800s and early 1900s, a period of widespread deforestation throughout the watershed that increased sediment and nutrient loading to surface waters (Hartig and Thomas, 1988; Meyers, 2003). Industrial and urban development resulted in uncontrolled discharges of sewage, industrial waste, organic contaminants (e.g., polychlorinated biphenyls, polyaromatic hydrocarbons, and dioxins), and heavy metals into the estuary (Dole and Wesbrook, 1907; MPCA and WDNR, 1992). Early water quality surveys reported sediment contamination from sawmill waste, tar substances, and organic matter, and episodic anoxia during summer (MSBH et al., 1929). These conditions virtually eliminated aquatic life in some areas of the estuary. Tertiary treatment of municipal and industrial wastewater began in 1978 with the establishment of the Western Lake Superior Sanitary District (WLSSD; MPCA and WDNR, 1992).

The Great Lakes Water Quality Agreement between the United States and Canada (http://epa.gov/grtlakes/glwqa/1978/annex.html#annex 2; site accessed 1/2015) designated 43 coastal ecosystems across the Great Lakes as areas of concern (AOC), defined as locations having significantly degraded chemical, physical, and biological attributes (referred to as beneficial use impairments, or BUIs). Nine BUIs were identified for the SLRE: restrictions on fish and wildlife consumption; degraded fish and wildlife populations; fish tumors and other deformities; degradation of benthos; restrictions on dredging; excessive loading of sediment and nutrients to Lake Superior; beach closings/body contact; degradation of

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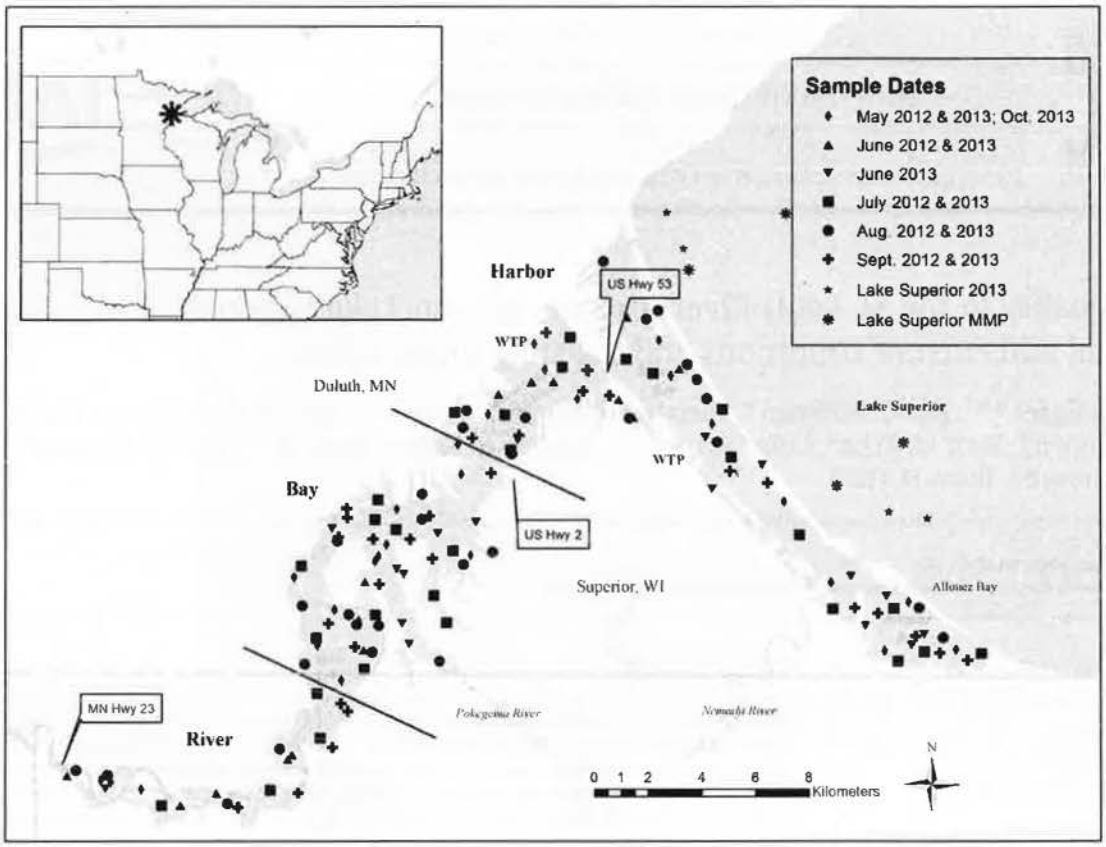

Fig. 1. Extent of the St. Louis River Estuary (SLRE) from the Fond du Lac Dam (upriver) to the western arm of Lake Superior. Milestone Monitoring Program (MMP) historic monitoring sites were at MN Hwy 23 ("Upper Estuary"), US Hwy 53 ("Lower Estuary"), and Lake Superior. Approximate zone delineations for the probabilistic sampling design applied in 2012 and 2013 are the head of Spirit Lake (River-Bay) and the US Highway 2 Bridge (Bay-Harbor). Lake Superior sites sampled in 2013 were randomly selected but meant to be near the MMP stations. Abbreviations: WTP = water treatment plant.

esthetics; and loss of fish and wildlife habitat (MPCA and WDNR, 1992; MPCA, 2013). To remove BUIs and delist an AOC, the U.S. Environmental Protection Agency (EPA) requires that condition indicators and delisting targets be established by local advisory groups through a remedial action plan (RAP) (Hartig and Thomas, 1988; US Policy Committee, 2001). Goals of the RAP include making recommendations of necessary remediation and restoration actions and developing BUI removal indicators and targets (MPCA and WDNR, 1992; US Policy Committee, 2001; MPCA, 2013).

One of the most significant developments toward water quality improvement in the SLRE that occurred before AOC listing was the establishment of the WLSSD in Duluth in 1978 (MPCA and WDNR, 1992). Phosphorus loadings from municipal treatment plants were reduced 80%, after which nuisance algal blooms became infrequent. However, there was still concern about water quality and nutrient loadings to Lake Superior, necessitating the water quality BUI listing (MPCA and WDNR, 1992). Following the Water Quality Agreement and RAP development, efforts to identify and remove impairments included: controlling storm water overflows, protection of existing forest and riparian areas, enhanced erosion control efforts, and implementation of agriculture and construction best management practices (MPCA and WDNR, 1992; MPCA, 2013). These efforts have improved water quality to the extent that nutrient and sediment loadings may have been reduced to concentrations consistent with removal of the excessive sediment and nutrient BUI. Loading of phosphorus has only been quantified from its major source, the WLSSD treatment plant (Fig. 1). However, the cumulative effect of the improvements and actions through time and on current conditions has yet to be assessed. The SLRE benefits from having a long history of water quality monitoring. In this paper, we analyze 60 years of water-quality data (1953–2013) from two fixed stations to determine whether nutrient and sediment concentrations and loads have changed in the SLRE.

We also describe current water quality conditions, compare concentration estimates with BUI removal criteria established by stakeholders, and estimate the proportion of the SLRE surface area below threshold (criterion) concentration. To estimate current conditions, we applied a probabilistic system-wide sample design that allowed us to assess seasonal as well as spatial variability in 2012 and 2013 (Crane et al., 2005; Messer et al., 1991). This study documents changes in BUI indicator values over time and it shows the utility of a spatially-balanced monitoring design for whole-system characterization necessary for BUI removal and AOC delisting.

Materials and methods

Study area

The 288-km long St. Louis River (9412-km^2 watershed) has an estimated mean daily discharge of 73 m^3 s^{-1} at the U.S. Highway 53 Bridge (Fig. 1). Our study area comprised the estuarine portion of the river (i.e., the reach of river subject to seiche-induced bi-directional flow; Stortz and Sydor, 1980), which extends from below the Minnesota Highway 23 Bridge, near the Fond du Lac Dam, to Lake Superior (including the Duluth–Superior Harbor), and the Lake Superior portion of the AOC, which extends approximately 16 km into the lake. The SLRE is situated behind a natural sand bar that restricts river-lake exchange to inlets at Duluth, MN, and Superior, WI. The upper SLRE ("river" section; Fig. 1) has a lotic character with generally intact riparian and floodplain

habitat. In contrast, the lower 20 km of the SLRE ("bay" and "harbor" sections; Fig. 1) has a lentic character and has been deepened by dredging, shoreline stabilization and hardening are extensive, and the riparian zone is highly developed. The SLRE is mostly shallow (mean depth ≈ 3.0 m) outside of the navigation channels (>7 m deep), and is generally well mixed. Estuarine waters have a slightly basic pH (ca. 7–8) and low transparency owing to high concentrations of dissolved organic carbon (DOC = 10–35 mg/L). Periodic seiche flows of about 8 h duration and weak semi-diurnal tides cause daily variation in water height in the SLRE of about 13 cm (Trebitz, 2006).

Historical water quality data

Long-term monitoring in the SLRE was conducted by the Minnesota Pollution Control Agency (MPCA) under the Milestone Monitoring Program (MMP). We accessed MMP data from the STORET database (www.epa.gov/storet; site accessed 1/2015). We analyzed data from the two fixed MMP stations within the SLRE with the longest periods of record: the Highway 23 Bridge ("upper estuary station") (1953–2008) and downriver at the Interstate 535/US Highway 53 John A. Blatnik Bridge ("lower estuary station") (1973–2008) (Table 1; Fig. 1). These two stations essentially bound the SLRE, providing data on nutrients and sediments imported from upriver and exported to Lake Superior via the harbor. The MMP generally included one surface sampling effort per month when the river was ice-free; if more than one sample was collected in a month, we averaged the data. For values below detection limits, we used one-half the minimum detection level as reported by MMP (TSS: 0.1 mg/L, nutrients: 0.01 mg/L; Table 1). We focused on status indicators for the St. Louis River BUI "Excessive loading of sediment and nutrients", which include concentrations of dissolved oxygen (DO), total phosphorus (TP), total nitrogen (TN), dissolved nitrate-N (NO_3^-), ammonium/ammonia-N (NH_4^+), and total suspended solids (TSS) (MPCA, 2013). Chlorophyll *a* (chl *a*) is also a status indicator for this BUI, but was not historically measured. To estimate loadings, we obtained mean daily discharge for the sampling dates from the nearest stream gage (Scanlon, MN; USGS gage #04024000), located 17 km upriver of the Fond du Lac Dam. To account for tributary inputs between the gage and MMP monitoring locations, we estimated cumulative downstream discharge to each station using the National Hydrography Dataset Plus (NHD Plus) unit runoff method (Research Triangle Institute, 2001). We calculated an associated discharge index for the upper and lower estuary monitoring stations (i.e., the estimated cumulative discharge at the station relative to the Scanlon stream gauge; 1.04 and 1.08, respectively), and corrected daily discharge values for each monitoring station by multiplying the Scanlon discharge by the respective index value. In addition, some along-thalweg DO data were available to depict historical changes in hypoxia (Hoffman et al., 2012; MDH, 1961; MSBH et al., 1929).

To characterize historical conditions in the Lake Superior portion of the AOC and compare them to present-day measures, we summarized MMP data from four stations in Lake Superior sampled between October 1974 and May 1975. The Lake Superior data are few, but they represent a period prior to improved wastewater treatment (Table 2; Fig. 1).

Current conditions survey sample design

Sites sampled in 2012 and 2013 were selected using a generalized random-tessellation stratified (GRTS) design which is based on the Environmental Monitoring and Assessment Program (EMAP; http://www.epa.gov/emap/; site accessed 1/2015; Crane et al., 2005; Messer et al., 1991). The site designation design provides unbiased area-weighted parameter estimates of condition across a defined sample "frame" (e.g., a region, watershed, or estuary) by generating random sample locations (Stevens and Olsen, 2003, 2004). The SLRE sample frame represented 4376 hectares (ha), or 90%, of the total surface area from the Fond du Lac Dam to Lake Superior. Across the sample frame, 150 unique sites (i.e., locations to conduct a sampling event) were identified and subsequently assigned to one of three zones with distinct hydrologic and geochemical character: the St. Louis River ("river" zone; 403 ha; n = 19), the central estuary ("bay" zone; 1482 ha; n = 57), and the lower estuary ("harbor" zone; 2491 ha; n = 74; Fig. 1). Sample site weights, in hectares, were determined by dividing the total number of sites within a zone by that zone's area (Stevens and Olsen, 2003). Thirty of the 150 sites were randomly selected and assigned to one of five months (May–September) and sampled (Fig. 1). Because we added a sixth sampling month in 2013, in October we resampled the 30 sites from May. Sampling typically occurred during the third week of each month. One sample was lost from the May 2012 sampling, and only 14 sites were sampled in June 2012 due to unsafe conditions following a 500-year recurrence interval flood (Czuba et al., 2012). Site weights were adjusted to ensure the entire sample frame was represented.

In 2013, we sampled the Lake Superior portion of the AOC at four haphazardly-selected sites near two of the MMP sites from the 1974–1975 survey (Fig. 1).

Table 1
Available historic data from the Minnesota Milestone Monitoring Program for the upper and lower estuary stations (Minnesota Highway 23 and US Highway 53, respectively; Fig. 1), and sample sizes for the Mann Kendall (M–K) test applied to both monthly concentration (conc.) data and annual load estimates with associated *p*-values (corrected for serial autocorrelation; see text for details). Abbreviations: TP = total phosphorus; NO_3^- = nitrate; NH_4^+ = ammonium; TSS = total suspended solids; DO = dissolved oxygen.

Station	Parameter	Available (missing)	n (non-detects)	Mean n/yr (range)	M–K test n, conc./load	M–K test*p*-value,conc./ oad
Minnesota Highway 23	TP	1958–2008 (1960–1961, 1966, 1997–2001, 2006)	571 (4)	14 (2–44)	369/43	<0.0001/<0.0001
	NO_3^-	1958–1996 (1961–1967)	334 (52)	7 (1–12)		
	NH_4^+	1960–2008 (1966, 1996, 2001, 2006)	548 (159)	12 (1–28)		
	TSS	1953–2008 (1957–1959, 1966, 1997–2001, 2006)	549 (11)	12 (1–22)	379/47	<0.0001/0.101
	DO	1953–2008(1960–1961, 1966, 1997, 1999, 2001–2002, 2004, 2007)	206 (0)	8 (2–12)		
US Highway 53	TP	1973–2008 (1995–1998, 2000–2001, 2006)	241 (1)	8 (1–15)	240/30	<0.0001/<0.0001
	NO_3^-	1976–2008 (1996, 2001, 2006)	228 (6)	7 (2–12)		
	NH_4^+	1973–2008 (1996, 2001, 2006)	267 (160)	8 (1–15)		
	TSS	1973–2008 (1995–1998, 2000–2001, 2006)	241 (0)	8 (1–15)	240/30	0.006/0.005
	DO	1973–2008(1996, 2001)	265 (0)	8 (1–12)		

Table 2
Mean water quality concentrations from the MPCA's Milestone Monitoring Program at four stations located in Lake Superior within the St. Louis River Area of Concern (Fig. 1). For all samples, data were available from three dates (12 October 1974, 24 October 1974, 13 May 1975) for multiple (generally three) depths at each station. Values shown are the daily means averaged across depths. Abbreviations: TP = total phosphorus; NO_3^- = nitrate; NH_4^+ = ammonium; TSS = total suspended solids.

Latitude (N)	Longitude (W)	TP (μg/L)	NO_3^- (μg/L)	NH_4^+ (μg/L)	TSS (mg/L)
46.7233	−92.0244	10.9	285.6	46.1	4.9
46.7331	−92.0022	13.7	382.8	55.6	4.6
46.7725	−92.0739	12.3	332.8	34.4	4.1
46.7853	−92.0414	9.2	317.8	62.2	2.5

Water sample collection and chemistry

At each site, we measured DO with a Hydrolab DataSonde (Hach Hydromet, Loveland, CO, USA). A 4-L surface water sample was collected and kept on ice for transport to the lab. Samples were stored at 4 °C and processed within 24 h. Water samples were analyzed for TSS by gravimetric analysis (APHA, 1998; method 2130B), and for chl *a* by flourometry (TD-700 Turner Designs, Sunnyvale, CA, USA) after extraction with magnesium saturated acetone (Welschmeyer, 1994). Total N, NO_x, NH_4^+, and TP were measured on a Lachat 8000 flow-injection analyzer (APHA, 1998; Lachat Quickchem methods, Lachat Instruments, Loveland, CO, USA). Unfiltered subsamples were digested by persulfate digestion for TP and TN (APHA, 1998). Total phosphorus was determined by the molybdate-ascorbic acid method (APHA, 1998; Lachat Quickchem method 10-115-01-1-B). Total N and dissolved NO_x (0.45-μm membrane filtered) were analyzed by the cadmium reduction method (APHA, 1998; Lachat Quickchem method 10-107-04-1-O). Ammonium was determined on filtered (0.45-μm pore membrane) samples on the Lachat analyzer with the phenolate method (APHA, 1998; Lachat Quickchem method 10-107-06-1-F; 2012 samples only) and salicylate method (Lachat Quickchem method 10-107-06-2-C; 2013 samples only). The methods were changed to reduce hazardous waste generation but were compared to ensure similar results. One NH_4^+ sample from July 2013, a TN and TP sample from August 2013, and a TP sample from September 2013 were lost or did not meet lab quality criteria and were not used in the final analyses.

Data analysis

We used the Mann–Kendall test to determine whether there was evidence for a monotonic decrease in TSS or TP monthly concentrations and annual loads over time. We used a one-tailed test with a continuity correction and the Hamed and Rao method (1998) to correct for serial autocorrelation. We used this non-parametric test because there were long periods for which data were unavailable (Table 1). We $\log_{10}$-transformed TSS and TP before analysis. Because TSS and TP concentrations demonstrate seasonality, we used a seasonal Mann–Kendall test using monthly data (Electronic Supplementary Material (ESM) Fig. S1). To compare trends between stations, we used the nonparametric Sen's slope estimator.

Annual TSS and TP loads were estimated using the ratio method (Preston et al., 1989): $L = (l / q_m)Q$, where L is the estimated annual load in metric tons (t), l is the average of daily loads, q_m is the average daily discharge on sample dates, and Q is the total annual discharge. The method is appropriate because we have a nearly complete discharge record, but only monthly nutrient and sediment concentration records (Quilbé et al., 2006). To account for the influence of lake water mixing at the lower estuary station due to seiches, we used a conservative mixing model to normalize lower estuary historical TSS and TP concentrations to 100% river water (i.e., $[TSS]_{river}$, where $[TSS]_{mixed} = f_{lake} \times [TSS]_{lake} + f_{river} \times [TSS]_{river}$, and $1 = f_{lake} + f_{river}$); we assumed a constant $[TSS]_{lake}$ of 1.93 mg/L and $[TP]_{lake}$ of 7 μg/L (Hoffman et al., 2012). The fraction river water (f_{river}) was estimated from mean daily discharge (q_d) using logistic regression ($f_{river} = a / (1 + (q_d / X_0)^b)$) to estimate f_{river} at the U.S. Highway 53 bridge at a given daily discharge level (ESM Fig. S2). The data for the logistic regression came from the 2012 and 2013 sampling; q_d data were from the Scanlon gage and f_{river} was estimated using measured magnesium (Mg) as a conservative tracer in a conservative mixing model ($[Mg]_{lake}$ was 2.9 mg/L, and $[Mg]_{river}$ was the value measured at the upper estuary station, which is above the mixing zone; Hoffman et al., 2012; Morrice et al., 2009). The regression met statistical assumptions of normality (Shapiro–Wilk test, $p = 0.3$) and constant variance ($p = 0.5$) and was significant ($p = 0.001$, $r^2 = 0.81$; $a = 1.07 \pm$ standard error [SE] 0.22, $b = -1.13 \pm$ SE 0.69, $X_0 = 16.63 \pm$ SE 7.59). Based on the regression, when Scanlon discharge is at 15 $m^3 s^{-1}$, water at the lower estuary station is of half lake and half river origin, whereas at a Scanlon discharge ≥130 $m^3 s^{-1}$, all the water is of river origin.

Temporal trends in DO (upper estuary only), NO_3^- ($\log_{10}(x + 1)$-transformed) and NH_4^+ ($\log_{10}(x + 1)$-transformed) concentrations were examined using local regression (LOESS; SigmaPlot 13.0, Systat Software, Inc., San Jose, CA, USA) because the time-series were not monotonic (i.e., entirely increasing or decreasing) and thus not suitable for the Mann–Kendall test. The local regressions were fit iteratively by varying the polynomial order (1–3) and sampling proportion (0.1–0.9) to generalize the data over a 5–10 year period. Monthly DO data were from late summer (July–September), when the annual seasonal low typically occurs; NO_3^- and NH_4^+ data were monthly data. For the lower estuary station, monthly NO_x and NH_4^+ concentrations were seiche-corrected, using the combined conservative mixing model and logistic regression approach previously described. At the lower estuary station this approach resulted in some negative concentration values, indicating the estuary was acting as a net N-sink relative to the inputs. For the mixing model, Lake Superior NO_3^- concentration increased through time, ranging from 0.28 to 0.36 mg/L (Finlay et al., 2007), and NH_4^+ concentration was constant, 0.1 mg/L, based on MMP data. Along-estuary thalweg DO data from summer months were compared among years when data were available (noted above: 1929, 1961, and 2007) to identify along-estuary changes.

For the 2012 and 2013 survey sampling, area-weighted mean TSS, TP, and chl *a* concentration estimates for each month, longitudinal zone, and summarized within a year across months and zones were compared with draft BUI targets derived for waters of Northern Minnesota (15 mg/L TSS, 30 μg/L TP, and 10 μg/L chl *a*; Heiskary et al., 2013; MPCA, 2013) using a one sample *Z*-test calculated as: $Z_{score} = (\overline{X}_w - T) / SE_w$ where $\overline{X}_w$ is the area-weighted mean, T is the BUI criteria, and SE_w is the area-weighted standard error. We differentiated monthly and zone water quality concentration differences by overlap in 95th percentile confidence intervals (CI). While the annual system-wide average concentration is most important for BUI removal, the other estimates revealed temporal changes and potential longitudinal variations in water quality across the SLRE. Lake Superior data were not based on a probability design and so monthly averages were compared using one-way ANOVA. Data were tested for normality using the Shapiro–Wilk test and were $\log_{10}$ transformed to approximate a normal distribution. Statistical analyses were conducted using Systat v.11.00.01 (Systat Software, Inc., San Jose, CA, USA).

Results

Long-term water quality trends

Annual mean TP concentrations and load to Lake Superior declined significantly and at similar rates at both stations over time (Table 1, Fig. 2A, B). For TP concentration, the decline (Sen's slope) was −0.017

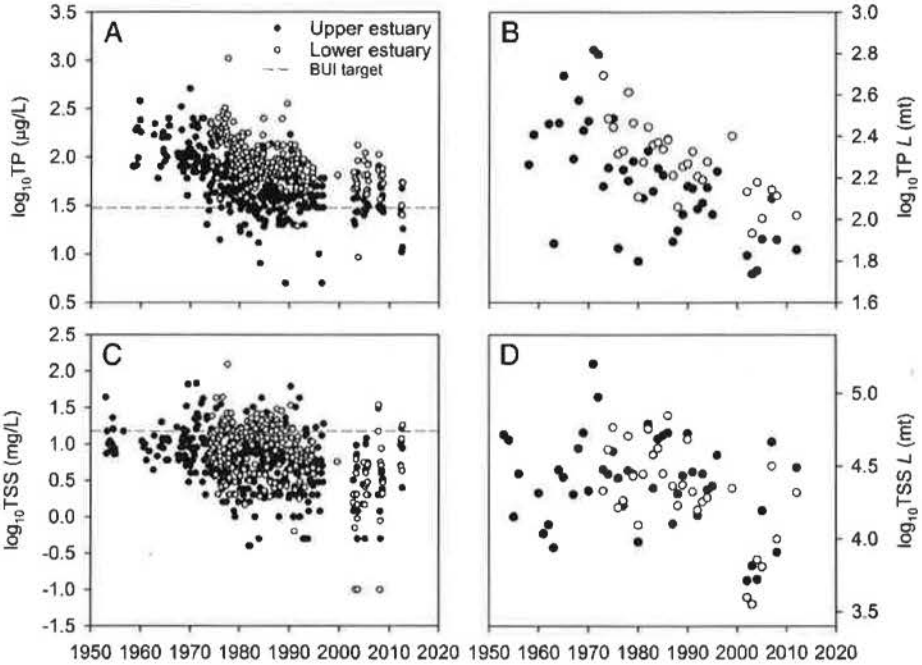

Fig. 2. Temporal trends in monthly total phosphorus (TP) and total suspended solids (TSS) concentrations panels A, C), and annual TP and TSS loads (panels B, D) measured at the upper (closed circle) and lower (open circle) estuary monitoring stations. There was a significant decline in all parameters except TSS loading at the upper estuary station (Mann Kendall test, $\alpha = 0.05$; see text for details). The dashed lines indicate the targets for Beneficial Use Impairment removal (30 µg/L TP, 15 mg/L TSS).

$\log_{10}$(TP, µg/L) yr^{-1} at the upper estuary station and -0.023 $\log_{10}$(TP, µg/L) yr^{-1} at the lower station. Though concentrations have declined, monthly and annual average TP concentrations for the two stations have exceeded the BUI criterion over the period of record at the two stations. For TP load, however, the declines (Sen's slope) were the same, ≈ -0.014 $\log_{10}$(TP L, t) yr^{-1}. The ratio between the mean annual load (l) and discharge (q_m), the mean mass per unit discharge, revealed a decline over time, indicating that the decline in TP load was the result of changes in TP concentration rather than discharge. Total P concentrations in the lower estuary were higher than in the upper estuary, implying there were internal TP sources (e.g., resuspension of sediment) or tributary additions. From 2002 to 2012, the estimated mean annual TP load was 76 t at the upper estuary station and 133 t at the lower estuary station, for an average annual net addition of 56 t.

Annual mean TSS concentrations significantly declined over time at both stations (Table 1, Fig. 2C and D). The decline (Sen's slope) was -0.016 $\log_{10}$(TSS, mg/L) yr^{-1} at the upper estuary station and -0.022 $\log_{10}$(TSS, mg/L) yr^{-1} at the lower station. Average annual TSS concentrations were above the BUI criterion three times prior to 1978, and monthly exceedances through 2008 were rare. After 2000, relatively low TSS concentrations (≤5 mg/L) were measured at both stations except for two instances (2007, 2012) in which elevated TSS concentrations (31.0 and 16.3 mg/L, respectively) coincided with large discharge events (354 and 120 $m^3\ s^{-1}$, respectively). Loading of TSS significantly declined at the lower estuary station, but not at the upper station (Table 1); and TSS loads to Lake Superior declined faster at the lower estuary station (Sen's slope -0.018 $\log_{10}$(TSS L, t) yr^{-1}) than the upper estuary station (-0.003 $\log_{10}$(TSS L, t) yr^{-1}; Fig. 2D). At the beginning of the time-series the estuary between stations was a source of TSS, whereas it is currently similar between stations (Fig. 2). As with TP loads, the ratio between the mean annual load (l) and discharge (q_m) declined over time, indicating that the change in TSS load was due to change in TSS concentration.

At the upper estuary station, late-summer (July–September) DO concentrations increased from 1953 to ca. 1990, after which it leveled-off and possibly declined slightly (Fig. 3A). Data from the lower estuary followed a similar pattern. Since 2000, monthly summer concentrations were always above 5.5 mg/L at both stations. At both monitoring stations, the last year of summer hypoxia (<2 mg/L) was 1964; values <5 mg/L were infrequent after 1975. The period for which hypoxia was present somewhere in the river was likely longer than the time-series suggest, however, because the available longitudinal DO concentration data indicate that the lowest DO concentrations in the river were typically located between the upper and lower estuary stations (i.e., between river km 20 and 35; Fig. 4).

Nitrate concentrations in the upper estuary were highest in the 1950s and declined until about 1970, after which they have been relatively constant (Fig. 3B). At the lower estuary station, for which we have a shorter record, NO_x concentrations have been more variable, possibly due to seiche-dominated inputs of NO_x from Lake Superior (Sterner, 2011), with sporadic low concentrations measured for 1988–2003. Ammonium concentrations were generally higher in the lower estuary than the upper estuary except from 1987 to 1997 when concentrations were similar (i.e., LOESS fits) between stations (Fig. 3C). There were numerous non-detections in the early part of the time-series, but there are no independent data to verify these observations.

2012 and 2013 survey summaries

System-wide TP concentrations ranged from 4.7 µg/L (May 2012 in the bay) to 195.4 µg/L (May 2012 in the harbor) with a median concentration across years of 28.7 µg/L (Table 3). The weighted mean TP concentration for 2012 (30.9 µg/L) and 2013 (30.7 µg/L) were not significantly different from the BUI criterion (30 µg/L; Tables 3 and 4). In July 2012 and in June and July 2013, weighted mean TP concentrations were significantly above the BUI criterion. May and August of 2012 and 2013 had TP concentrations significantly less than the BUI criterion (Tables 3 and 4). Total P concentrations in the bay in 2012 were significantly above 30 µg/L; the river in 2012 was significantly below the criterion.

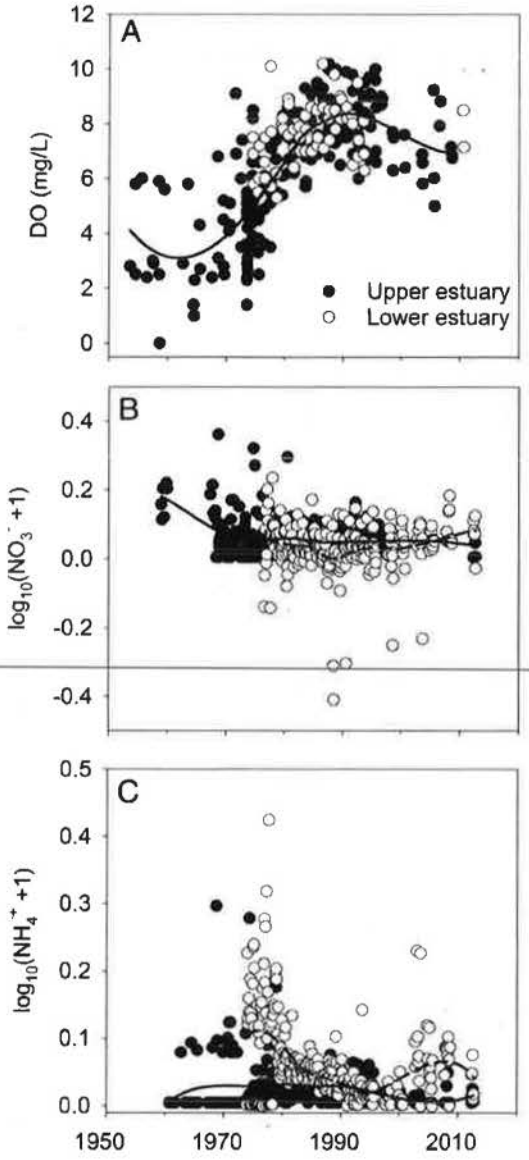

Fig. 3. Temporal trends in dissolved oxygen (DO; A), nitrate (NO_3^-; B) and ammonium (NH_4^+; C) measured in the upper (closed circle) and lower estuary (open circle) monitoring stations and LOESS regressions (lines). For A, the solid line is the LOESS fit to the upper estuary station only (3rd order, proportion = 0.8); lower estuary station data are added for a visual comparison. For B, the solid line is the LOESS fit to the upper estuary station (3rd order, proportion = 0.7) and the dashed line is the LOESS fit to the lower estuary station (3rd order, proportion = 0.6). For C, the solid line is the LOESS fit to the upper estuary station (3rd order, proportion = 0.6) and the dashed line is the LOESS fit to the lower estuary station (3rd order, proportion = 0.7).

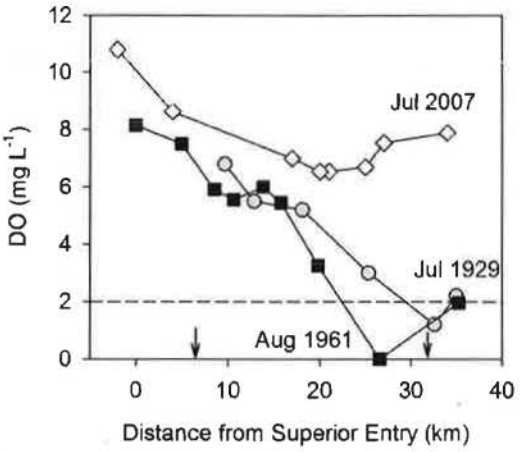

Fig. 4. Historical late-summer thalweg dissolved oxygen (DO) concentrations (MSBH et al., 1929 [closed circles], MDH, 1961 [closed squares]) compared to a 2007 survey (open diamonds; J. Hoffman, unpublished data). Arrows indicate locations of upper estuary and lower estuary monitoring stations. Where the River flows into Lake Superior (inlet near Allouez Bay) is at km 0.

In both years, about 60% of SLRE area was below the BUI removal criterion (Fig. 5A).

Dissolved oxygen concentration in the SLRE ranged from 4.5 mg/L (July 2012 in the bay) to 13.3 mg/L (October 2013 in the river), with a median of 8.3 and 9.5 mg/L in 2012 and 2013, respectively (Table 3). Lowest weighted mean DO concentration (6.4 mg/L) was measured in July 2012. Concentrations differed among months in both years. In 2013, there were significant differences among zones (Table 3).

Total N concentrations ranged from 399 to nearly 4300 μg/L (Table 3), and had a median concentration across years of 990 μg/L. Lowest concentrations were measured in August and September (Table 3). Total N did not differ among zones in either year. Between years, NO_x ranged from 1.6 to 418.7 mg/L. Median concentrations for the SLRE were 153.4 and 135.4 μg/L in 2012 and 2013, respectively. In both years, greater NO_x concentrations were measured in the harbor than elsewhere. Total NH_4^+ ranged from 7.0 to 3645.0 μg/L (Table 3). Greatest NH_4^+ concentrations were measured in June and July, at sites nearest the WTPs (Fig. 1), and differed among zones.

Total suspended solid concentrations varied from 2.3 mg/L (October 2013 in the bay) to 71.4 mg/L (September 2012 in the harbor) (Table 3) with a median concentration across years of 8.6 mg/L. The weighted mean TSS concentration for 2012 (12.0 mg/L) and 2013 (9.9 mg/L) was significantly below the BUI criterion of 15 mg/L (Tables 3 and 4). Total suspended solid concentration was above the BUI criterion in July and September 2012 and June 2013 (Tables 3 and 4). Weighted means within zones were significantly below the BUI criterion in both years. In 2013, there were significant differences among zones. At least 85% of the area of the SLRE had TSS concentrations below 15 mg/L in both years (Fig. 5B).

Chlorophyll *a* concentrations ranged from 0.6 μg/L (October 2013 in the harbor) to 49.9 μg/L (September 2012 in the harbor) (Table 3). The weighted mean chl *a* concentrations in 2012 and in 2013 were significantly below the BUI criterion of 10 μg/L (Tables 3 and 4). In both years, chl *a* concentrations were greatest in August and September (Table 3). Chlorophyll *a* concentrations exceeded the BUI criterion in September 2012 and August 2013. Between zones across all months, chl *a* concentrations in the bay and river were significantly below the BUI removal criterion (Tables 3 and 4). For both years, over 70% of the area of the SLRE had chl *a* concentrations below the criterion (Fig. 5C).

In 2013, average TSS concentration at Lake Superior sites was 4.4 mg L, ranging from 0.4 to 15.1 mg/L (Table 5). Chlorophyll *a* concentration was greatest in June (4.5 mg/L) and averaged 2.7 mg/L. Total phosphorus concentrations were greatest in June and averaged 12.7 μg/L. Dissolved oxygen was always at or near saturation; concentration for the season averaged 12.2 mg/L. Total N concentration averaged 606.9 μg/L with 2–5% as NH_4^+ and 35–65% as NO_x (Table 5).

Discussion

Documentation of system recovery is required for BUI removal and AOC delisting. In this study, we utilized long-term fixed station data

(1953–2008) and data from a system-wide monitoring study (2012–2013) to show the remarkable improvement in water quality in the St. Louis River estuary over the past 60 years, driven primarily by changes in land-use practices within the watershed and improved wastewater treatment. The system-wide surveys provide area-weighted estimates of system condition relative to BUI criterion, and detail spatial variability in water quality across the SLRE. The condition estimates derived from the probability design show that most of the area of the SLRE was similar in condition or better than conditions defined by BUI removal criteria, and that the other parameters measured were not indicative of eutrophic conditions.

Long-term change in water quality

Data from the two fixed monitoring stations demonstrate that sediment and nutrient inputs to Lake Superior have declined over time. In the Laurentian Great Lakes region, eutrophication of coastal systems from increased delivery of soil-derived phosphorus began in the mid-1800s, and anthropogenic eutrophication from soil- and wastewater-derived nutrients increased until 1965–1975, after which changes to nutrient management were implemented basin-wide (Meyers, 2003). In Minnesota, logging activity was greatest from 1860 to 1931 (White and Mladenoff, 1994). These timelines are consistent with changes in the SLRE, particularly the gradual improvements in DO concentration and declines in TSS and TP concentrations during 1960–1970. As logging declined throughout the first half of the 20th century, loading of TSS (and associated TP) from the watershed and the discharge of sawmill waste to the SLRE declined (Smith and Moyle, 1944). The relative difference between the two monitoring stations in the change in annual TSS load suggests that changes in organic waste inputs were more influential than changes in watershed-based inputs. Lower loadings of organic waste likely contributed to a reduction in biological oxygen demand and hypoxic conditions, with recovery of DO beginning around 1966; by 1976 hypoxia was rare. Improved wastewater treatment since the 1970s are estimated to have contributed to an 80% reduction in TP loadings to the estuary compared to the years before the WLSSD (MPCA and WDNR, 1992).

Nitrate concentration at the upper SLRE station was relatively stable over time; variability in NO_x was greater at the lower estuary station. Low NO_x values measured during summer months from 1987 to 2004 suggest periods of high primary production, which is consistent with declines in NH_4^+ at both stations. Corroborating estimates of primary productivity or chl *a* are lacking, however. The increase in NH_4^+ after 2001 at the lower estuary station was coincident with the implementation of a biosolids program at WLSSD (http://www.wlssd.com/wastewater_biosolids.php), the result of which was increased ammonium concentration in plant discharge necessary to manage the quality of the biosolid product. Lake Superior is also a substantial source of NO_x to the lower SLRE (Bellinger et al., 2014; Sterner, 2011). Because of lake inputs of NO_x, the highest denitrification rates in the system occur in the harbor (Bellinger et al., 2014). Algal biomass was greater in the harbor than elsewhere (Table 3), presumably owing to higher N-availability. Nitrogen concentrations in the lower SLRE should continue to be monitored because elevated NH_3/NH_4^+ and NO_x concentrations can negatively affect aquatic organisms and are expensive to remove through wastewater treatment (Compton et al., 2011).

Current conditions

The 2012–2013 surveys document that most of the SLRE water quality was at or below impairment criteria designated in the AOC RAP (MPCA, 2013). Between sample years, we found agreement in our system-wide seasonal and monthly water quality parameter estimates. Spatial and seasonal variation of water quality was evident in the SLRE, as it is in other Great Lakes coastal embayments (Hiriart-Baer et al., 2009; Steinman et al., 2008). In light of the monthly and longitudinal differences in water quality parameter concentrations, consideration must be given to sampling frequency as well as site selection as part of a system-monitoring plan.

The size and location of the SLRE present unique challenges for monitoring because of the extent of the mixing zone with Lake Superior water, the variability in tributary watershed geology (Bartsch et al., 2015), and the uneven distribution of riparian development (Crane et al., 2005; MPCA, 2013). The GRTS design was spatially stratified *a priori* in an effort to capture the longitudinal heterogeneity of the SLRE and to identify potential outliers. For example, Allouez Bay and the adjacent Nemadji River are naturally turbid because of the clay-dominated soils of their watersheds. By weighting sites, which decreased the influence of Allouez Bay on the mean, estimated mean TSS concentration across all months was 28% lower in the harbor zone than the unweighted arithmetic mean. The sample design provides flexibility as it can be modified to increase monitoring intensity or frequency in specific zones (e.g., those consistently above a criterion or being in the highest 5% of all parameter concentrations).

Although most of the SLRE area (>50%) was below criterion values for each parameter (Fig. 5), whether or not this is sufficient justification for BUI removal is up to local stakeholders and EPA. We have shown how a probability site selection process enables estimation of the proportion of a system above or below target condition criteria (Jackson et al., 2000; Messer et al., 1991); this is information that cannot be derived from repeated measures at fixed stations. A similar probabilistic survey has been used in the SLRE for characterization of sediment contaminants and invertebrate communities (Crane et al., 2005). The authors concluded that the EMAP survey design is an effective means of tracking system health and condition.

Total P concentrations in 2012–2013 were lowest in the river zone, and increased in the zones downriver. The spatially-comprehensive survey corroborated the long-term trends observed between the upper estuary station (MN Highway 23) and lower estuary station (Highway 53 Bridge). Greater TP concentrations in the central and lower estuary could be due to internal loading by sediment resuspension or tributary sources. For example, the Pokegama River is a naturally turbid system, with average TP and TSS concentrations of 60 μg/L and 18 mg/L, respectively (B.J. Bellinger, unpublished data). Non-point sources of phosphorus to the lower SLRE have been estimated to represent 50–90% of total inputs (MPCA and WDNR, 1992). Watershed management efforts and benchmarks continue to be pursued and prioritized by local agencies in order to reduce TP and TSS loads (MPCA, 2013).

Dissolved oxygen concentrations from the 2012–2013 survey corroborated observations at the long-term monitoring stations, indicating that hypoxia ($DO < 5$ mg L^{-1}) was not wide-spread in the SLRE (<1% of all stations were hypoxic at time of sampling). However, we did not sample all sites early in the morning to capture lowest possible concentrations. Periodic, localized hypoxia is also known to occur in small embayments along the Wisconsin shoreline of the SLRE (R. Garono, personal communication). Those small embayments and other similar backwaters comprise a relatively small area of the SLRE, and therefore had a low probability of sampling under the probabilistic sampling design. However, they have physicochemical characteristics important to aquatic organisms and nutrient cycling functions of the SLRE (Johnston et al., 2001). Their absence in our dataset highlights the difficulty of monitoring complex coastal systems and in the application of a randomized probability design (Crane et al., 2005).

The elevated concentration of most water quality parameters (TP, TSS, TN, and NH_4^+) and the decrease in DO in July 2012 were most likely due to a 500-year-recurrence flood that struck the lower St. Louis River watershed in mid-June (Czuba et al., 2012; Minor et al., 2014). However, TP and TSS concentrations in June and July 2013 were also elevated

Table 3
Summary statistics of water quality parameters identified in the original Remedial Action Plan (RAP) and in the RAP update (MPCA, 2013) as being impaired, measured in the St. Louis River Estuary (SLRE) in 2012 and 2013. Abbreviations: TP = total phosphorus; DO = dissolved oxygen; TN = total nitrogen; NO_x-N = nitrate/nitrite-N; NH_4-N = ammonium-N; TSS = total suspended solids; Chl *a* = chlorophyll *a*; CI = confidence interval; SE = standard error; na = not available.

			Month						Zone			
Parameter	Year	Statistic	May	June	July	August	September	October	Harbor	Bay	River	All observations
TP (μg/L)	2012	Range	4.7–195.4	6.0–31.0	23.6–102.5	11.0–66.2	6.1–93.7	na	6.1–195.4	4.7–102.5	6.0–54.0	4.7–195.4
		Median	20.2	24.8	45.4	24.2	28.5	na	26.3	31.0	18.5	26.9
		Weighted Mean ± SE	26.5 ± 4.1	23.1 ± 1.8	46.1 ± 2.0	26.3 ± 1.7	27.3 ± 2.2	na	30.1 ± 2.1	35.0 ± 2.4	21.0 ± 2.3	30.9 ± 1.5
		Weighted 95th CI	18.5–34.5	19.6–26.7	42.2–50.0	23.0–29.6	23.1–31.5	na	26.0–34.2	30.4–39.6	16.5–25.6	28.1–33.8
	2013	Range	15.1–52.9	15.5–66.5	10.0–66.6	20.1–37.6	22.9–65.7	23.9–58.5	10.0–65.7	16.4–66.6	15.5–66.5	10.0–66.6
		Median	21.2	32.5	40.2	24.2	29.6	29	28.1	31.8	26.0	29.0
		Weighted Mean ± SE	24.6 ± 1.9	34.3 ± 1.3	39.2 ± 1.5	25.2 ± 0.6	32.9 ± 1.2	31.1 ± 1.3	30.3 ± 0.8	32.0 ± 1.2	28.1 ± 1.6	30.7 ± 0.6
		Weighted 95th CI	20.9–28.2	31.7–36.8	36.2–42.2	24.1–26.3	30.6–35.2	28.6–33.6	28.7–32.0	29.8–34.2	25.0–31.2	29.4–31.9
DO (mg/L)	2012	Range	6.6–11.9	7.0–10.0	4.5–8.5	6.0–9.0	8.2–12.0	na	4.8–12.0	4.5–11.3	6.8–11.9	4.5–12.0
		Median	11.1	8.0	6.5	7.5	10.6	na	8.2	7.9	8.6	8.3
		Weighted Mean ± SE	10.9 ± 0.1	8.1 ± 0.1	6.4 ± 0.1	7.6 ± 0.1	10.4 ± 0.1	na	8.9 ± 0.3	8.4 ± 0.3	9.1 ± 0.3	8.7 ± 0.2
		Weighted 95th CI	10.7–11.2	7.8–8.4	6.2–6.7	7.4–7.8	10.1–10.7	na	8.3–9.4	7.9–8.9	8.5–9.8	8.4–9.1
	2013	Range	7.1–12.9	7.6–11.0	7.7–10.8	7.3–9.8	6.4–9.6	8.7–13.3	6.4–12.7	7.3–12.9	7.8–13.3	6.4–13.3
		Median	11.9	9.5	9.2	8.9	8	11.6	9.5	9.0	10.3	9.5
		Weighted Mean ± SE	11.7 ± 0.2	9.5 ± 0.1	9.2 ± 0.1	9.0 ± 0.1	8.4 ± 0.1	11.5 ± 0.1	10.0 ± 0.1	9.6 ± 0.2	10.2 ± 0.4	9.9 ± 0.1
		Weighted 95th CI	11.3–12.1	9.2–9.8	9.0–9.3	8.9–9.1	8.2–8.5	11.2–11.7	9.7–10.2	9.3–10.0	9.5–10.9	9.7–10.1
TN (μg/L)	2012	Range	746–4274	1036–1222	798–1580	399–1213	449–1358	na	399–4274	612–1580	491–1381	399–4274
		Median	1004	1142	1285	799	739	na	922	970	1004	963
		Weighted Mean ± SE	1104 ± 70	1138 ± 12	1273 ± 25	787 ± 19	746 ± 20	na	1006 ± 43	996 ± 34	943 ± 75	997 ± 28
		Weighted 95th CI	968–1240	1114–1163	1224–1322	751–824	707–785	na	922–1091	928–1062	795–1092	943–1051
	2013	Range	655–4390	436.0–2057	760–1515	406–918	467–4149	691–379	466–4390	666–1277	406–1800	406–4390
		Median	919	1193	1035	57	711	919	903	907	912	905
		Weighted Mean ± SE	1066 ± 113	1202 ± 34	1018 ± 16	765 ± 15	853 ± 80	1035 ± 97	1019 ± 37	900 ± 14	867 ± 43	965 ± 22

(continued on next page)

Table 3 (*continued*)

Parameter	Year	Statistic	Month						Zone			All observations
			May	June	July	August	September	October	Harbor	Bay	River	
		Weighted 95th C.I.	844–1289	1136–1268	987–1049	735–794	696–1009	846–1224	946–1091	873–927	782–952	922–1008
NO_x-N (μg/L)	2012	Range	8.5–418.7	143.8–212.7	44.6–275.9	3.8–387.5	1.6–238.3	na	1.6–418.7	2.3–180.5	4.0–180.5	1.6–418.7
		Median	134.7	173.5	177.7	137.9	12.0	na	193.8	121.0	113.9	153.4
		Weighted Mean ± SE	183.2 ± 14.3	178.7 ± 5.3	173.1 ± 7.6	213.5 ± 14.5	78.8 ± 11.4	na	214.1 ± 8.7	98.2 ± 8.5	96.7 ± 22.7	164.0 ± 6.0
		Weighted 95th CI	155.2–211.2	168.2–189.1	158.2–187.9	185.0–242.0	56.5–101.1	na	197.0–231.1	81.6–114.8	52.3–141.1	152.3–175.7
	2013	Range	87.0–351.4	41.6–261.9	25.7–311.8	4.7–309.7	4.0–255.9	59.4–251.9	23.0–351.4	4.0–261.9	4.7–225.3	4.0–351.4
		Median	143.7	133.9	190.8	89.6	24.0	108.5	169.8	101	87.3	135.4
		Weighted Mean ± SE	177.4 ± 6.1	142.6 ± 4.8	201.3 ± 11.4	147.5 ± 15.4	96.5 ± 7.7	150.9 ± 8.5	189.4 ± 6.3	97.0 ± 7.3	91.1 ± 11.1	149.0 ± 4.4
		Weighted 95th C.I.	165.5–189.3	133.2–152.1	178.9–223.7	117.4–177.7	81.4–111.5	134.2–167.6	177.0–201.7	82.8–111.3	69.1–112.8	140.4–157.7
NH_4-N (μg/L)	2012	Range	9.7–110.9	34.3–142.9	30.8–180.3	11.0–52.7	7.0–78.5	na	7.0–180.3	8.2–89.2	10.3–67.1	7.0–180.3
		Median	22.6	68.9	73.7	22.8	12.4	na	34.8	25.2	18.6	29.5
		Weighted Mean ± SE	42.5 ± 5.2	80.9 ± 11.3	85.1 ± 6.6	24.3 ± 1.5	15.2 ± 1.5	na	56.7 ± 6.4	35.0 ± 3.5	26.8 ± 4.2	46.6 ± 3.9
		Weighted 95th CI	32.3–52.8	58.7–103.1	72.1–98.1	21.5–27.2	12.4–18.0	na	44.2–69.3	28.1–41.9	18.5–35.1	39.1–54.2
	2013	Range	5.0–3629.0	16.5–174.8	10.0–123.9	4.9–129.6	2.0–3645.0	2.02–2875.0	2.0–3645.0	4.5–77.4	9.1–207.9	2.0–3645.0
		Median	17.4	47.1	33.2	17.2	10.6	51.8	35.5	23.7	24.7	26.6
		Weighted Mean ± SE	170.2 ± 117.7	79.7 ± 6.8	50.3 ± 5.2	30.7 ± 7.2	126.0 ± 76.8	194.5 ± 92.9	134.3 ± 28.8	25.8 ± 1.7	36.4 ± 7.5	88.7 ± 16.5
		Weighted 95th CI	0.0–400.9	66.4–93.0	40.1–60.5	16.6–44.8	0.0–276.6	12.5–376.6	77.9–190.6	22.5–29.2	21.7–51.1	56.4–121.0
TSS (mg/L)	2012	Range	3.7–51.3	3.6–13.7	7.7–62.7	2.5–18.5	6.0–71.4	na	3.3–71.4	4.2–28.8	2.5–33.3	2.5–71.4
		Median	6.5	10.1	13.4	6.4	13.6	na	10.5	10.8	9.7	10.1
		Weighted Mean ± SE	11.1 ± 1.3	8.7 ± 1.0	16.3 ± 1.3	7.0 ± 0.5	15.1 ± 1.3	na	13.0 ± 0.8	10.9 ± 0.6	10.2 ± 1.4	12.0 ± 0.5
		Weighted 95th CI	8.6–13.6	6.8–10.6	13.8–18.8	6.0–8.0	12.5–17.7	na	11.3–14.6	9.7–12.2	7.4–13.0	11.0–13.1
	2013	Range	3.2–55.6	5.0–40.5	2.7–26.5	3.7–19.3	2.7–17.3	2.3–31.7	4.3–55.6	2.3–36.7	1.8–17.3	2.3–55.6
		Median	7.9	12.8	10.3	6.6	7.7	6.0	9.3	7.3	6.5	8.0
		Weighted Mean ± SE	14.1 ± 1.8	15.1 ± 1.2	12.4 ± 0.6	7.0 ± 0.4	7.4 ± 0.4	8.9 ± 1.0	11.0 ± 0.5	8.6 ± 0.6	7.9 ± 1.3	9.9 ± 0.4
		Weighted 95th CI	24.9–41.4	22.8–33.8	24.2–31.6	11.3–13.8	15.0–18.2	26.2–37.1	10.0–12.1	7.3–9.8	5.3–10.4	9.2–10.7
Chl *a* (μg/L)	2012	Range	2.3–13.5	1.1–5.5	1.4–41.6	2.6–19.4	4.3–49.9	na	1.1–49.9	1.4–41.6	2.1–21.3	1.1–49.9
		Median	4.6	3.2	4.9	6.3	15.9	na	6.3	5.5	5.5	6.0
		Weighted Mean ± SE	5.0 ± 0.3	3.0 ± 0.3	7.5 ± 1.3	8.4 ± 0.7	17.2 ± 1.3	na	9.6 ± 1.0	8.1 ± 0.8	6.4 ± 1.1	8.8 ± 0.7
		Weighted 95th CI	4.3–5.6	2.4–3.6	5.0–10.0	7.1–9.7	14.6–19.8	na	7.6–11.7	6.5–9.8	4.3–8.5	7.5–10.1
	2013	Range	3.0–8.9	3.5–19.5	3.9–28.1	5.1–23.1	2.3–21.4	0.6–6.7	0.6–26.0	1.5–28.1	1.8–17.3	0.6–28.1
		Median	5.2	7.1	7.8	13.6	7.4	2.5	7.1	6.3	5.10	6.3
		Weighted Mean ± SE	5.4 ± 0.2	8.2 ± 0.5	9.0 ± 0.8	14.6 ± 0.6	10.0 ± 0.8	2.7 ± 0.2	9.1 ± 0.6	7.2 ± 0.5	5.7 ± 0.6	8.1 ± 0.4
		Weighted 95th CI	5.0–5.8	7.4–9.1	7.4–10.6	13.4–15.7	8.4–11.5	2.4–3.0	7.9–10.3	6.3–8.2	4.5–7.0	7.4–8.9

Table 4
Summary statistics comparing BUI criterion concentrations with weighted means (Z-test) for each month, zone, and for all observations within a sampling year. Arrows indicate whether the mean was above (↑) or below (↓) the BUI criterion. Abbreviations: TP = total phosphorus; TSS = total suspended solids; Chl *a* = chlorophyll *a*; NS = not significantly different from criterion.

		Month						Zone			
Year	Parameter	May	June	July	August	September	October	Harbor	Bay	River	All observations
2012	TP	NS	$\downarrow -3.8^{***}$	$\uparrow 8.1^{***}$	$\downarrow -2.2^{*}$	NS	na	NS	$\uparrow 2.1^{*}$	$\downarrow -3.9^{***}$	NS
	TSS	$\downarrow -3.0^{***}$	$\downarrow -6.3^{***}$	NS	$\downarrow -16.0^{***}$	NS	na	$\downarrow -2.5^{*}$	$\downarrow -6.8^{***}$	$\downarrow -3.2^{**}$	$\downarrow -6.0^{***}$
	Chl *a*	$\downarrow -16.7^{***}$	$\downarrow -23.3^{***}$	NS	$\downarrow -2.3^{*}$	$\uparrow 5.5^{***}$	na	NS	$\downarrow -2.4^{*}$	$\downarrow -3.3^{**}$	NS
2013	TP	$\downarrow -2.8^{***}$	$\uparrow 3.3^{***}$	$\uparrow 6.1^{***}$	$\downarrow -8.0^{***}$	$\uparrow 2.4^{*}$	NS	NS	NS	NS	NS
	TSS	NS	NS	$\downarrow -4.3^{***}$	$\downarrow -20.0^{***}$	$\downarrow -19.0^{***}$	$\downarrow -6.1^{***}$	$\downarrow -8.0^{***}$	$\downarrow -10.7^{***}$	$\downarrow -5.5^{***}$	$\downarrow -12.8^{***}$
	Chl *a*	$\downarrow -23.0^{***}$	$\downarrow -3.6^{***}$	NS	$\uparrow 7.7^{***}$	NS	$\downarrow -36.5^{***}$	NS	$\downarrow -5.6^{***}$	$\downarrow -7.2^{***}$	$\downarrow -4.8^{***}$

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

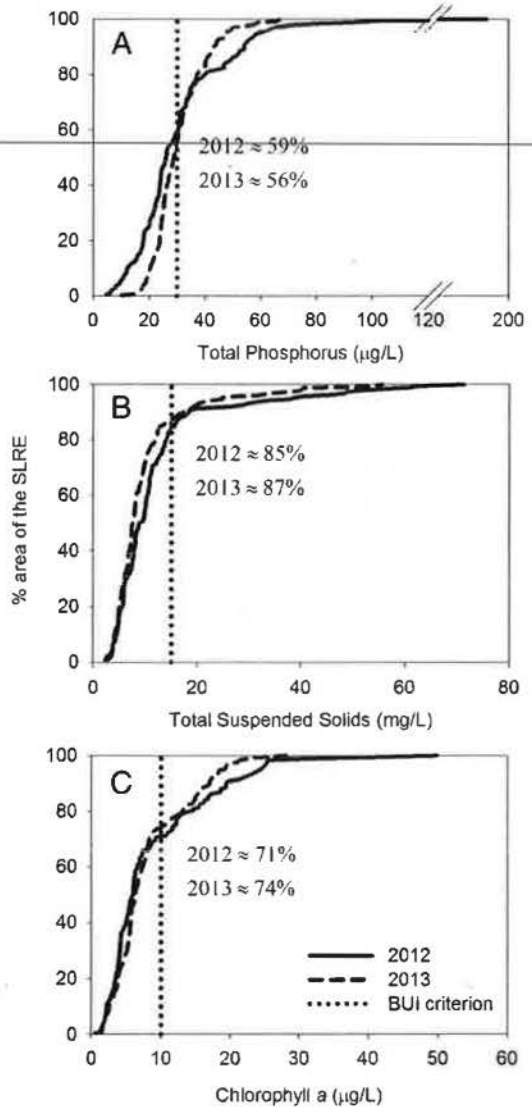

Fig. 5. Cumulative area functions for A) total suspended solids; B) total phosphorus; and C) Chlorophyll *a*. The x-axis is the analyte concentration versus the y-axis which is the cumulative percentage of area-weighted sites sampled in 2012 (solid line) and 2013 (dashed line) with 100% being the total surface area of the St. Louis River Estuary (i.e., 4376 ha). Vertical dotted lines are BUI criterion concentrations; percentages in each figure indicate the proportion of the system surface area below the criterion concentration.

relative to other months, indicating a recurring seasonal pattern. Factors include coupled rainfall mobilization of sediments and nutrients from the watershed and a lack of robust aquatic vegetation early in the season. Phytoplankton biomass and sediment-adsorbed P contribute to TP concentrations, and most P in the SLRE is particulate rather than dissolved (e.g., as orthophosphate; B.J. Bellinger, unpublished data). Additionally, vegetation coverage in the SLRE was reduced after the flood (Angradi et al., 2013). Resuspension of flood-transported sediment may still be elevating TSS and nutrient concentrations in the SLRE over pre-flood concentrations.

St. Louis River flood-pulse effects on water quality in the western arm of Lake Superior were apparently short-lived (Minor et al., 2014). The lake portion of the AOC had water quality conditions in 2013 consistent with oligotrophic conditions, satisfying the established BUI criteria (MPCA, 2013). Comparing findings from the 1974–1975 surveys with 2013, the water quality of western Lake Superior has remained consistently oligotrophic suggesting no enrichment from the estuary. Rather, Lake Superior is an N source to the estuary, especially of nitrate (Hiriart-Baer et al., 2009; Sterner, 2011). Elevated mean NH_4^+ concentrations in the harbor zone (Table 5) reflect samples collected near municipal wastewater treatment facilities (Fig. 1). Despite loadings of NH_4^+ and TP to Lake Superior, low concentrations in the open lake are maintained by phytoplankton and microbial processes (e.g., nitrification; Finlay et al., 2007; MPCA and WDNR, 1992; Munawar and Munawar, 1978).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jglr.2015.11.008.

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Table 5
Lake Superior water quality summary statistics data collected in 2013. Arithmetic means were compared between months and zones with one-way ANOVA. Significant pair-wise differences were identified with Scheffe's post hoc test and are indicated by different letters. Abbreviations: TP = total phosphorus; DO = dissolved oxygen; TN = total nitrogen; NO_x-N = nitrate/nitrite-N; NH_4-N = ammonium-N; TSS = total suspended solids; Chl *a* = Chlorophyll *a*; SE = standard error; NS = not significantly different.

			Month							
Parameter	Year	Statistic	May	June	July	August	September	October	ANOVA ($F_{5,18}$)	All Observations
TP (μg/L)	2013	Range	11.4–13.9	21.6–25.7	17.1–22.5	4.4–19.1	3.4–23.5	4.0–5.7		3.4–25.7
		Unweighted mean ± SE	12.2 ± 0.6	24.4 ± 0.9	19.0 ± 1.3	9.8 ± 4.7	8.6 ± 5.0	4.7 ± 0.4	5.2**	12.7 ± 1.7
DO (mg/L)	2013	Range	14.4–15.9	11.3–13.1	12.1–12.5	10.7–14.2	9.7–10.3	11.1–11.2		9.7–15.9
		Unweighted mean ± SE	15.1 ± 0.3^{c}	12.3 ± 0.4^{ab}	12.2 ± 0.1^{ab}	12.5 ± 0.9^{a}	10.1 ± 0.1^{b}	11.1 ± 0.1^{ab}	15.3***	12.2 ± 0.4
TN (μg/L)	2013	Range	467.1–742.4	531.4–887.2	523.8–576.7	309.3–651.1	493.8–714.1	513.4–824.3		309.3–887.2
		Unweighted mean ± SE	594.3 ± 56.6	784.0 ± 84.9	556.6 ± 11.5	526.6 ± 77.2	573.5 ± 48.8	606.1 ± 73.2	NS	606.9 ± 28.8
NO_x-N (μg/L)	2013	Range	304.1–358.9	268.5–283.0	315.9–326.0	325.0–351.4	307.4–337.3	311.1–364.1		268.5–364.1
		Unweighted mean ± SE	339.6 ± 12.2^{a}	274.5 ± 3.2^{b}	322.7 ± 2.3^{a}	333.0 ± 6.2^{a}	324.9 ± 7.2^{a}	334.3 ± 11.1^{a}	9.0***	321.5 ± 5.4
NH_4-N (μg/L)	2013	Range	2.0–42.1	28.1–47.7	13.5–26.1	2.0–30.6	6.2–17.8	2.0–30.7		2.0–47.7
		Unweighted mean ± SE	18.9 ± 9.0	39.3 ± 4.3	21.6 ± 2.9	10.3 ± 6.9	13.3 ± 2.6	14.0 ± 6.8	3.2*	19.6 ± 2.9
TSS (mg/L)	2013	Range	2.1–15.1	1.1–6.0	2.8–3.8	0.4–3.0	4.0–7.7	2.8–6.0		0.4–15.1
		Unweighted mean ± SE	6.6 ± 3.0	4.1 ± 1.2	3.1 ± 0.2	1.7 ± 0.6	6.2 ± 0.9	4.6 ± 0.8	NS	4.4 ± 0.6
Chl *a* (μg/L)	2013	Range	1.8–2.6	3.7–4.8	2.1–3.5	1.7–6.7	1.2–3.7	1.2–1.6		1.2–6.7
		Unweighted mean ± SE	2.1 ± 0.2^{ab}	4.5 ± 0.3^{a}	3.1 ± 0.3^{ab}	3.0 ± 1.2^{ab}	2.4 ± 0.5^{ab}	1.4 ± 0.1^{b}	4.4**	2.7 ± 0.3

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

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Appendix 2

Paleolimnology of the St. Louis River Estuary
And
Paleolimnology of a freshwater estuary
to inform Area of Concern nutrient delisting efforts
(Pertaining to management action 6.03.)

Paleolimnology of the St. Louis River Estuary

May 2016
The Minnesota Pollution Control Agency

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Executive Summary to the Beneficial Use Impairment Actions
Associated with Investigations of
Paleolimnology and Historic and Current Water Quality Condition
in the St. Louis River Area of Concern

Introduction

This executive summary provides an overview of the following investigations in relation to the St. Louis River Area of Concern's (SLRAOC) excessive loading of sediment and nutrients Beneficial Use Impairment (BUI) removal objectives:

Paleolimnology research results are organized into three attached reports structured for submission to peer-reviewed literature:

- Attachment A - Paleolimnology of the St. Louis River Area of Concern using algal microfossils including diatoms, pigments and basic sediment composition;
- Attachment B - Paleolimnology of wild rice and other plants in the St. Louis River Area of Concern using phytoliths and pollen;
- Attachment C - Historic Analysis of Various Chemical Constituents in Sediment Cores from the St. Louis River Area of Concern.

Purpose

The St. Louis River Watershed which drains to the St. Louis River and its associated estuary near Lake Superior has more than 150 years of human development history since Euro-Americans first settled there, resulting in critical water quality impacts. In 1987, the U.S. Environmental Protection Agency designated the St. Louis River as an Area of Concern primarily due to that history which entailed inappropriate discharge of untreated wastewater and debris from poor industrial and community practices. The organic matter loading from inadequate treatment of sewage and paper mill products along with the dumping of woody debris from sawmills contributed to low oxygen levels in the river. The result included devastating impacts to the entire food web from the bacteria to vegetation to invertebrates to fish. Concurrently, poorly managed stormwater runoff from this post-logged, barren landscape contributed excessive loading of suspended sediments resulting in increased turbidity and nutrient concentrations (e.g., phosphorus, nitrogen) to the river. Since then, government and private entities have taken action to restore the water quality in the St. Louis River Estuary, and to eventually remove the eight remaining SLRAOC BUIs. This summary focuses on the research documenting water quality changes over time associated with the excessive loading of sediment and nutrients BUI.

The historical magnitude and extent of sediment and nutrient impacts during the years preceding systematic long-term monitoring of water quality (pre-1953-1973, depending on location) is not well understood, and therefore, a paleolimnology study of the St. Louis River Estuary (SLRE) was initiated to close the knowledge gap. To help understand this history seven cores from the SLRE were evaluated for retrospective analyses by the Natural Resources Research Institute under Dr. Euan Reavie. The primary goal especially related to the excessive loading of sediment and nutrients BUI was to determine pre-

European settlement water quality conditions and to track through time the anthropogenic impacts followed by the extent of remedial progress. In order to do this, sediments in the core samples were dated using isotopic analyses and fossil remains (diatoms, pigments, pollen, phytoliths) were identified in concordance with other stratigraphic indicators (organic and inorganic materials, contaminants, sedimentation rates) to reconstruct the history of the system from 1850 to the present.

Historic and current water quality conditions were also evaluated to compare concentration estimates with BUI removal targets established by SLRAOC stakeholders. For the historic component, 60 years of water quality data (1953 – 2013) from two fixed stations were used to determine whether nutrient and sediment concentrations and loads have changed in the SLRE. In addition, current water quality condition was assessed both seasonally and spatially using data collected in 2012 and 2013. This work was supported by U.S. Environmental Protection Agency – Office of Research and Development under Dr. Joel Hoffman and Dr. Brent Bellinger.

SLRAOC BUI Overview

This summary is intended to assist Minnesota, Wisconsin, and the Fond du Lac Band of Lake Superior Chippewa in assessing the BUI targets for this particular action and determine if the BUI removal objectives are being met. The SLRAOC's Remedial Action Plan (RAP, 2015) describes the 1992 rationale for listing this BUI as follows:

> *Prior to the improvements in wastewater treatment in the late 1970s, water quality and biological investigations characterized the St. Louis River estuary (SLRE) as low in dissolved oxygen and high in total phosphorus and total suspended solids. At that time, the Western Lake Superior Sanitary District (WLSSD) treatment plant was built and the Superior wastewater treatment plant was upgraded. Since then, many indicators of trophic status have shown improvements. For instance, concentrations of total phosphorus have decreased and dissolved nitrogen has shown variable decline in St. Louis Bay. The loading of phosphorus to the estuary from point sources has been reduced substantially. At the time of AOC listing, further work was needed to ascertain the effects of nonpoint source loadings to the system and to Lake Superior. Despite the reductions in point source loadings, phosphorus concentrations in the estuary remained at levels where eutrophic conditions might be expected. Algal biomass was lower than would be expected, however, given these high phosphorus concentrations. Chlorophyll α concentrations measured in the estuary were similar to levels found in mesotrophic or oligotrophic waters. Several investigators proposed that reduced light penetration caused by turbidity and color may be a limiting factor for algal growth in the estuary. Although persistent water quality problems associated with eutrophication were not observed in the estuary, the high levels of nutrients and sediments being delivered to Lake Superior were determined to be an important concern. Therefore, the RAP used a modification of the International Joint Commission eutrophication criterion to reflect local conditions.*

BUI removal target

The BUI removal target, as established by stakeholders in 2008, is:

Nutrient and sediment levels have not been shown to impair water quality and habitat, and do not restrict recreation, including fishing, boating, or body contact in the estuary and within western Lake Superior based on the following criteria:

1. *All federal, state, and local point source and nonpoint source discharge permits in the AOC are in compliance with regard to controlling sources of nutrients (particularly nitrogen and phosphorous), organic matter, and sediment; and*
2. *Total phosphorus concentrations in the Lake Superior portion of the AOC do not exceed 0.010 mg/l (upper limit of oligotrophic range); and*
3. *There are no exceedances of the most protective water quality standard for either state in the western basin of Lake Superior due to excessive inputs of organic matter or algal growth attributed to loadings from wastewater overflows into the St. Louis River; and,*
4. *Total phosphorus concentrations within the St. Louis River portion of AOC do not exceed an interim guide of 0.030 mg/l (upper limit of mesotrophic range) or the most restrictive water quality standards. This ensures that anthropogenic sources and activities in the St. Louis River AOC do not result in excessive productivity and nuisance conditions within the St. Louis River Estuary.*

The removal of this BUI (RAP, 2015) will be justified when:

1. All federal, state, and local point source and nonpoint source discharge permits in the AOC are in compliance with regard to controlling sources of nutrients (particularly nitrogen and phosphorus), organic matter, and sediment.
2. Assessment of current water quality data for the Lake Superior and the St. Louis River estuary portions of the AOC indicate that water quality meets the water quality goals for a mesotrophic estuary and an oligotrophic lake.
3. Watershed management objectives for the Nemadji River watershed, as established by the Nemadji Basin Plan (NRCS, 1998), have been adopted and progress towards implementing the objectives is being made.

This work addresses the removal justification for #2 underlined above. It is important to keep in mind that there were fewer data to rely on when these targets were established. Therefore, the partners and stakeholders used the state established phosphorus criteria for inland lakes and rivers; these water bodies are very different than the western arm of Lake Superior and the St. Louis River Estuary. This work reviewed a suite of water quality parameters as specified in the RAP (2015) to determine whether legacy related impairments are influencing a eutrophic classification in the St. Louis River Estuary or a mesotrophic classification in Lake Superior. The parameters include:

- Chemical - total phosphorus, un-ionized ammonia, dissolved oxygen
- Biological – chlorophyll α
- Physical - total suspended solids (TSS) and turbidity or other loading metric based on tons of sediment

Paleolimnology study as a component of the BUI removal strategy

The BUI removal target contains several actions which should be completed in order to evaluate and document if the SLRAOC meets the objectives necessary to remove the BUI. Past efforts, particularly in regard to improved treatment of point source discharges and the construction of the Western Lake Superior Wastewater Sanitary District facilities in the late 1970s resulted in substantial water quality improvements in the estuary. This paleolimnological investigation report was completed in order to better understand historical water quality conditions prior, during, and following Euro-American settlement and development of the harbor. This study was designed to help clarify long-term nutrient trends prior to water quality monitoring in the estuary and to better understand the status of primary producers (e.g., phytoplankton, algae) and the potential for eutrophication in the estuary.

Paleolimnology Findings in relation to the BUI

Diatoms in relation to water quality

Diatom assemblages were assessed from sediment intervals and these assemblages were used to infer trophic conditions using a regional diatom-based model for Great Lakes coastlines. Interpretations were based on diatom-based models that contain known species responses to water quality, which are applied to fossil assemblages. The diatom records indicate varying ecological histories and trajectories depending on the location within the estuary. Deeper core locations (e.g., within or close to the federal navigation channel, Lake Superior) indicate water quality improvement from past periods of higher total phosphorus concentrations and algal productivity, and that current, prevailing concentrations of phosphorus do not exceed the SLRAOC BUI removal phosphorus target. However, the near-coastal (e.g., North Bay, Pokegama Bay, Allouez Bay) reconstructions reveal a recent increase in inferred phosphorus. At these locations, the inferred phosphorus levels based on the diatom species model would be in exceedance of the BUI removal target (see BUI Removal Target above). It is noteworthy that the earliest dated concentrations (~1850) are also inferred to be above the target, reflecting the natural productivity of these systems at that time.

Geochemistry in relation to water quality and nutrient loading

Algal pigment concentrations in the sediment profiles concur with diatom-based inferences. Main channel cores do not indicate recent increases in algal abundance, however the increasing presence of cyanobacterial pigments in two bays (North Bay, Billings Park – the only bays characterized for pigments) indicate increases in potentially undesirable algae, which are an indicator of increasing nutrients in those locations.

Historical sediment accumulation rates (organic and inorganic) indicate that recent sediment loads to the estuary remain higher than loads estimated around 1850. However, three sites (Lake Superior, Allouez Bay and Billings Park) exhibit reduced sediment loads since the peak period of development in the mid-20th century.

Paleolimnology Findings in relation to the overall history of the St. Louis River and its impairments

Diatoms and water quality

Overall, paleolimnological results from Lake Superior and the main stem of the estuary indicate improvements in nutrient loads or discontinuation in the enrichment trends that were observed through the 1970s. Based on similar observations in degrading systems (e.g., western Lake Erie, southern Lake of the Woods [Minnesota-Ontario]), the nearshore eutrophication observed in the estuary may be due to factors such as periodic recycling of stored sedimentary phosphorus (regulated by the extent and duration of oxygen depletion coupled with intermittent wind mixing events). These conditions may be further aggravated by climate change related to increased winds and stormwater runoff and stronger thermal stratification in the ice-free season. These factors are poorly understood and require further studies based, in part, on comprehensive long-term water quality monitoring.

Geochemistry

This work was intended to better understand general trends of sediment contaminants and to see if the science behind the analysis can provide a line of evidence that supports improvement of these historic contaminations through time. Mercury is a marker of human activities such as mining, burning of fossil fuels, and untreated sewage disposal. Sediment mercury concentrations peaked in the mid-1900s but more recently declined to near pre-impact concentrations, indicating recent decreases in some combination of direct atmospheric deposition, watershed runoff, and point source domestic and industrial discharges. There were distinct mid-1900s peaks in cadmium, zinc, lead, tin, antimony and magnesium, likely resulting from watershed disruptions that exposed materials to erosion and runoff and/or industrial discharges. With improved regulation of these activities there has been a concurrent reduction in sedimentary concentrations. Sedimentary organic contaminants (PCBs, VOCs, PAHs) analyzed from a single core from the harbor had concentrations below the detection levels.

Wild Rice/Vegetation Historical Patterns

Pollen distributions in the cores generally reflected the historical reduction in conifers due to deforestation through the late 1800s and early 1900s and the more recent increase in birch trees. Sedimentary remains of wild rice (phytoliths and pollen) do not indicate a reduction in wild rice abundance in the SLRAOC in modern times relative to pre-industrialization of the harbor, but it was clear the core locations were not optimal for assessing historical wild rice communities. Wild rice restoration is occurring in the estuary and future work outside of the SLRAOC program is recommended at core locations within or close to known or past wild rice beds to help better understand expectations for restoration.

Conclusions in Relation to BUI Status

Paleolimnology Investigation

Clear improvements in TP concentrations in the water column, as inferred from paleo-diatom analyses from mid-channel cores, have resulted from remedial efforts (largely wastewater treatment and stormwater management) in the SLRAOC over the past ~40 years. Increasing nutrient loads are inferred

in the three nearshore locations. In terms of nearshore phosphorus, the study generated evidence that pre-European impact concentrations of phosphorus likely exceeded the BUI removal target criteria by approximately 10 – 15 µg TP/L. Also, it is likely nearshore changes in water quality are a result of phenomena (such as climate change, sediment phosphorus recycling, and perhaps other indirect mechanisms) that were not included in the rationale for listing this BUI. A more detailed paleolimnology investigation, including speciation of phosphorous and development of a nutrient budget for the system would be needed to determine the factors influencing the nearshore areas.

These data indicate BUI removal objectives are being met in over fifty percent of the estuary. It is interesting to note the BUI target is lower than some of the earliest inferred phosphorus conditions. It may be appropriate to support BUI removal based on overall improvement. As we move forward from legacy to present issues, studies are recommended to better understand the main drivers of algal production (i.e., a direct cause of eutrophication) in nearshore areas of the SLRE and portions of the St. Louis and Nemadji watersheds. Efforts to minimize pollutant loads should continue and a comprehensive monitoring program with periodic re-evaluation of these data should be established.

ATTACHMENT A

Paleolimnology of a freshwater estuary to inform Area of Concern nutrient delisting efforts

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Key words: Diatoms; Great lakes; Lake Superior; Beneficial use impairment; St. Louis River; Minnesota; Wisconsin

Abstract

The St. Louis River Estuary (SLRE), a freshwater estuary bordering Duluth, MN, Superior, WI, and the most western point of Lake Superior (46.74°, -92.13°), has a long history of human development since Euro-American settlement ~200 years ago. Due to degradation from logging, hydrologic modification, industrial practices, and untreated sewage, the SLRE was designated an Area of Concern in 1987. Action has been taken to restore water quality including the installation of the western Lake Superior Sanitary District (WLSSD) in 1978 to help remove beneficial use impairments (BUIs). A better understanding of historical impacts and remediation is necessary to help document progress and knowledge gaps related to water quality, so a paleolimnological study of the SLRE was initiated. Various paleolimnological indicators (pigments, diatom communities, and diatom-inferred phosphorus) were analyzed from six cores taken throughout the SLRE and another from western Lake Superior. Reductions in eutrophic diatom taxa such as *Cyclotella meneghiniana* and *Stephanodiscus* after 1970 in certain cores suggest an improvement in water quality over the last 40 years. However, in cores taken from estuarine bay environments, persistence of eutrophic taxa such as *Cyclostephanos dubius* and *Stephanodiscus binderanus* indicate ongoing nutrient problems. Sedimentary pigments also indicate cyanobacteria increases in bays over the last two decades. Diatom model-inferred phosphorus and contemporary monitoring data suggest some of the problems associated with excess nutrient loads have been remediated, but modern conditions (internal phosphorus loading, changing climate) may be contributing to ongoing water quality impairments in some locations. The integrated record of biological, chemical, and physical indicators preserved in the sediments will aid state and federal agencies in determining where to target their resources.

Introduction

Natural and anthropogenic history

The St. Louis River (SLR; Figure 1) flows 288 km through northeastern Minnesota draining an area of 9412 km^2. On average, it delivers 73.3 m^3/s of water to western Lake Superior, making it the largest tributary to Lake Superior in the United States. The drainage basin land cover consists of forests (61%), wetlands (24%), and grasslands (7%); the remaining 8% is developed (MPCA 2013). The most downstream portion (after Minnesota Highway 23) of the river before it joins Lake Superior is the St. Louis River Estuary (SLRE). In contrast to a mostly rural upstream, the dominant land use surrounding the SLRE is urban. The estuary is bordered by two major cities—Duluth, MN and Superior, WI—which have a combined population of 113,000 people (United States Census Bureau 2010).

Proximity to water provided access to the eastern United States and beyond, expansive timber stands, and iron-rich rock allowed for settlement of the region. Since European settlement the SLRE became one of the most impacted ecosystems in the Laurentian Great Lakes. In the late 1890s the SLRE became a major shipping port. The estuary was first dredged in 1867 and the Duluth Shipping Canal was completed by 1871 (MPCA and WDNR 1992). Approximately 1600 hectares of open water and shoreline were filled with dredged material since 1861 (Devore 1978). Dredging is still routine to maintain the port for commercial shipping.

The "cutover" period from the late 1800s to early 1900s, when most of the watershed was clear cut by loggers, resulted in a dramatic impact on land cover, leading to increased runoff of both water and soils. In order to control the transportation of timber downstream, 50 to 100 dams were installed on the river. The SLRE was home to many sawmills, pulp mills, and paper mills. These were a major source of pollution to the estuary, discharging large quantities of sawdust and slab wood (MPCA and WDNR 1992).

The population of Duluth and Superior grew from an estimated 600 in 1865, to 100,000 by 1930 (MPCA and WDNR 1992). The region lacked proper stormwater and wastewater infrastructure, so untreated wastewater (e.g. human waste and high-phosphorus detergents) introduced nutrients and organic matter into the SLRE, leading to episodic hypoxia (Hargis 1983; Carlson and Thomas 1984).

In 1966, the Federal Water Pollution Control Administration Great Lakes Regional Office made recommendations on strategies to reduce pollution. This included installing wastewater and stormwater treatment facilities to reduce nutrient loading (FWPCA 1966). In response, the western Lake Superior Sanitary District (WLSSD) opened in 1978 to treat wastewater from a 1,375 km^2 region including Duluth and surrounding communities (MPCA and WDNR 1992). A study comparing concentrations of various metals, nutrients, and physical parameters before and after 1978 showed significant water quality improvement in the SLRE including a decrease in total phosphorus, turbidity, total coliform, lead, and copper and an increase in dissolved oxygen (McCollor 1990).

The International Joint Commission, a governing body including representatives from Canada and the United States regulating international waters, wrote and passed the Great Lakes Water Quality Agreement in 1972 in order to "to restore and maintain the chemical, physical, and biological integrity of the Waters of the Great Lakes" (IJC 1972). The legislation was amended in 1987 to designate degraded areas throughout the Great Lakes as Areas of Concern (AOC) based on one or more of 14 beneficial use impairments (BUIs) (IJC 1987). The SLRE

was listed as one of 43 (26 in the US) AOCs and was defined by 9 BUIs: restrictions on fish consumption, degraded fish and wildlife populations, fish tumors and other deformities, degradation of benthos, restrictions on dredging, excessive loading of sediments and nutrients, beach closings and body contact restrictions, degradation of aesthetics, and loss of fish and wildlife habitat (MPCA and WDNR 1992). In 1992, the first Remedial Action Plan (RAP) was released which outlined the degradation of the SLRE and described the BUIs (MPCA and WDNR 1992). Since then, one BUI was removed (degradation of aesthetics).

Agencies hope to remove all BUIs and delist the AOC by 2025. To achieve this, there has been an estuary-wide effort to remediate contaminated sediments and restore aquatic habitat. Agencies have developed removal targets for BUI 6 (excessive loading of sediment and nutrients). No doubt best management practices within the watershed and the installation of wastewater treatment facilities helped to reduce the flux of nutrients to the SLRE (MPCA and WDNR 2013), but the extent of recovery is poorly understood. A phosphorus dataset beginning in 1953 exists for the SLRE and consists of monthly measurements. These data show a recovery in phosphorus concentrations in the main estuary channel (Bellinger et al. 2016). Still, measurements of nutrient concentrations and sediment loading, and biological responses to these changes, are absent for most of the SLRE's past, especially pre-settlement conditions.

A paleolimnological study of the SLRE was initiated to help to provide insight on long-term environmental impacts and remediation while considering the known human history of the watershed. We hypothesized that paleolimnology could would provide valuable retrospective data in support of the AOC delisting process. Subfossil diatoms (Bacillariophyceae) and algal pigments were the primary indicators used. To examine the historical environmental conditions of the SLRE, relative abundances of diatom taxa before, during, and after European settlement were characterized. Changes in diatom assemblages and inferred environmental information were correlated with anthropogenic activities and validated with additional data, including fossil pigments and sediment organic content. The extent of degradation and recent AOC remediation was clarified, and management recommendations relevant to AOC BUI removal are provided herein.

Methods

Site selection and coring

Sediment cores were taken from areas believed to have undisturbed sediments and continuous depositional environments. Maps and hydrological data were used along with consultation with the US Army Corps of Engineers in order to avoid areas previously impacted by dredging or shipping activities. Sites were chosen to represent a variety of environments including different hydrologic regimes (bays, harbor, Lake Superior) and varying anthropogenic impacts (formerly polluted versus purportedly less impacted). A total of seven cores were collected; six cores from discrete locations in the lower SLRE and one core from western Lake Superior (Figure 1).

At each coring location we attempted to collect at least 200 years of sediment in order to evaluate the SLRE's anthropogenic record related to European settlement. SLRE cores were collected in winter of 2014. A piston corer designed for sampling recent lake sediments was used (Glew et al. 2001). For each core, approximately one meter of sediment was collected and sectioned into 1-cm intervals (0.25 cm intervals for the first 10 cm and then 0.5 cm intervals for Lake Superior). The core taken from western Lake Superior was collected in May 2014 from the

US Environmental Protection Agency's research vessel *Lake Guardian* by use of a multicorer (methods described by Shaw Chraïbi et al. 2014). Samples were extruded in intervals using a close-sectioning extruder and kept refrigerated for later analyses.

Sediment chronology

To determine age and sediment accumulation rates for the past 150 to 200 years, sediment cores were analyzed for ^{210}Pb activity. Lead-210 activity was measured from its daughter product, ^{210}Po, which is considered to be in secular equilibrium with the parent isotope. Aliquots (0.5-3.0 g) of freeze-dried sediment were spiked with a known quantity of ^{209}Po (~4 pCi/g) as an internal yield tracer and the isotopes distilled at 550°C after treatment with concentrated HCl. Polonium isotopes were then directly plated onto Au planchets from a 0.5 N HCl solution. Activity was measured for 1-3 x 10^5 s using an Ortec alpha spectrometry system. Supported ^{210}Pb was estimated by mean activity in the lowest core samples and subtracted from upcore activity to calculate unsupported ^{210}Pb. Core dates and sedimentation rates were calculated using the constant rate of supply model (Appleby and Oldfield 1978, Appleby 2001). Dating and sedimentation errors represented first-order propagation of counting uncertainty (Binford 1990). For cores with problematic decay profiles (e.g., North of Clough Island), gamma spectrometry was used to measure supported ^{210}Pb and identify the distribution of ^{137}Cs in the core (Ritchie and McHenry 1973). Activity was measured using an Ortec-EGG (Oak Ridge, TN) High-Purity, Germanium Crystal Well, Photon Detector (Well Detector) coupled to a Digital Gamma-Ray Spectrometer (Dspec).

Inorganic and organic chemistry

Loss on ignition analysis to determine inorganic and organic content followed Dean (1974). Water content was determined by heating sediment samples to 100 °C for 24 hours. Samples were heated to 550 °C for two hours to determine organic content and the remaining material was brought to 1000 °C to calculate clastic composition. All content was calculated from weight lost after heating.

Pigments

On a subset of four cores, pigments (carotenoids and chlorins) were analyzed to examine historical algal communities according to methods in Alexson (2016) based on Reuss (2005) and Reuss and Conley (2005). The pigments analyzed represented total algae (chlorophyll *a*, pheophytin *a*, and *β*-carotene), diatoms (diatoxanthin and fucoxanthin), and dinoflagellates (fucoxanthin), cryptophytes (alloxanthin), and cyanobacteria (aphanizophyll and myxoxanthophyll). Pigments were extracted from the freeze-dried sample material using acetone/water mixture (90/10 by volume). After extraction, the material was quantitatively analyzed using a Shimadzu High Performance Liquid Chromatographer equipped with a photodiode array detector.

Diatoms

For each core interval analyzed for diatoms, approximately 1 g of wet sediment was subsampled and digested with strong acid to remove all organic material and isolate siliceous microfossils. Samples were then rinsed with distilled water to neutralize acid and applied to coverslips quantitatively using the Battarbee (1986) method. This method allowed for the quantitative analysis of diatom abundance and accumulation rates. Coverslips were mounted to microscope slides with Naphrax for identification and enumeration.

Diatoms were identified and enumerated by use of oil immersion on a light microscope (1,250X magnification). Diatoms on each slide were identified along random transects until at least 400 diatom valves were enumerated. Each diatom was identified to species level according to Reavie and Kireta (2015), Krammer and Lange-Bertalot (1989 - 1991), Patrick and Reimer (1966, 1975), and several other taxonomic works. Phytoliths, sponge spicules, chrysophyte scales and stomatocysts, and testate amoebae plates were also counted when observed. These siliceous remains can be used to infer environmental conditions and may provide additional insight on the ecological condition of the SLRE (Smol et al. 2001).

Cluster analysis

We aimed to characterize the stratigraphic zonation which may reflect historical events leading to reorganization of the diatom community. Relative abundance was calculated for each diatom species for all core intervals. For common taxa (at least five occurrences with at least 5% abundance in one or more samples), a depth-constrained cluster analysis was done using the "chclust" function in R with the "rioja" package (Juggins 2014) to identify temporally constrained diatom assemblage zones. The CONISS algorithm (Grimm 1987) was used to perform clustering constrained to vertical stratigraphy, based on dissimilarity in squared Euclidian distances among samples. The embedded function "bstick" was used to perform a broken-stick analysis and determine the minimum number of significant zones (Bennett 1996). In addition to zones determined by the broken stick analysis, zones were delineated based on major changes in abundance of characteristic species.

Ordination

In order to better assess the similarities among cores and track temporal trajectories, non-metric multidimensional scaling (NMDS) analyses were performed. NMDS is an ordination technique allowing for visualization of highly dimensional data in lower dimensional space. Multidimensional scaling examines distances between observations (e.g. samples or species); shorter distances indicate similarity. The statistical software R with the vegan package (R Core Team 2014; Oksanen et al. 2015) was used to create an NMDS plot from diatom relative abundance data. Species with a maximum relative abundance less than 5% were omitted to reduce analytical artifacts from rare species.

Diatom-inferred modeling

Diatom-inferred (DI) modeling translates fossil diatom data into quantitative profiles of water quality variables. To develop the DI models, diatom species in a training set of samples were

related to total phosphorus (TP) measurements and species coefficients (phosphorus optima) were calculated. These species-specific coefficients were applied to the diatom assemblages in cores, and TP was inferred based on the relative abundances of fossil diatom taxa. Models were developed from two Great Lakes training sets: (1) open water (Reavie et al. 2014; used for the Lake Superior core) and (2) coastal embayments, wetlands, and high-energy areas (Reavie et al. 2006; used for the six estuary cores).

A set of analyses verified the efficacy of both models' ability to reconstruct phosphorus. An analog analysis determined similarities between diatom assemblages in the models and fossil assemblages. Using the R package analogue (Simpson and Oksanen 2015) assemblages from the model were matched to fossil assemblages following Flower et al. (1997) and Simpson et al. (2005). Analogs were determined using Bray-Curtis dissimilarity (Bray and Curtis 1957). Dissimilarities between fossil and modern samples were examined to determine how well fossil assemblages were represented in model assemblages. A constrained canonical correspondence analysis (CCA) was done to examine the relationship between modern phosphorus and diatom assemblages, and then fossil samples were ordinated passively to determine goodness of fit. Using the R packages vegan and analogue (Oksanen et al. 2015; Simpson and Oksanen 2015) a CCA defined residual distances of fossil assemblages (i.e. sample scores) and TP gradient (i.e. constrained CCA axis 1). Fossil residual distances within the 95 % confidence interval of the modern sample distances were considered to have good fit to TP.

Analyses according to Reavie et al. (2014) were completed to determine if TP was related to changes in fossil species assemblages. Using the R package vegan (R Core Team 2014; Oksanen et al. 2015), each set of fossil data in a given core was distilled using principal components analysis (PCA) to derive axis scores representing the primary gradient of variation in the diatom assemblage data (Juggins and Birks 2012). A correlation coefficient (r) was calculated for historical diatom inferred total phosphorus (DI-TP) versus the axis 1 PCA scores. If |r| was high and significant, it was likely changes in fossil diatom assemblages in cores were at least in part determined by TP, and so use of the DI-TP model was considered appropriate.

Results

Sediment chronology

Exponential decay of ^{210}Pb with sediment depth was used to determine the validity of chronological profiles. With the exception of North of Clough Island, cores showed a consistent record of sediment accumulation and were dateable (Alexson 2016). ^{210}Pb data from North of Clough Island suggested recent disturbance, likely due to increased sedimentation from a 500-year flood that affected the SLRE in 2012 (Czuba et al. 2012). Unsupported (excess) ^{210}Pb data were relatively monotonous with depth, aside from an uppermost section above ~35 cm depth with higher concentrations. Supplementary dating using ^{137}Cs characterized high concentrations of that isotope around 1963 due to nuclear weapons testing (Krishnaswami and Lal 1978). Based on a peak in ^{137}Cs at 60 cm depth, we assigned a rough, recent chronology based on knowing the 1963 interval, acknowledging dates since 1963 are highly uncertain due to flood disturbance.

With the exception of North of Clough Island, cores showed increased sedimentation rates in the early 1900s or just prior (Figure 2, left-most panels). Several cores demonstrated a recovery in sedimentation rates: Allouez Bay and Billings Park had peaks around 1920 - 1930 and subsequently fell to sedimentation rates of 0.35 to 0.15 g/cm^2/y and 2.0 to 0.2 g/cm^2/y

respectively. In western Lake Superior, sedimentation rates peaked around 1970 at 0.12 g/cm^2/y with a secondary peak at 0.11 g/cm^2/y in 1900; rates recovered to near pre-settlement conditions around 2000. This trend is similar to North Bay where there was a secondary peak in 1930 (0.3 g/cm^2/y) and a peak in 1970 (0.4 g/cm^2/y). Rates declined to 0.2 g/cm^2/y by 2000, but rates remained higher than pre-settlement. In contrast, sedimentation rates continued to increase in cores from Minnesota Point and Pokegama Bay. At Minnesota Point, accumulation rates rose from less than 0.02 g/cm^2/y to 0.14 g/cm^2/y, with the greatest rate of change occurring in the last 40 years. Sedimentation rates at Pokegama Bay increased from 0.05 g/cm^2/y to 0.25 g/cm^2/y with two peaks occurring at 1960 and 1990 (both around 0.35 g/cm^2/y). The accumulation profile for North of Clough Island was based on a single ^{137}Cs date, so we have great uncertainty about recent accumulation rates. Overall, differences in average sediment accumulation rates among cores reflected their physical settings, such as the lower rates in the more lacustrine areas (Minnesota Point and Lake Superior).

Sediment Content

All cores indicated decreasing water content with depth due to compaction (Figure 2). Cores from Allouez Bay and North Bay had the most distinct changes in organic content with a peak in the 1930s and a concomitant increase in % inorganic material. An increase in organic content was also seen at North of Clough Island since 2000, and due to uncertainty in dating may reflect a depositional layer from the 2012 flood (Czuba et al. 2012).

Accumulation rates of organic, inorganic, and carbonate components largely followed total sedimentation rates, although there were some anomalies. There was a period of very low carbonate accumulation from ~1970 through ~1995 in North Bay and from 1910 – 1940 in western Lake Superior. In North of Clough Island, there was lower accumulation of organic material from 1970 – 2000 and heightened accumulation from 2000 until present.

Pigments

Pigments in four estuary cores (North Bay, Billings Park, North of Clough Island, and Minnesota Point; Figure 3) tracked historical shifts in algal groups. Pigments representative of total algae (chlorophyll *a*, pheophytin *a*, and *β*-carotene) in all cores showed temporary heightened productivity in recent sediments since ~1990 in North Bay and Billings Park. Fucoxanthin and diatoxanthin were higher in North Bay and Billings Park. Fucoxanthin (diatoms and dinoflagellates) increased rapidly in more recent intervals, since 1990 in North Bay and 2005 in Billings Park, whereas diatoxanthin (diatoms) showed a gradual increase since 1980. Pigments from cyanobacteria (aphanizophyll and myxoxanthophyll) have increased in both North Bay and Billings Park in the last 20 years. Although there was a strong peak in alloxanthin (representing cryptophytes) around 1970, since ~1980 pigment concentrations in Minnesota Point and North of Clough Island cores remained relatively low and steady. To account for the possibility of some pigments degrading with time (e.g. chlorophyll *a* tends to have low stability; Leavitt and Hodgson 2001), we note recent increases occur in pigments with known reliability in long-term preservation in sedimentary records (pheophytin *a*, fucoxanthin, diatoxanthin, aphanizophyll, and myxoxanthophyll, and especially *β*-carotene).

Diatoms

A total of 654 diatom taxa were observed from 88 genera. In SLRE cores, both benthic and planktonic diatoms were common whereas the species composition in Lake Superior was mostly planktonic. Diatom accumulation rates in North Bay, Pokegama Bay, Billings Park, and Allouez Bay peaked in the mid-20th century (Figure 4) whereas accumulation rates were highest around 2000 in Minnesota Point and North of Clough Island. North Bay and Allouez Bay shifted to centric-dominated (i.e. planktonic) assemblages (~1900 and ~1940 respectively) and had mostly consistent proportions of pennates to centrics. Chrysophyte stomatocyst to diatom ratio was higher in earlier intervals of the North Bay, Pokegama Bay, Billings Park, Minnesota Point, and Allouez Bay cores. Chrysophytes are more competitive in oligotrophic environments; therefore, higher ratios of chrysophyte cysts to diatoms are associated with lower nutrients (Smol 1985). Long-term trends showed a decrease in chrysophyte stomatocyst to diatom ratio indicating increased nutrient loading. In North Bay, Allouez Bay, and Billings Park this ratio continued to decline, while it stabilized in Minnesota Point and Pokegama Bay (around 1970 and 1950 respectively). Accumulation rates of stomatocysts generally had similar stratigraphies to those of diatoms.

Several estuary cores (North Bay, Pokegama Bay, Minnesota Point, and Allouez Bay) transitioned (~1850 - 1900) from benthic diatoms (*Staurosira* Ehrenberg and *Staurosirella* Williams and Round) to communities dominated by planktonic *Aulacoseira* Thwaites (Figure 5). This suggests a transition to a more lacustrine environment resulting from damming (associated with the lumber industry) taking place during this time period. Because of unique assemblages among cores, we present diatom results separately for each core. Stratigraphic zones representing periods of major assemblage changes, as determined by cluster analysis, were used to guide interpretation of historical trends.

North Bay

North Bay had two significant zones determined by the broken stick analysis, pre and post-1900. However, based on apparent shifts in diatom assemblages, we delineated three zones in the core: (A) pre-1900, (B) 1900 - 1945, and (C) post-1945 (Figure 5a). The core was made up of mostly planktonic diatoms (*Aulacoseira*, *Fragilaria* Lyngbye, and *Stephanodiscus* Ehrenberg), but was also accompanied by some benthic genera (*Staurosira*, *Achnanthidium* Kützing, *Cocconeis* Ehrenberg, and *Navicula* Bory de Saint-Vincent). *Staurosira construens* var. *venter* (Ehrenberg) Hamilton and *Staurosirella pinnata* (Ehrenberg) Williams & Round dominated Zone A (combined ~20 %). These are epipsammic and epipelic diatoms indicating a low-nutrient, benthic-dominated community (Estepp and Reavie 2015; Morales 2010a). In the early 1900s (Zone B) many species comprising the modern assemblage increased in abundance while *S. construens* var. *venter* and *S. pinnata* declined. Higher-nutrient indicators *Aulacoseira ambigua* (Grunow) Simonsen, *Stephanodiscus parvus* Stoermer& Håkansson, *Cyclotella meneghiniana* Kützing, and *Stephanodiscus hantzschii* Grunow (Stoermer et al. 1985; Stoermer and Yang 1970; Stoermer and Håkansson 1984) appeared in greater abundance in Zone C. Since their initial increase, some species (*C. meneghiniana* and *S. hantzschii*) declined in the last decade, although a few nutriphilic taxa (*Aulacoseira granulata* (Ehrenberg) Simonsen and *S. parvus*) became more abundant during the last decade. Also in Zone C, benthic and epiphytic taxa like *Cocconeis placentula* Ehrenberg (Round et al. 1990), and *Navicula gregaria* Donkin (Round et al. 1990)

increased in abundance, reflecting a probable, local increase in macrophyte habitat. *Fragilaria vaucheriae* Petersen (Morales 2010b) and *Fragilaria mesolepta* Rabenhorst (Potapova and Spaulding 2013) were also higher in Zone C.

Pokegama Bay

Centric diatoms dominated the core from Pokegama Bay (Figure 5b). While only four zones were determined to be significant by broken stick analysis, we interpreted five zones based on the cluster analysis: (A) pre-1830, (B) 1830 - 1910, (C) 1910 - 1970, (D) 1970 - 1980, and (E) post-2000. The historical community in Zone A consisted of *S. pinnata* (benthic; Estepp and Reavie 2015), *Achnanthidium minutissimum* (Kützing) Czarnecki (epiphytic; Potapova 2009), and *Aulacoseira pusilla* (Meister) Tuji & Houk (planktonic; Potapova 2010). In Zone B, *A. pusilla,* joined by *A. granulata* and *A. ambigua* increased in abundance to dominate the assemblage. *A. ambigua* and *A. granulata* continued to increase in Zone C until their peak (35 % in 1940, 45 % in 1960) after which they generally declined. Eutrophic *S. parvus* also increased in this period until it declined in Zone D, though its abundance was still higher than pre-settlement. In Zone E, *Aulacoseira* still dominated (~40 %) but was partly replaced by another eutrophic diatom, *Cyclostephanos dubius* (Fricke) Round (Hickel and Håkansson 1987), whose abundance grew to around 10 % of the diatom assemblage.

North of Clough Island

The diatom record from North of Clough Island only extended back to 1940 so pre-impact conditions cannot be determined (Figure 5c). The broken stick analysis determined at least three temporal zones were significant. In Zone A (pre-1970) eutrophic indicators *C. meneghiniana* and *A. granulata* peaked in the late 1960s (20 % abundance) and then rapidly returned to earlier conditions (1 - 4 %) in Zone B (1970 - 1990). During Zone B *S. parvus* also increased, but abundance decreased around 1990 (the start of Zone C). The modern assemblage had high diversity and consisted of both pelagic and benthic diatoms. Common genera were *Cyclotella* (Kützing) Brébisson, *Cocconeis*, *Aulacoseira*, *Achnanthidium*, *Staurosira*, and *Staurosirella*. We again note great uncertainty in the timing of changes in this core due to the recent flood, which may have deposited allochthonous material in an undetermined layer near the core surface. We have confidence the uppermost ~2 intervals represent post-flood deposition, and the assemblage (*S. pinnata*, *A. minutissimum*, and *A. ambigua*) indicates lower nutrients than pre-1970 taxa.

Billings Park

The core from Billings Park was dominated by planktonic diatoms, especially species from the genus *Aulacoseira* (Figure 5d). The core had three significant zones; however, we determined five zones showed important changes in assemblages: (A) ~1900 - 1940, (B) 1940 - 1970 (C) 1970 - 2000, (D) 2000 - 2010, and (E) post-2010. The assemblage was made up of largely *A. pusilla*, A. *granulata*, and *A. ambigua* in Zone A, but shifted to *A. granulata* and *A. ambigua* dominance in Zone B. Nutrient-tolerant diatoms *S. parvus*, *S. hantzschii*, *Stephanodiscus binderanus* (Kützing) Krieger, and *C. meneghiniana* increased in abundance in Zone C, followed by a partial decline as they were replaced by small, benthic species (e.g. *S. construens* var.

venter, *S. pinnata*, and *Pseudostaurosira brevistriata* (Grunow) Williams & Round) in Zone D. Zone E shifted back to an *Aulacoseira*-dominated assemblage similar to before ~1970.

Minnesota Point

The core from Minnesota point was largely made up of centric and araphid planktonic diatoms with a smaller proportion of benthic species (Figure 5e). Three zones (two were determined significant by broken stick analysis) were identified: (A) pre-1850, (B) 1850 - 1980, and (C) post-1980. *Staurosira construens* Ehrenberg, *S. pinnata*, *S. construens* var. *venter*, *A. granulata*, and *A. ambigua* dominated Zone A, which existed as far back as ~1700. In Zone B, *Staurosira* and *Staurosirella* decreased and there was some growth in the already dominant *Aulacoseira* population. Eutrophic *S. parvus* increased and reached a maximum abundance (<10 %) in ~1965 and returned to near pre-settlement abundances (<5 %) in Zone C. Also in Zone C *A. granulata*, *A. ambigua*, and *P. brevistriata* increased.

Allouez Bay

Allouez Bay consisted of mostly planktonic diatoms (Figure 5f). Although the broken stick analysis only found two significant zones, we delineated four zones based on apparent changes in diatom assemblages. The historical assemblage (Zone A, pre-1880) was very diverse, including the phytoplankton *Aulacoseira subarctica* (O. Müller) Haworth, *A. pusilla*, *A. ambigua*, *A. granulata*, *Stephanodiscus* sp. #10, the epiphytic *A. minutissimum* and *Eunotia incisa* Smith ex Gregory, and the benthic *S. construens* var. *venter* (each ~5 %). In Zone B (1880 - 1940) *A. ambigua* and *A. granulata* grew to dominate the assemblage, indicating greater planktonic dominance and probable nutrient enrichment. They continued to rise and reached a maximum (together 40 % of the assemblage) in Zone C (1940 - 1960). In Zone D (post-1960), eutrophic indicators *S. binderanus*, *C. meneghiniana*, and *C. dubius* (Stoermer et al. 1987; Hickel and Håkansson 1987) began to increase in abundance, each occupying ~5 – 10 % of the assemblage in the upper intervals.

Lake Superior

The species assemblage in Lake Superior was dominated by planktonic, centric diatom species (*Lindavia* (Schutt) De Toni & Forti, *Cyclotella*, *Stephanodiscus*, and *Aulacoseira*) (Figure 5g). Zone A (pre-1910) was dominated by *S.* sp. #10, *Lindavia ocellata* (Pantocsek) T.Nakov et al., *Cyclotella atomus* var. 1, and *Lindavia comensis* (Grunow in Van Heurck) T.Nakov et al., taxa generally reflecting low nutrients. *Stephanodiscus conspicueporus* Stoermer, Håkansson & Theriot, *A. subarctica*, *A. islandica*, and *A. ambigua*, mesotrophic diatoms indicating higher nutrients in oligotrophic Lake Superior (Stoermer 1993) increased in Zone B (1900 - 1970) but decreased in Zone C (1970 - 1985). Small centric diatoms, *L. comensis* and *L. comensis* var. "rough center with process" (*Lindavia* cf. *delicatula* (Hustedt) T.Nakov et al.; Reavie and Kireta 2015) began increasing in Zone C and increased to a combined abundance of ~40 % in Zone D (post-1985). These low-nutrient taxa may be related to climate-driven physical changes in the lake (Shaw Chraïbi et al. 2014, Reavie et al. 2016).

Ordination

Based on an initial ordination of diatom samples from all cores, Lake Superior was highly dissimilar to SLRE cores, indicating substantial differences in common taxa between the lake and SLRE (Figure 6a). Therefore, the analysis was repeated to examine (1) all cores, (2) Lake Superior, and (3) SLRE cores to better visualize historical trajectories in NMDS ordinations.

NMDS of Lake Superior (Figure 6b) reflected a constant reorganization of diatom assemblage, from a pre-1900 assemblage dominated by *S.* sp. #10, *L. ocellata*, and *L. comensis*, followed by an increase in higher nutrient taxa (e.g. *A. subarctica* and *A. islandica*) in the upper right quadrant. Migration to the left reflected current conditions dominated by small centrics such as *L. comensis* and *L.* cf. *delicatula*.

With the exception of Billings Park, the oldest intervals of each SLRE core fell within the lower, right quadrant (Figure 6c), indicating consistent assemblage baselines of *S. construens*, *S. construens* var. *venter*, and *S. pinnata*. Into the 20th century assemblages migrated to the upper, left quadrant, representing assemblage shifts associated with higher nutrients (e.g. *C. meneghiniana* and *S. parvus*). The most recent sample scores in Billings Park, Pokegama Bay, and Allouez Bay were especially constrained to the left of the ordination in accordance with higher relative abundances of *C. dubius, S. binderanus*, and *Aulacoseira* spp. In general, fossil assemblages in the SLRE exhibited consistent reorganization, and there was little evidence recent diatom communities have returned to pre-impact assemblages.

Diatom-inferred modeling

Based on model validation, there was a significant relationship between changes in TP and diatom assemblages in all cores (i.e. DI-TP strongly correlated with the primary gradient of variation in assemblages in each core). Further, analog analyses showed good fit between fossil assemblages and model training sets in all cases. Details of these validations are provided by Alexson (2016).

DI-TP results (Figure 7) indicated western Lake Superior had much lower concentrations of TP (3 - 6 µg/L) than the SLRE (15 - 80 µg/L). DI-TP increased during the mid-20th century in open water cores (Lake Superior, Minnesota Point, and North of Clough Island), followed by a decline in western Lake Superior and North of Clough Island cores and stabilization in the Minnesota Point core. Cores taken from SLRE bays (North Bay, Billings Park, Allouez Bay, and Pokegama Bay) generally showed increasing DI-TP since the mid-20th century.

DI-TP in western Lake Superior suggested phosphorus loading in the early 1900s contributed to a maximum of TP of 5.5 µg/L around 1930, and a secondary peak (5 µg/L) around 1970. After 1970, TP decreased and stabilized around pre-settlement concentrations (~3 µg/L), similar to observations in other Lake Superior cores (Shaw Chraïbi et al. 2014).

In the SLRE, the open-water environments (Minnesota Point and North of Clough Island) showed stabilization or decrease of TP. The DI-TP from North of Clough Island showed an increase from 25 µg/L to 65 µg/L, peaking around 1970. Recovery to lower concentrations occurred later and the earlier concentration of ~25 µg/L was reached in the upper intervals. Because North of Clough Island's record did not extend before 1940, it was not possible to compare pre- and post-settlement conditions. The North of Clough Island reconstruction indicated an increase in the late 1900s from 25 µg/L to 35 µg/L TP. After a peak in 1980, DI-TP

stabilized around 30 µg/L. Again, due to uncertainty in accumulation, we inferred higher nutrients in the 1960s and lower nutrients today, but timing of transitions are ambiguous.

Cores from bay environments showed modern conditions of increasing DI-TP. Cores from Allouez Bay and Pokegama Bay both remained at near-constant concentrations of DI-TP (30 and 45 µg/L, respectively) until ~1950, after which TP concentration increased to as high as ~80 µg/L. DI-TP began to increase around 1920 at North Bay and rose from ~50 to ~60 µg/L in modern intervals. In Billings Park DI-TP increased from ~1950 to ~2000 (from ~20 to 38 µg/L), followed by two modern intervals with lower DI-TP (~20 µg/L).

Compared to historical measured TP from a location in the lower estuary (at Blatnik Bridge; Bellinger et al. 2016), DI-TP concentrations were lower (Figure 13); however, the general trend of declining monitored TP in recent decades was similar to DI-TP from North of Clough Island and Lake Superior cores. The monitoring dataset spanning 1953 to 2014 showed a peak of TP in ~1980 (~180 µg/L) and afterward a steady decrease in TP concentration to approximately 40 µg/L (based on the lowess smoothing), which is a fair match with modern DI-TP of ~30 µg/L from Minnesota Point and North of Clough Island cores.

Discussion

These paleolimnological data describe the history of anthropogenic influence on the SLRE and western Lake Superior and reveal where remediation may be occurring. As previously detailed by Reavie and Edlund (2010), paleolimnology in lotic environments can be challenging. We believe we have overcome these limitations through application of multiple fossil indicators and careful selection of core locations.

Early impacts from logging and subsequent modifications of the drainage basin and the SLR were prevalent in the paleorecord. When logging was at its peak (~1850 - 1900), a transition in SLRE diatom communities from benthic genera (*Staurosira* and *Staurosirella*) to centric, planktonic diatoms (e.g. *Aulacoseira*) suggested a physical transformation to a more lacustrine (but still fluvial) system as a result of hydrological manipulation by damming and dredging of the SLR.

By the 1930s, with growing industries and a growing population to support them, the SLRE's ecology changed. Increased sedimentation rates, greater abundance of eutrophic diatom species, and higher DI-TP dominated the paleorecord. This was likely due to the combined effects of untreated wastewater and runoff from a landscape transformed by logging. With the construction of Fond du Lac Dam (upstream of all core locations) in 1924, decreased sedimentation rates were expected due to the retention effect of the new reservoir. However, it is clear other factors (algal production and watershed disruptions leading to increased erosion) contributed to increased sediment loads at some locations. Since 1970, sedimentation decreased in all cores with the exception of Minnesota Point and Pokegama Bay, and nutrient trajectories varied among locations. Cores from SLRE open-water environments suggested a remediation or stabilization of environmental quality, while in SLRE bays phosphorus loading may be continuing. Fossil pigments corroborate this recent trend, with increased concentrations of pigments from total algae and those from cyanobacteria in two bay locations.

Changes in legislation such as the Clean Water Act in 1972 accompanied by restoration efforts are associated with recovery we observed in some cores. The recovery is defined partly by a decrease in nutrient-tolerant diatoms—*Aulacoseira* spp. and *S. conspicueporus* in western Lake Superior, *Aulacoseira* spp. and *C. meneghiniana* in North of Clough Island, and *S. parvus*

in Minnesota Point. This was affirmed by a decrease in DI-TP—a reduction in Lake Superior and North of Clough Island and apparent stabilization in Minnesota Point.

Results from these cores mostly agreed with monitoring data from Bellinger et al. (2016). Though the overall measured TP trend matched DI-TP (especially North of Clough Island), concentrations found by Bellinger et al. were much higher than those inferred by the model. This discrepancy may be due to the natural variability in the SLRE as the nearest coring location (Minnesota Point) is ~3.5 km away. Additionally, DI-TP produces values representing a more integrated dataset (spatially and temporally) rather than the episodic TP measurements reported by Bellinger et al., so DI-TP may integrate nutrient information from unmonitored times.

Fossil data from four cores taken from bay environments suggest continued high nutrients in these parts of the SLRE. Higher populations of all algae groups (notably cyanobacteria), a growth in abundance of nutrient-tolerant diatoms (*C. dubius*, *C. meneghiniana, S. parvus*, and *S. binderanus*), and increased DI-TP all support this conclusion. Recent persistence of high concentrations of nutrients in parts of the SLRE may be due to more localized nutrient sources, potentially from recent development and continued presence of industry, or enhanced internal loading of sedimentary nutrient pools. But, contemporary anthropogenic issues facing other water bodies such as those reported in Lake Erie—internal phosphorus loading and higher runoff from high-intensity rain events associated with climate change (Kane et al. 2009, Matisoff et al. 2016)—may also be responsible. Such possible drivers need additional study in the SLRE.

There is little doubt efforts to remediate the SLRE reduced the flux and concentration of nutrients in the SLRE (Bellinger et al. 2016). To meet BUI removal targets, the portion of Lake Superior in the AOC must have TP concentrations below 10 μg/L, the upper limit for oligotrophic designation, and the estuary must be below 30 μg/L, the upper limit for mesotrophic designation (MPCA and WDNR 2013). According to DI-TP, western Lake Superior easily falls within passing criteria, and always has. Minnesota Point, North of Clough Island, and Billings Park (at least according to the most recent interval) have TP concentrations around or below 30 μg/L, whereas North Bay, Pokegama Bay, and Allouez Bay exceed desired concentrations. Pre-impact concentrations of DI-TP at North Bay and Pokegama Bay (40 - 45 μg/L) surpass delisting criteria, so a criterion of 30 μg/L may be unrealistic for these areas as they appear to be naturally productive. Delisting goals may need reconsideration in order to accommodate the natural state of and variability within the estuary. The higher DI-TP at these locations may be due to legacy pollution, but it is also possible these recent increases are instead a result of more modern stressors like climate change and internal phosphorus loading. For instance, enhanced stratification due to warmer atmospheric temperatures has aggravated hypoxia and increased sediment phosphorus releases in lakes (North et al. 2014). Such phenomena were understood at the time of the AOC listing and RAP development. Managing agencies may choose to remove the nutrient BUI with the intention of addressing these modern issues driving water quality in the estuary.

Presently, only four American and three Canadian AOCs have been delisted, leaving 36 remaining. Although there have been paleolimnological studies in AOCs in the past (Reavie et al. 1998; Yang et al. 1993), there have been few studies done intentionally to advise AOC programs. In a similar study to ours, Dixit et al. (1998) examined metals, accumulation rates, and diatom taxa to understand the anthropogenic influence in the Spanish Harbor of Lake Ontario to inform a RAP. They found similar anthropogenic activities facing the SLRE (paper mills, iron smelting, and untreated wastewater) led to increased metal concentrations and nutrients. As

demonstrated here, paleolimnological investigations can be useful in not only developing RAPs, but also gauging the extent of remediation in AOCs to aid in their eventual delisting.

Acknowledgements

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Figure 1 (A) Map indicating the location of the St. Louis River estuary (SLRE) area of concern (AOC) relative to all Great Lakes AOCs (orange) and their associated watersheds (red hash) (Environment Canada, EPA, IJC 2013) (B) Map of the St. Louis River drainage basin and the boundary (red) of the AOC (C) Map of coring locations in the SLRE. WTP = locations of water treatment plants

Figure 2 Results from of inorganic and organic content analyses of seven sediment cores from the SLRE and Lake Superior. *The North of Clough core demonstrated a poor ^{210}Pb record, so we provide a very rough estimate of dates and accumulation rates based on ^{137}Cs data that indicated the ~1963 interval. Note the x-axis (analyte) scales are different for each core

Figure 3 Recent concentrations of various algal pigments determined by HPLC in four SLRE cores

Figure 4 Diatom accumulation rates, % pennates, % centrics, ratio of chrysophyte stomatocysts to diatoms, and chrysophyte stomatocyst accumulation rates of seven cores in the SLRE and Lake Superior. Chrysophyte stomatocysts were not in great enough abundance in Lake Superior to be plotted. Note that x-axis scales vary among cores to better illustrate temporal trends

Figure 5 Relative abundances of the most common taxa in the core taken from (a) North Bay, (b) Pokegama Bay, (c) North of Clough Island, (d) Billings Park, (e) Minnesota Point, (f) Allouez Bay, and (g) Western Lake Superior. The labeled zones represent changes in assemblages determined by cluster analysis

Figure 6 NMDS analysis of diatom species assemblages (>5 % relative abundance) in (a) all seven cores from the SLRE and Lake Superior (stress of 0.1258), (b) Lake Superior (stress of 0.0889), and (c) the SLRE (stress of 0.1663)

Figure 7 Diatom-inferred total phosphorus from all cores. The purple line represents a lowess model of total phosphorus measurements (black dots) from the Blatnik Bridge from 1958 to 2012 as reported in Bellinger et al. (2016)

Figures

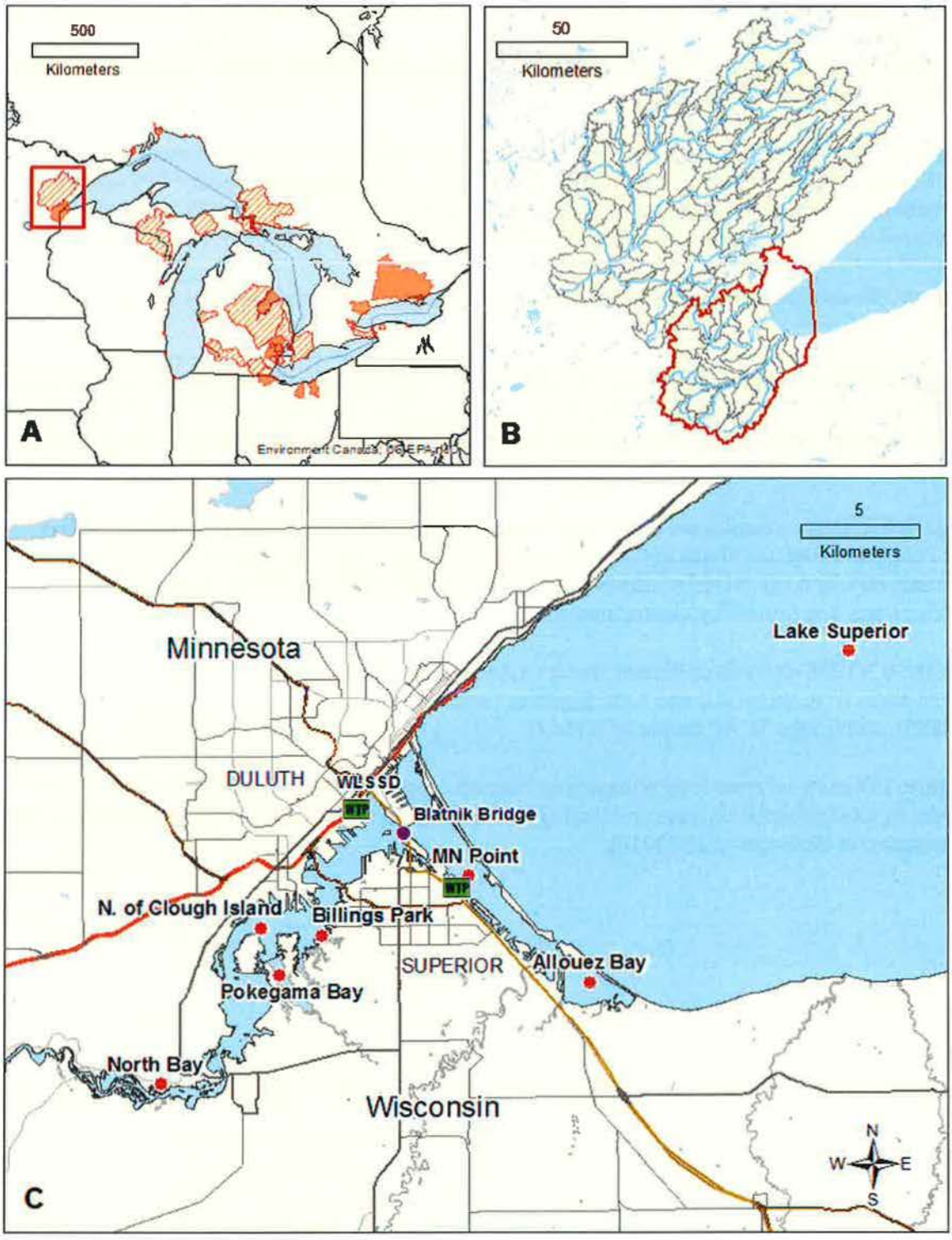

Figure 1

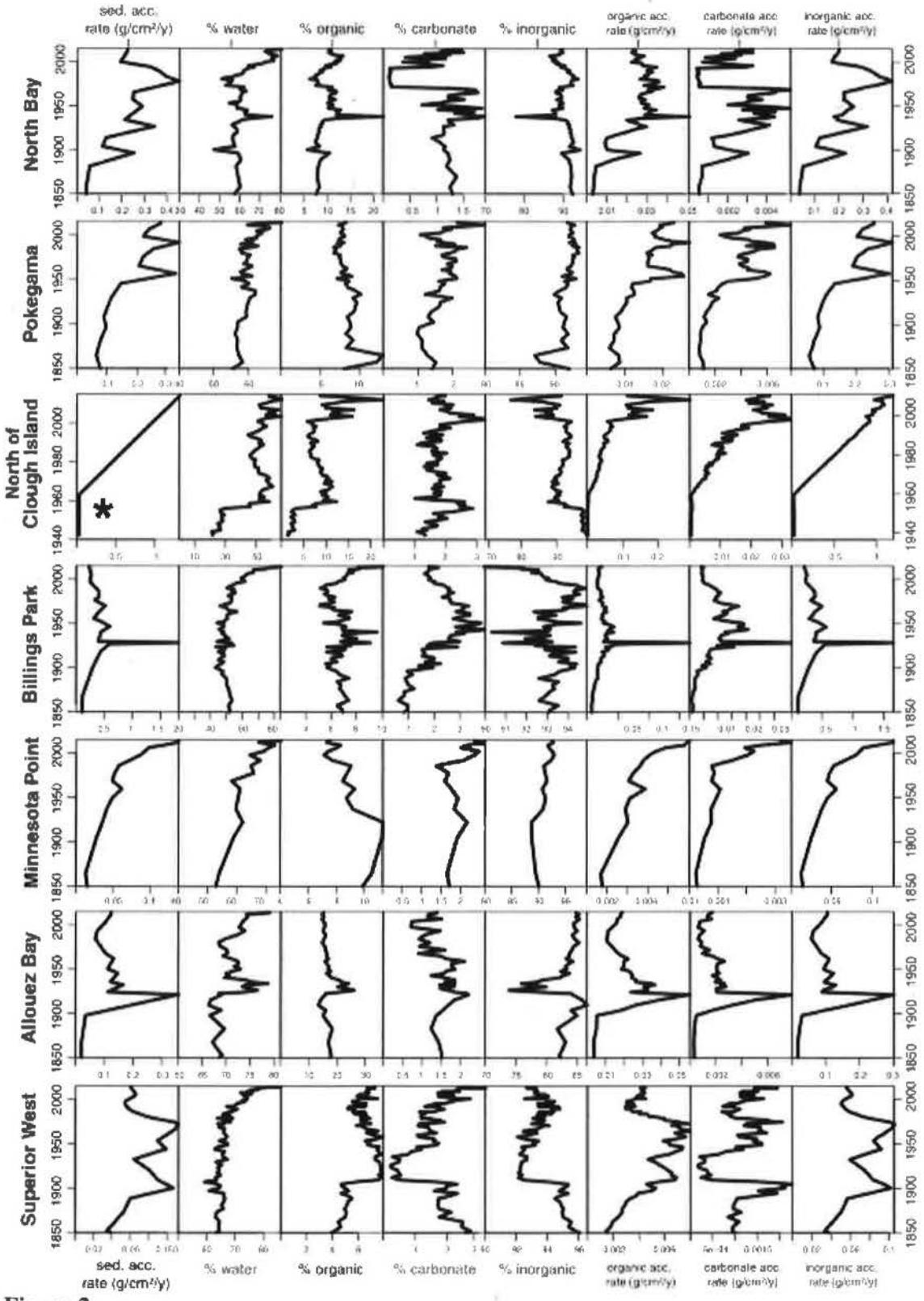
sed. acc. rate (g/cm²/y)
% water
% organic
% carbonate
% inorganic
organic acc. rate (g/cm²/y)
carbonate acc. rate (g/cm²/y)
inorganic acc. rate (g/cm²/y)
North Bay
Pokegama
North of Clough Island
Billings Park
Minnesota Point
Allouez Bay
Superior West
*

Figure 2

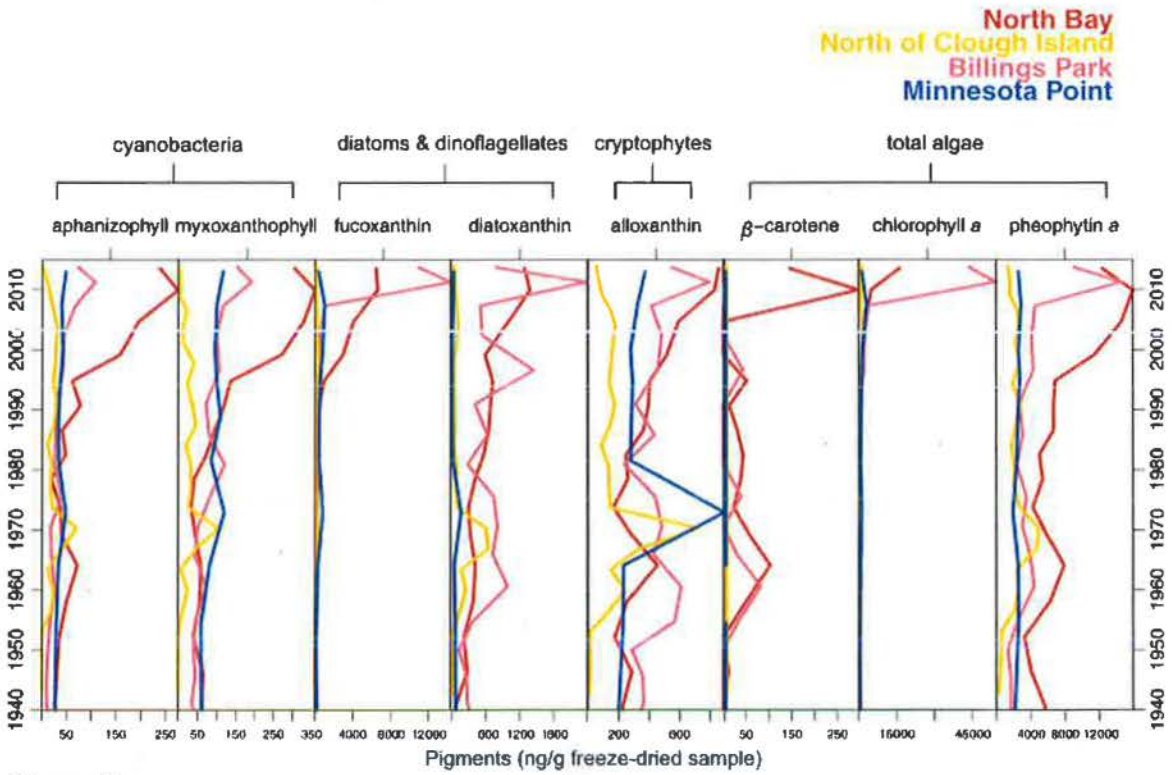
North Bay
North of Clough Island
Billings Park
Minnesota Point
cyanobacteria
diatoms & dinoflagellates
cryptophytes
total algae
aphanizophyll
myxoxanthophyll
fucoxanthin
diatoxanthin
alloxanthin
β–carotene
chlorophyll a
pheophytin a
1940
1950
1960
1970
1980
1990
2000
2010
Pigments (ng/g freeze-dried sample)

Figure 3

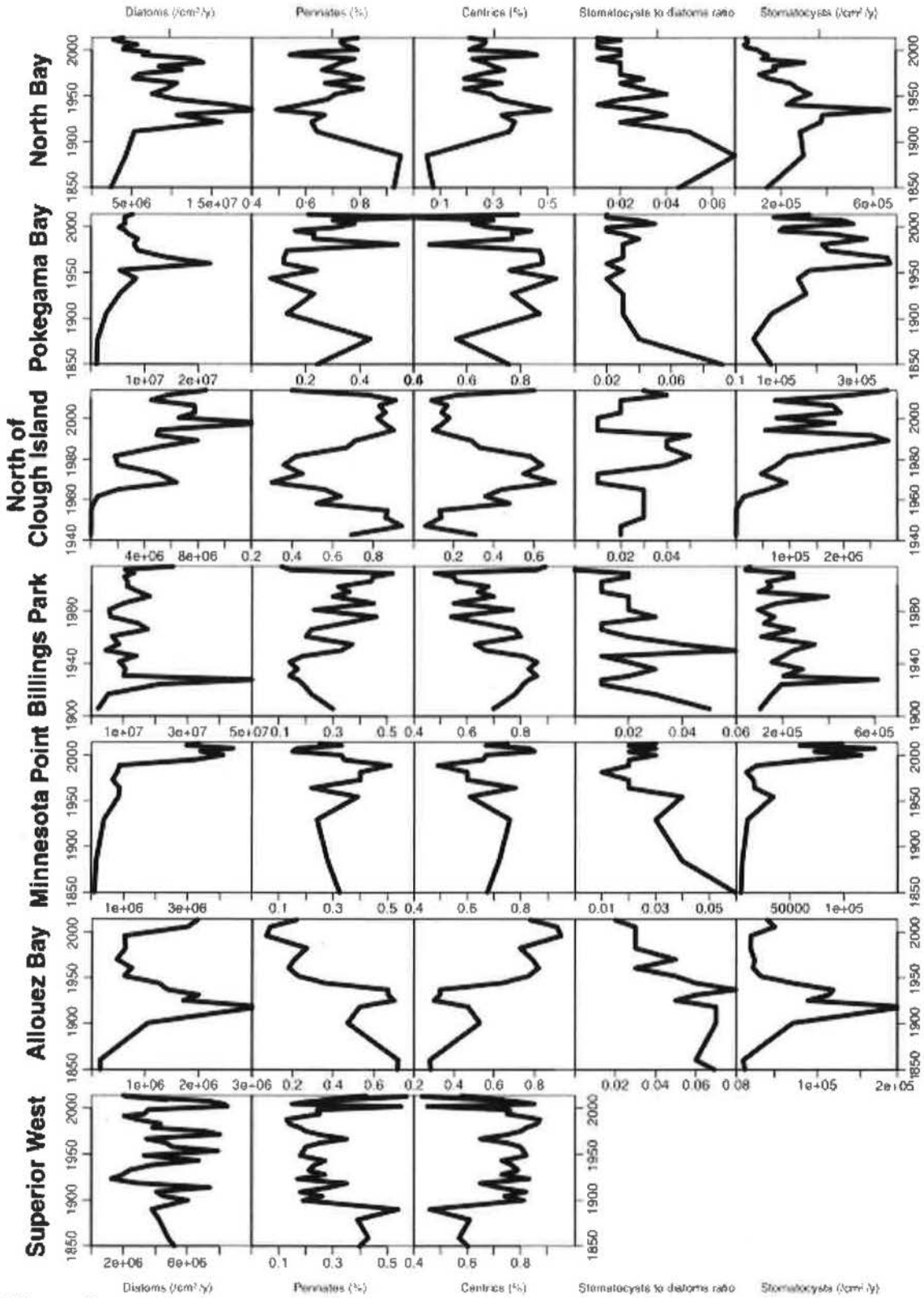
Diatoms (/cm²/y)
Pennates (%)
Centrics (%)
Stomatocysts to diatoms ratio
Stomatocysts (/cm²/y)
North Bay
Pokegama Bay
North of Clough Island
Billings Park
Minnesota Point
Allouez Bay
Superior West

Figure 4

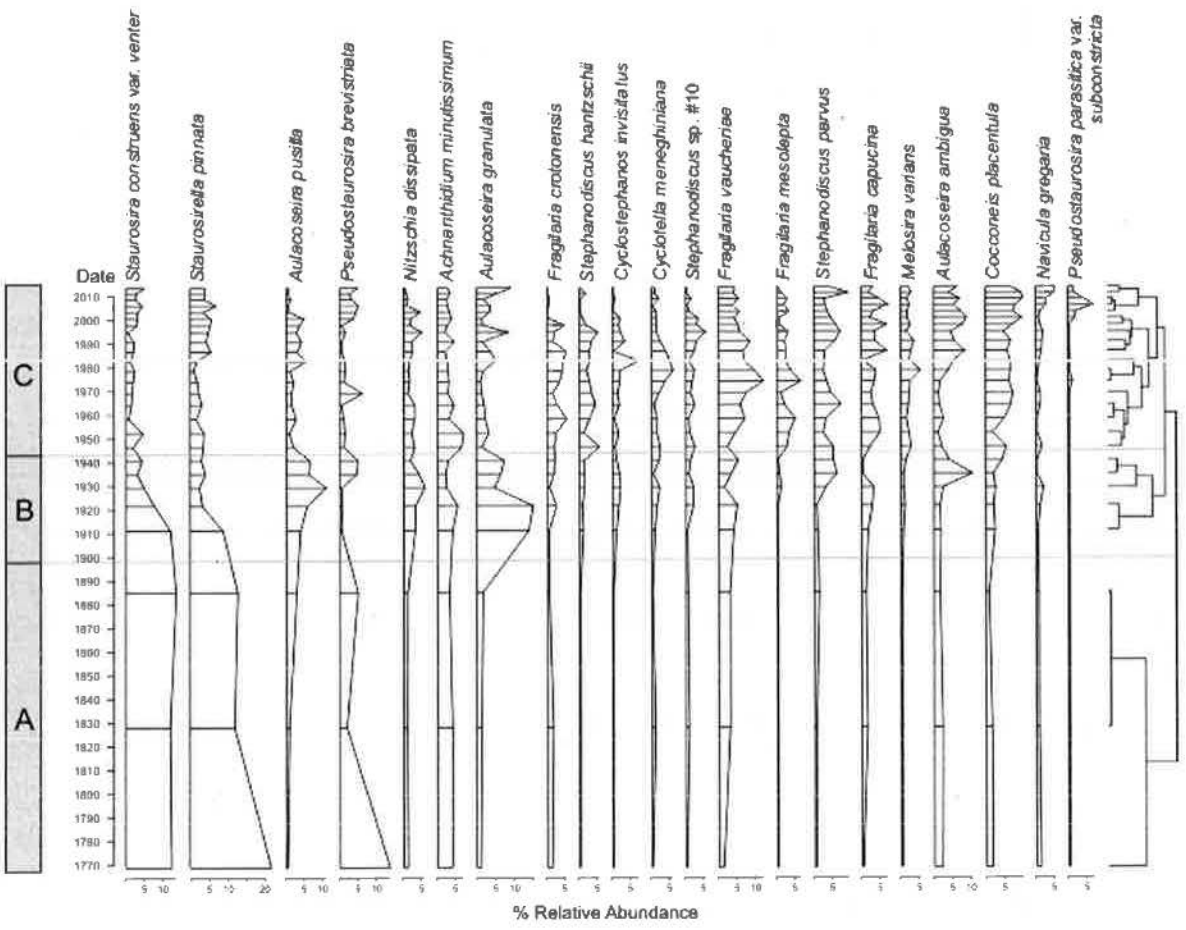
Date
C
B
A
Staurosira construens var. venter
Staurosirella pinnata
Aulacoseira pusilla
Pseudostaurosira brevistriata
Nitzschia dissipata
Achnanthidium minutissimum
Aulacoseira granulata
Fragilaria crotonensis
Stephanodiscus hantzschii
Cyclostephanos invisitatus
Cyclotella meneghiniana
Stephanodiscus sp. #10
Fragilaria vaucheriae
Fragilaria mesolepta
Stephanodiscus parvus
Fragilaria capucina
Melosira varians
Aulacoseira ambigua
Cocconeis placentula
Navicula gregaria
Pseudostaurosira parasitica var. subconstricta
% Relative Abundance

Figure 5a

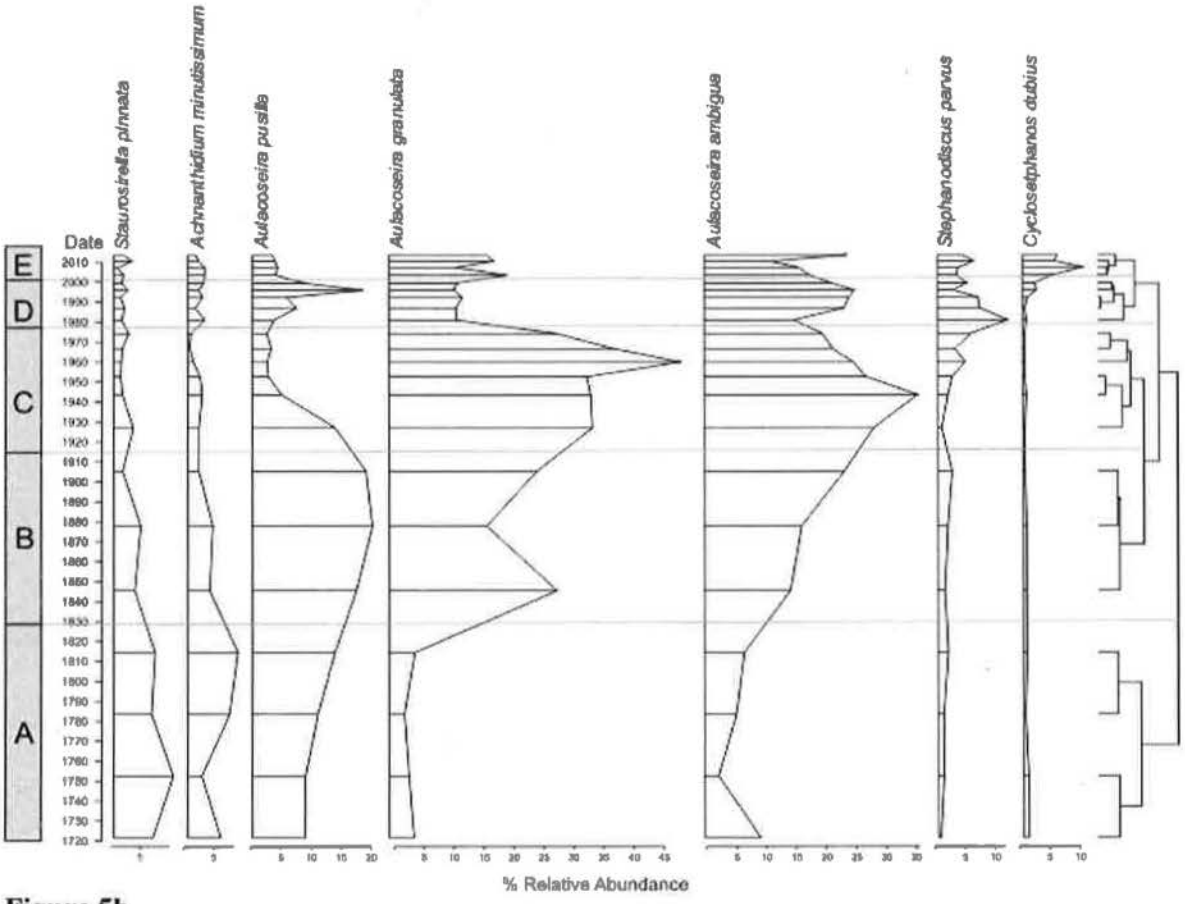
Date
Staurosirella pinnata
Achnanthidium minutissimum
Aulacoseira pusilla
Aulacoseira granulata
Aulacoseira ambigua
Stephanodiscus parvus
Cyclostephanos dubius
E
D
C
B
A
% Relative Abundance

Figure 5b

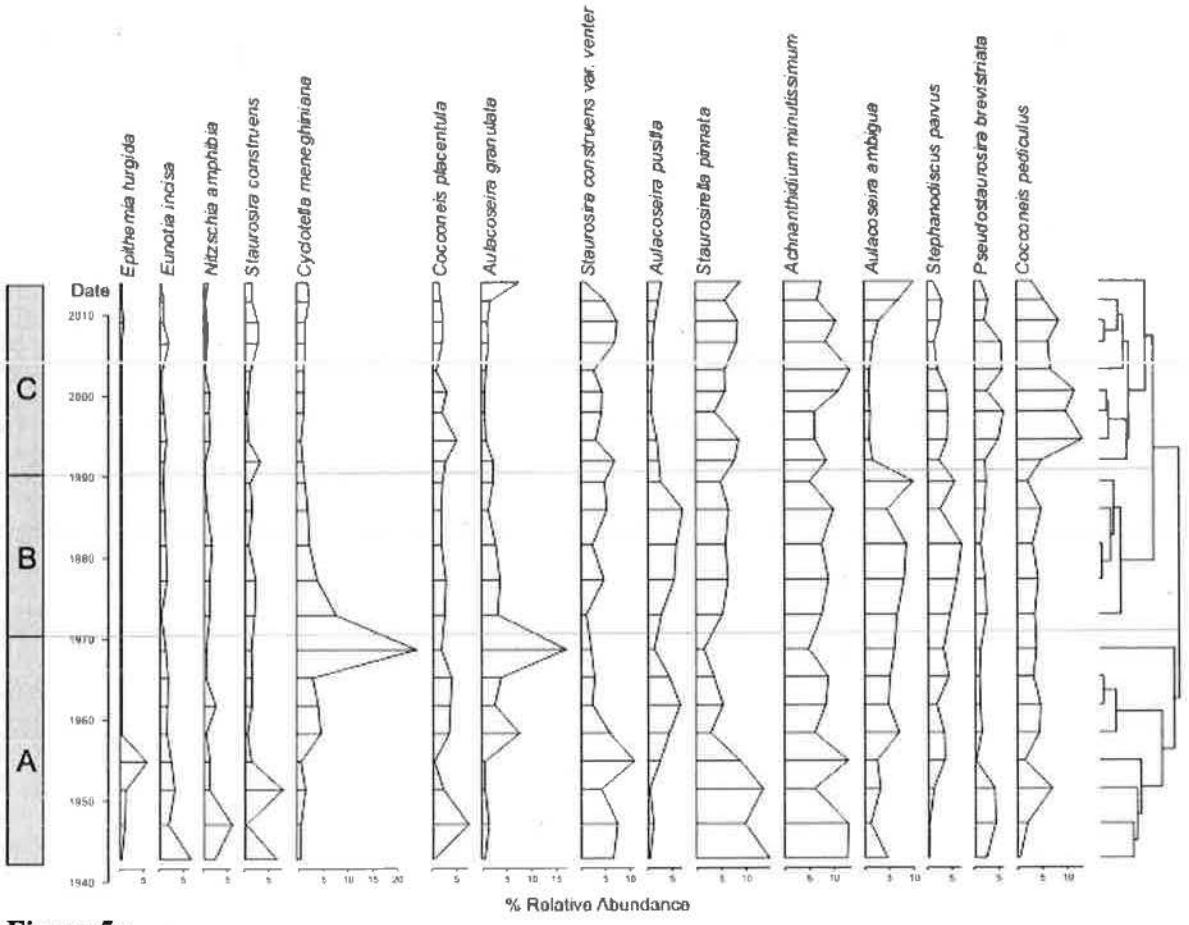
Epithemia turgida
Eunotia incisa
Nitzschia amphibia
Staurosira construens
Cyclotella meneghiniana
Cocconeis placentula
Aulacoseira granulata
Staurosira construens var. venter
Aulacoseira pusilla
Staurosirella pinnata
Achnanthidium minutissimum
Aulacoseira ambigua
Stephanodiscus parvus
Pseudostaurosira brevistriata
Cocconeis pediculus
Date
2010
2000
1990
1980
1970
1960
1950
1940
A
B
C
% Relative Abundance

Figure 5c

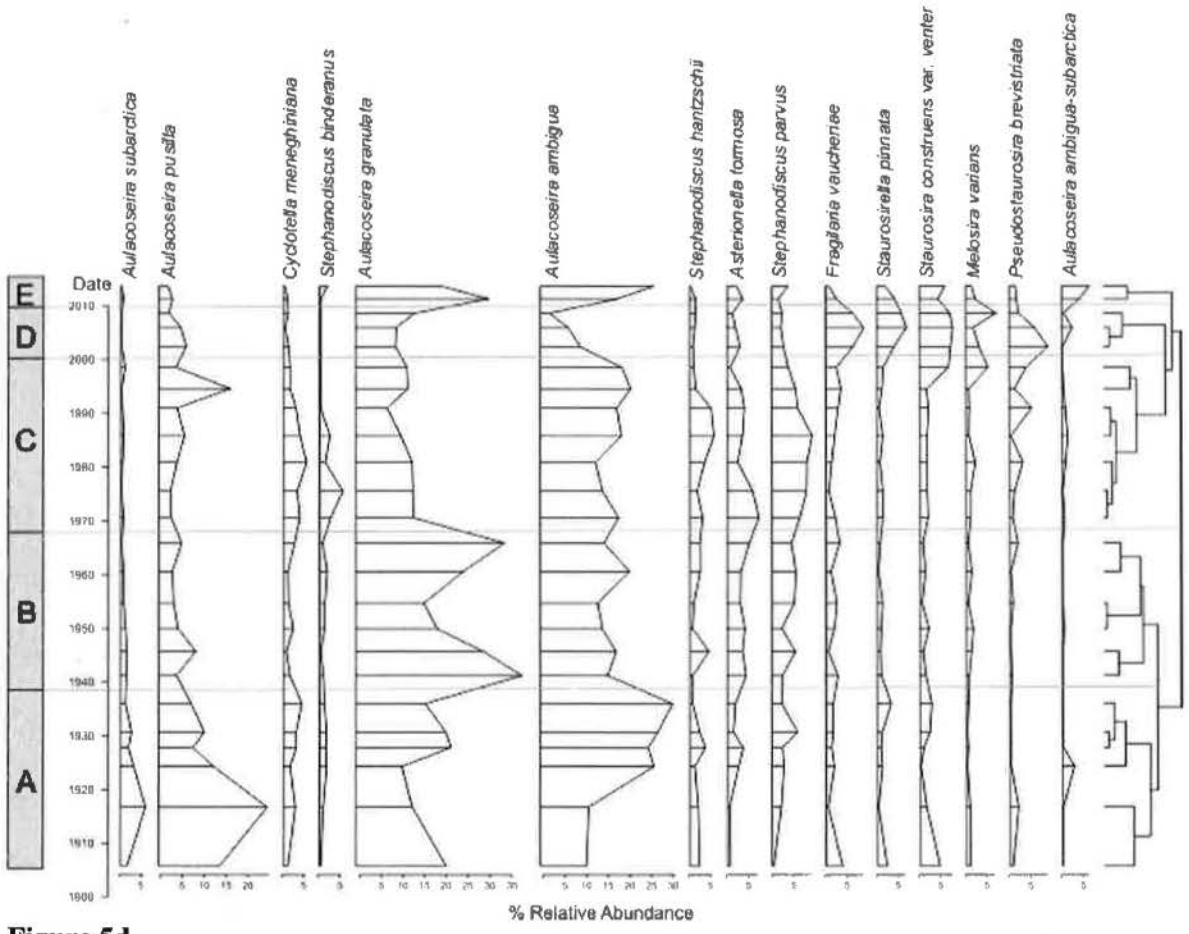
E
D
C
B
A
Date
2010
2000
1990
1980
1970
1960
1950
1940
1930
1920
1910
1900
Aulacoseira subarctica
Aulacoseira pusilla
Cyclotella meneghiniana
Stephanodiscus binderanius
Aulacoseira granulata
Aulacoseira ambigua
Stephanodiscus hantzschii
Asterionella formosa
Stephanodiscus parvus
Fragilaria vaucheriae
Staurosirella pinnata
Staurosira construens var. venter
Melosira varians
Pseudostaurosira brevistriata
Aulacoseira ambigua-subarctica
% Relative Abundance

Figure 5d

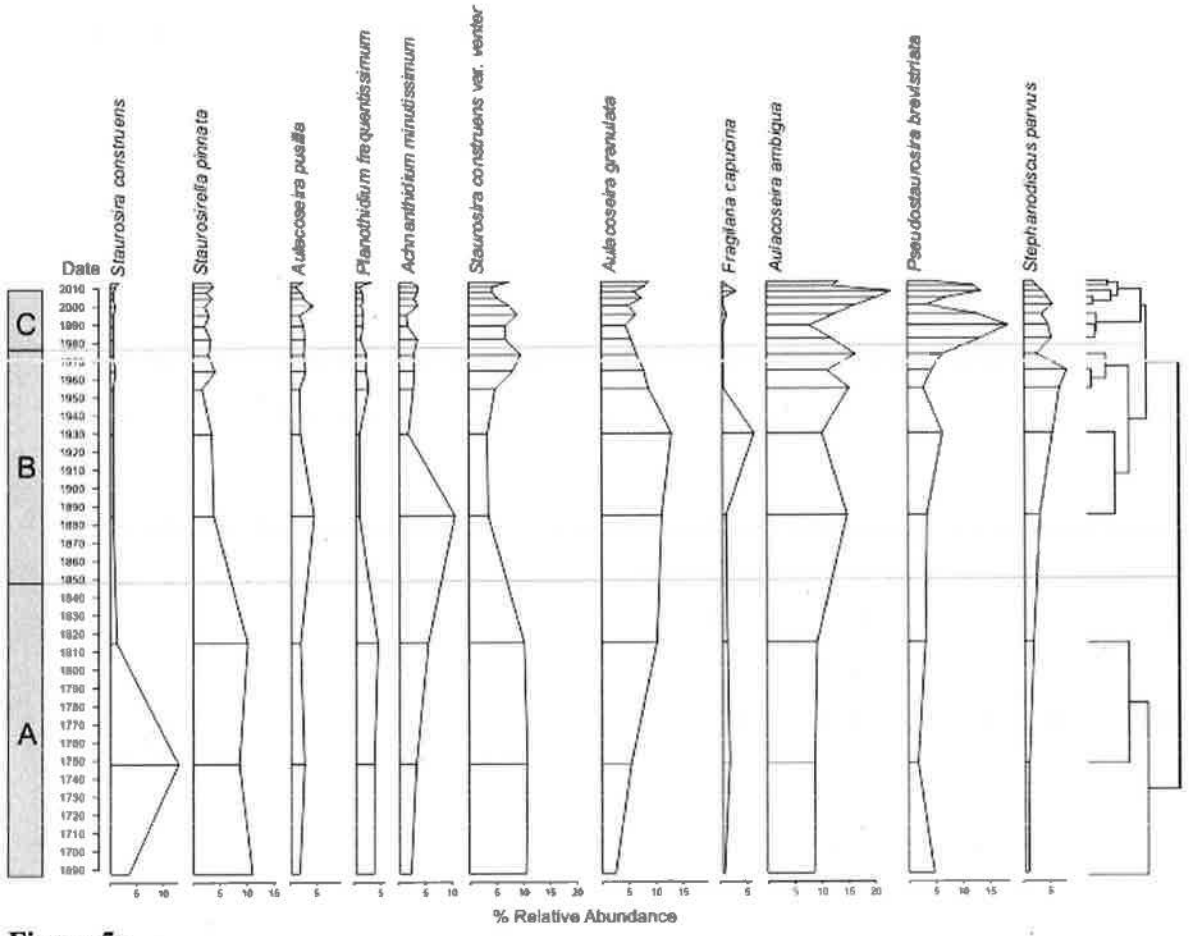
Date
Staurosira construens
Staurosirella pinnata
Aulacoseira pusilla
Planothidium frequentissimum
Achnanthidium minutissimum
Staurosira construens var. venter
Aulacoseira granulata
Fragilaria capucina
Aulacoseira ambigua
Pseudostaurosira brevistriata
Stephanodiscus parvus
C
B
A
% Relative Abundance

Figure 5e

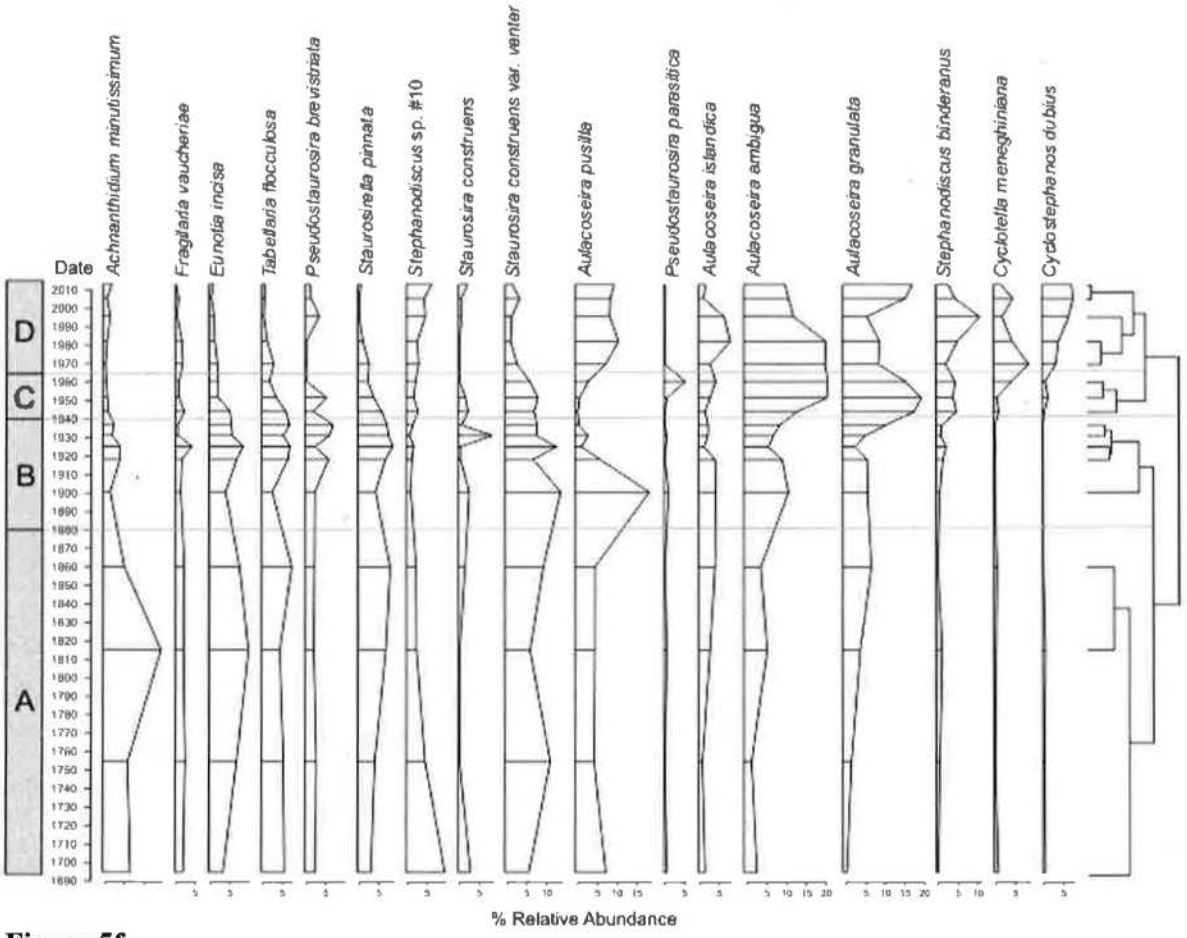
Date
Achnanthidium minutissimum
Fragilaria vaucheriae
Eunotia incisa
Tabellaria flocculosa
Pseudostaurosira brevistriata
Staurosirella pinnata
Stephanodiscus sp. #10
Staurosira construens
Staurosira construens var. venter
Aulacoseira pusilla
Pseudostaurosira parasitica
Aulacoseira islandica
Aulacoseira ambigua
Aulacoseira granulata
Stephanodiscus binderanus
Cyclotella meneghiniana
Cyclostephanos dubius
D
C
B
A
% Relative Abundance

Figure 5f

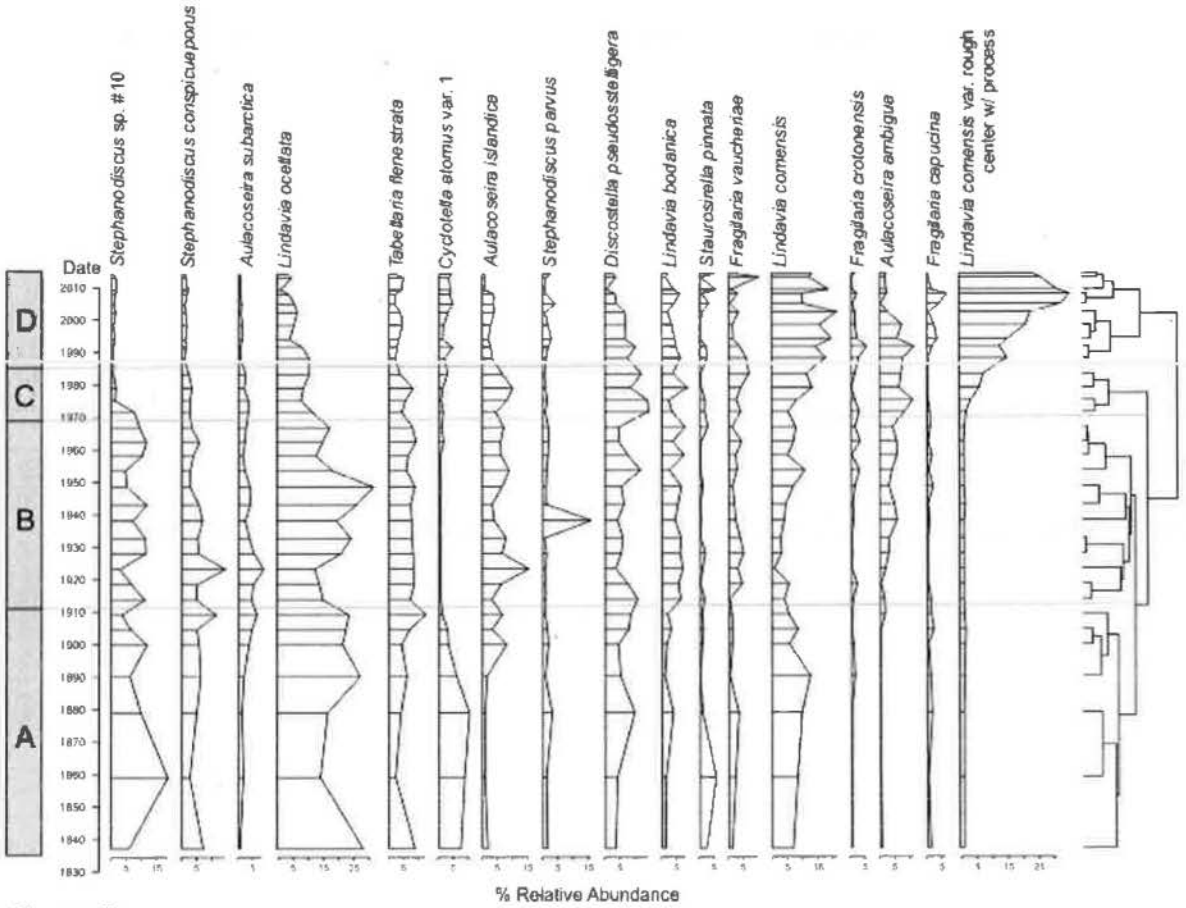
Date
D
C
B
A
Stephanodiscus sp. #10
Stephanodiscus conspicueporus
Aulacoseira subarctica
Lindavia ocellata
Tabellaria flenestrata
Cyclotella atomus var. 1
Aulacoseira islandica
Stephanodiscus parvus
Discostella pseudostelligera
Lindavia bodanica
Staurosirella pinnata
Fragilaria vaucheriae
Lindavia comensis
Fragilaria crotonensis
Aulacoseira ambigua
Fragilaria capucina
Lindavia comensis var. rough center w/ process
% Relative Abundance

Figure 5g

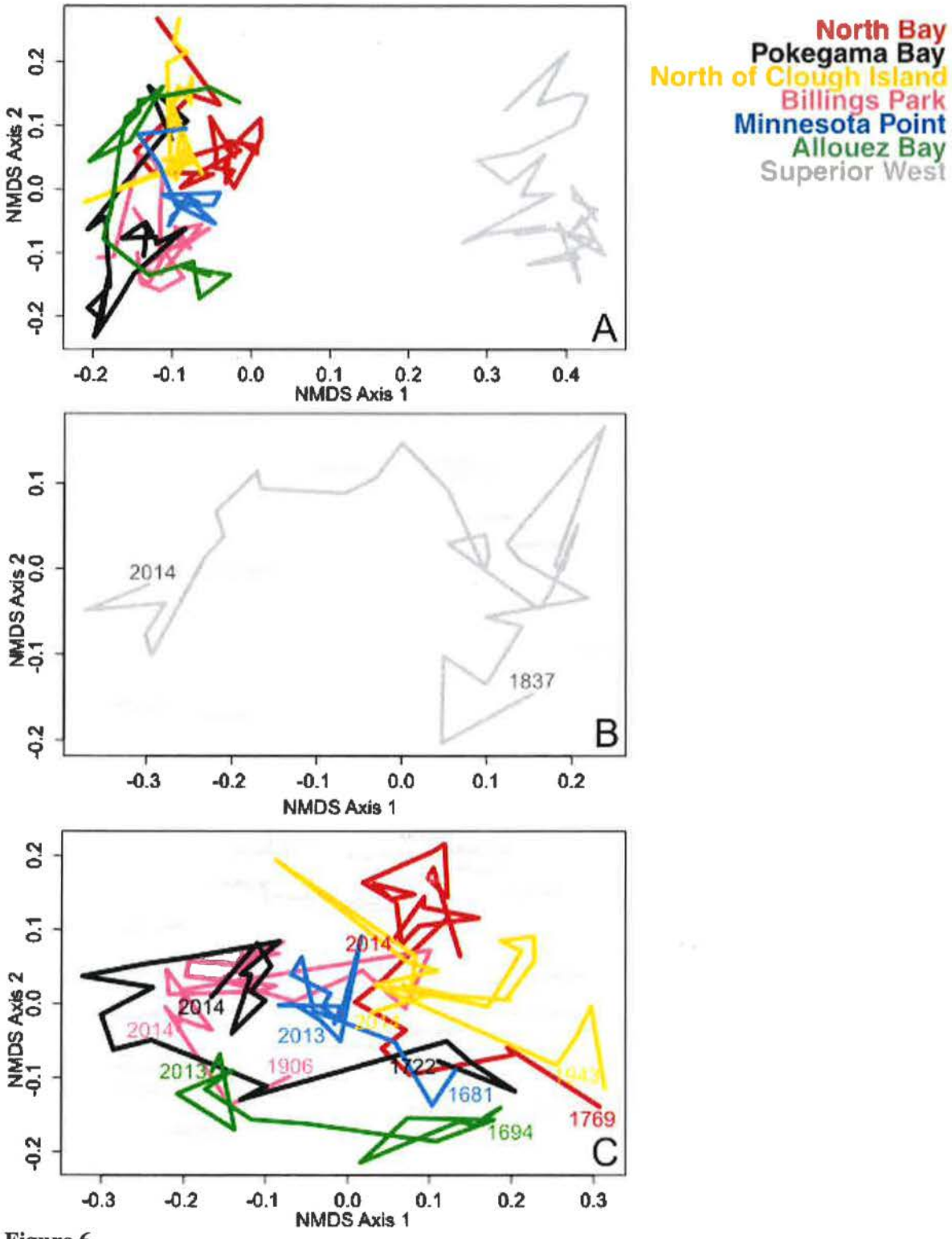
North Bay
Pokegama Bay
North of Clough Island
Billings Park
Minnesota Point
Allouez Bay
Superior West
NMDS Axis 2
NMDS Axis 1
A
2014
1837
B
2014
2014
2014
2013
2013
1906
1722
1681
1769
1694
C

Figure 6

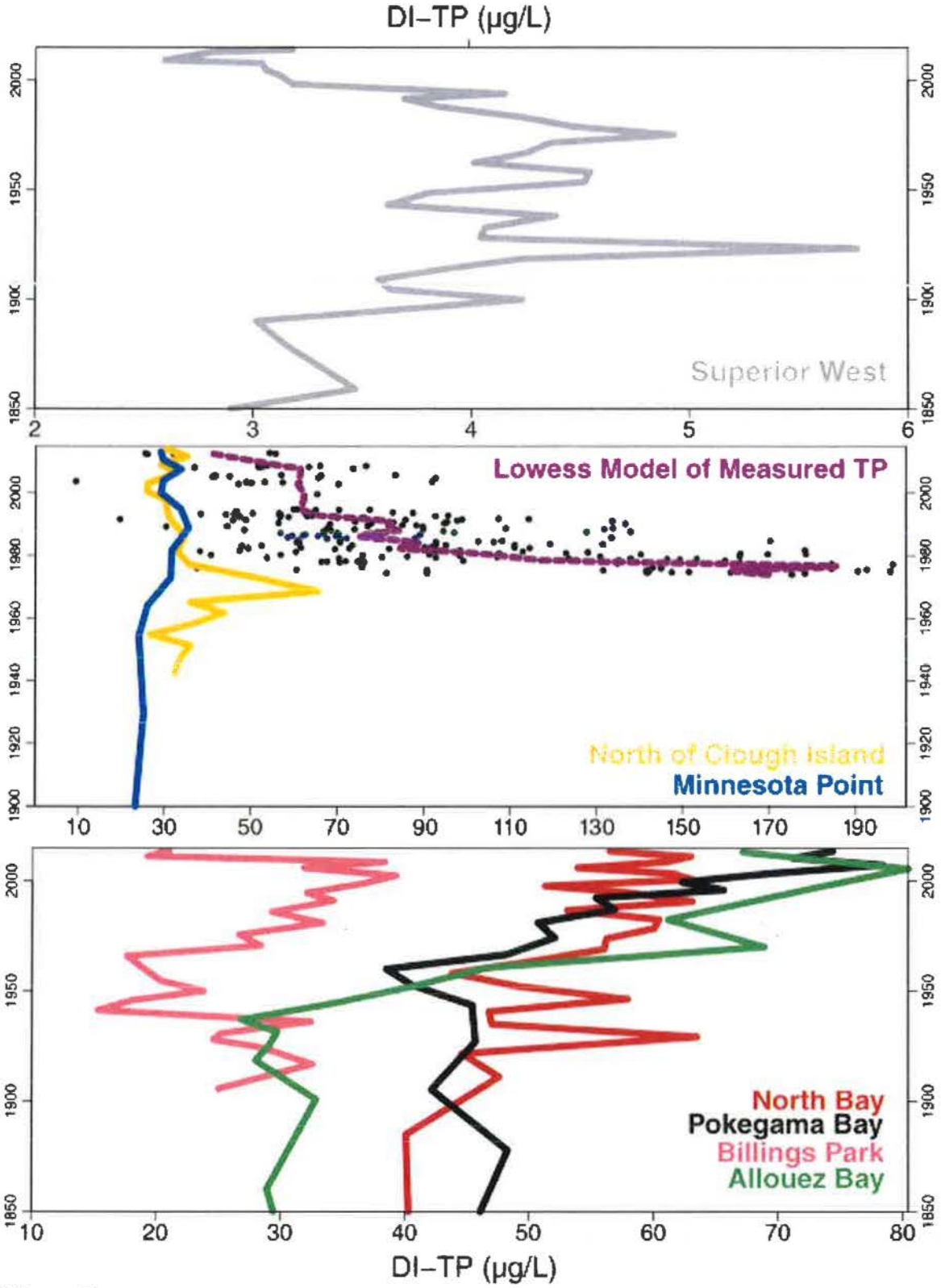
DI–TP (µg/L)
Superior West
Lowess Model of Measured TP
North of Clough Island
Minnesota Point
North Bay
Pokegama Bay
Billings Park
Allouez Bay
DI–TP (µg/L)

Figure 7

ATTACHMENT B

Pollen and phytolith paleoecology in the St. Louis River estuary, Minnesota, USA, with special consideration of *Zizania palustris* L.

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Abstract

Development of the catchment of the St. Louis River Estuary (SLRE) since Euro-American settlement has resulted in quantifiable impacts to the aquatic ecosystem. *Zizania palustris* L. (northern wild rice) is an important food commodity for the state of Minnesota and the SLRE contains several wild rice stands. Known threats to SLRE wild rice stands include excessive sediment and nutrient loading. This paleoecological study combined pollen and phytolith analyses from five sediment cores from the SLRE to investigate three objectives: (1) how to enhance identification procedures by distinguishing *Z. palustris* pollen from other aquatic and upland grasses present in the fossil record; (2) the ability to reconstruct the 250-year history of upland and aquatic plants with a particular focus on wild rice; (3) the utility of these indicators for paleolimnology studies of plant communities in a lotic system. Pollen and phytolith analyses

of sediment samples yielded paleoenvironmental data that confirmed that wild rice microfossils can be conclusively identified from sedimentary records, and *Zizania palustris* has been continuously present in the SLRE since the mid 1700s. Although substantial reorganization of plant communities occurred, there is little evidence to suggest human development, beyond shifts in water level, significantly reduced wild rice stands. However, it is apparent our sampling strategy was not ideal for assessment of wild rice history and we recommend that retrospective studies for wild rice be based on sediment collected within or closer to littoral environments that can support wild rice stands.

Keywords:

Zizania; St. Louis River Estuary; wild rice; pollen; phytoliths; paleolimnology

1. Introduction

Zizania palustris L. (northern wild rice) was an important food source for pre-contact and historic Indigenous peoples living in the Great Lakes region (Boyd et al., 2013). Today, wild rice is a major food commodity for the state of Minnesota. The St. Louis River Estuary (SLRE) contains several wild rice stands that are an important traditional food for the local Fond du Lac band of Lake Superior Chippewa. Ongoing threats to SLRE wild rice stands include excessive sediment and nutrient loading and stand degradation from powerboat propeller backwash and wakes. Anecdotal observations and modeling of physical limnological characteristics for wild rice growth (Minnesota Department of Natural Resources, 2014) suggest historic losses of wild rice stands have occurred since Euro-American settlement.

Z. palustris, a member of the Poaceae (grass) family, grows in the littoral zones of lakes and rivers in the southern regions of the North American boreal forests. *Z. palustris* is

47 monoecious, with the female flowers situated above the male flowers on the inflorescence (Fig.
48 2). Anthers open to release pollen during warm, dry weather of late July and early August
49 (Faegri and Van Der Pijl, 1971). *Zizania* pollen is wind distributed. Controlled experiments with
50 *Zizania texana* Hitchc. indicated that greatest pollen distribution occurs within 1.5 meters of the
51 plant (Oxley et al., 2008). Successful pollination is more likely to occur where *Zizania* plants
52 grow close together and competing vegetation does not block wind transport. Rain washes the
53 pollen out of the air (Faegri and Pijl, 1971), further reducing the area of pollination.
54 Taxonomic differences in pollen of closely related taxa are often subtle, as is the case
55 within the grass family Poaceae. Poaceae pollen grains are spherical, ovoid, or ellipsoid and
56 range in size from 20 microns (μm) (emergent littoral species *Phragmites australis* (Cav.) Trin.
57 *ex* Steud. and upland annual bluegrass *Poa annua* L.) to 100 μm for *Zea mays* L. (corn). The
58 pollen exine is thin with psilate (*Zizania*), scabrate (*Calamagrostis*), or rugulate (*Elymus,*
59 *Koeleria*) sculpturing. All Poaceae pollen has a single pore surrounded by a raised annulus. The
60 pore is circular and covered by an operculum, although the operculum is often absent in fossil
61 pollen.
62 Differentiation of *Zizania* from other grass pollen in paleoecological studies is typically
63 based on pollen grain size and abundance in the sediment (e.g. Geisler, 1945; McAndrews, 1969;
64 Huber, 2000; Koster et al., 2007). Lee et al. (2004) found that fossil *Zizania* pollen did not
65 always fit into the same size range as modern *Zizania* pollen, and small size differences existed
66 between *Zizania* and frequent companion grasses *Glyceria borealis* (Nash) Batch. (northern
67 manna grass), *Glyceria canadensis* (Michx.) Trin. (rattlesnake manna grass), and *Phalaris*
68 *arundinacea* L. (reed canary grass).
69 Hansen and Cushing (1973), Lindbladh et al. (2002), and Barton et al. (2011) speciated

70 southwestern *Pinus* (pine), northeastern *Picea* (spruce), and New England diploxylon *Pinus*
71 respectively using statistical analysis of numerical classification of morphologic traits coupled
72 with measurements of cell features. Because slight genetic variation in widely scattered
73 populations can produce subtle differences in pollen morphology, pollen speciation based on
74 taxonomic features is often restricted to the geographic region of interest.

75 *Zizania* pollen is difficult to distinguish from the pollen of other aquatic and emergent
76 grasses (McAndrews, 1969; Kohler and Lange, 1979; Huber, 2000; Lee et al., 2004; Boyd et al.,
77 2013) found in the SLRE. *Calamagrostis canadensis* (Michx.) Nutt (Canada blue joint) is a
78 common perennial grass. *C. canadensis* and Cyperaceae (sedges) form dense vegetative stands
79 along the SLRE estuary shoreline. While the invasive *Phragmites australis* is not yet prominent
80 throughout the estuary, United States Department of Agriculture (2016) documented its native
81 cousin *Phragmites americanus* (Saltonstall, P.M. Peterson, & Soreng) along the Wisconsin shore
82 of Lake Superior and into the Superior, Wisconsin, estuary drainage. *Glyceria canadensis* grows
83 in shallow water along shorelines. United States Department of Agriculture (2016) reported
84 *Phalaris arundinacea* and *Glyceria borealis* within the estuary watershed. These emergent and
85 aquatic grasses produce large stands with ecologies similar to *Zizania*. Hence, any
86 comprehensive paleoecological study of *Zizania palustris* requires identification of unique
87 *Zizania* pollen characteristics.

88 Many terrestrial vascular plants absorb soluble silica from the groundwater. The silica is
89 deposited within the cellular structure as uniquely shaped silica particles known as phytoliths.
90 When the plant dies and decays, the inorganic phytoliths remain in the sediment as microscopic
91 silica particles of varying sizes and shapes. A single plant can produce several different phytolith
92 morphotypes diagnostic of the plant family, genus, or species. The Poaceae family is a prolific

93 producer of distinct phytoliths. Certain phytolith morphotypes are more commonly found in or
94 are restricted to specific portions of a plant, e.g. inflorescence, leaves, stalks, or rhizomes. The
95 most diagnostic phytolith form produced by *Z. palustris* is the Inflorescence Type 1 rondel (see
96 Fig. 3A-C; Yost and Blinnikov, 2011) produced in the spikelet, lemma, and palea (inflorescence
97 bracts enclosing the seed). Although this portion of the plant comprises only a small amount of
98 the dry weight and phytolith extract weight overall, *Z. palustris* inflorescence contributes the
99 largest number of short cell phytoliths from the dense arrangement of indents on the spikelet
100 surface. For *Zizania*, inflorescence rondels account for 37% of the total phytolith morphotype
101 assemblage produced by the entire plant (Yost and Blinnikov, 2011). Rondels are also produced
102 in the sheath (5.2% occurrence) and culm material (6.9% occurrence) (Yost and Blinnikov,
103 2011).

104 Because mature *Z. palustris* seeds drop and quickly sink below the water surface, we
105 expected that a larger proportion of inflorescence-type phytoliths would comprise the phytolith
106 assemblage recovered in sediments. We also anticipated that stable and sizable *Zizania*
107 populations would deposit large numbers of inflorescence rondels into estuary sediment. In fact,
108 wave and current winnowing of this material complicates interpretation of samples containing
109 both floating and non-floating leaf-type phytoliths. Decaying plant material may be preferentially
110 aggregated in shallow bays or pushed up onto distant shorelines by ice-out events coupled with
111 strong winds and currents. In a riverine system, post-deposition transportation of material is even
112 greater. Investigation of two independent proxies for *Z. palustris* populations at multiple sites -
113 pollen and phytolith deposition - minimized the confounding problem of sediment redistribution.

114 We explored three main hypotheses in this study: (1) It is possible to distinguish *Z.*
115 *palustris* pollen from other aquatic and upland grasses present in the fossil record by coupling

116 statistical techniques with morphologic characters; (2) Reconstruction of the ~250-year history
117 of upland and aquatic plants as recorded by pollen and phytoliths in sediment cores reflects pre-
118 and post-impact conditions and more recent remediation of plant communities, with a particular
119 focus on *Z. palustris*; (3) Pollen and phytolith analyses of sediment samples from five cores from
120 the SLRE yields paleoenvironmental data useful in reconstructing past vegetation within the
121 watershed as well as the aquatic area of the core site, thereby supporting future work using these
122 indicators.

123 **2. Site Description**

124 The St. Louis River (SLR) flows 288 km through northeastern Minnesota, draining an
125 area of 9412 square kilometers and delivering 73.3 square meters per second of water to Lake
126 Superior (Fig. 1). The SLR empties into Lake Superior's westernmost point. The downstream
127 portion of the river before it joins Lake Superior is the SLRE. The estuary is bordered by two
128 major cities, Duluth, MN, and Superior, WI, with a combined population of approximately
129 113,000 people (Minnesota Pollution Control Agency and Wisconsin Department of Natural
130 Resources, 1992).

131 Proximity to a body of water that provides access to eastern United States seaports,
132 expansive tracts of valuable timber stands, and iron-rich rock provided ample incentive for
133 industry and settlement of the region (Baeten et al., 2016). The estuary was first dredged in 1867,
134 and the Duluth Shipping Canal was completed by 1871 (Minnesota Pollution Control Agency
135 and Wisconsin Department of Natural Resources, 1992). Extensive logging in the early 1900s
136 dramatically impacted the northern Great Lakes states, including the portion of Minnesota
137 drained by the SLR. During this time virtually all old growth forested areas were cleared. The
138 change in land cover of the once heavily-forested drainage basin led to increased runoff and soil

139 erosion. Lumber from the region supplied several saw mills, pulp mills, and paper mills on the
140 SLRE. These mills were a major source of pollution, discharging both chemicals used in
141 processing and wood waste into the estuary (Minnesota Pollution Control Agency and Wisconsin
142 Department of Natural Resources, 1992). By 1928, the Minnesota State Board of Health noticed
143 degradation of the SLR and declared the river as "pollutional" (Minnesota State Board of Health,
144 1929), followed by confirmed untreated sewage and other sources of pollution-related hypoxia,
145 sludge deposits, and a funny taste in fish (Minnesota State Board of Health, 1948). Since
146 increased awareness of these problems, initiation of the Clean Water Act (Great Lakes Water
147 Quality Agreement, 1972), and sewage treatment starting in 1978, the condition of the SLRE
148 improved significantly, including decreases in total phosphorus (Bellinger et al., 2016), turbidity,
149 total coliform count, lead, and copper and an increase in dissolved oxygen (McCollor, 1990).

150 **3. Materials and Methods**

151 *3.1. Site Selection*

152 We chose core sites to represent a variety of environments, including different hydrologic
153 regimes. Five cores were collected from discrete locations in the lower SLRE (Fig. 1) with two
154 core sites (Pokegama Bay and North Bay) located near-downstream from active *Z. palustris*
155 stands. SLRE cores were taken in the winter of 2014. Approximately a meter of sediment was
156 collected for each location with the goal of obtaining at least 250 years of estuary history. Details
157 of sediment sampling procedures are provided in Alexson et al. (unpublished data) / Alexson
158 (2016)[1].

159 *3.2. Sediment chronology*

[1] This work is currently in review with the *Journal of Paleolimnology* and will be updated as necessary.

160 We used lead-210 (^{210}Pb) isotopic analysis to determine sediment core chronological
161 profiles and to calculate sedimentation rates. All ^{210}Pb analyses were completed by the Science
162 Museum of Minnesota's St. Croix Watershed Research Station. Methods followed Schelske et al.
163 (1994) including calculations from Appleby and Oldfield (1978).

164 *3.3. Poaceae pollen differentiation*

165 Preliminary to the investigation of *Z. palustris* population dynamics in the SLRE, we
166 expanded the pollen morphology differentiation to five characteristics of *Z. palustris* pollen
167 discernable with light microscopy (exine sculpture, width of annulus, polar length, equatorial
168 length, Polar/Equatorial (P/E) ratio). *Z. palustris* pollen was examined from herbarium samples
169 from four sites in Minnesota (Vermillion River, Rainy Lake, Pokegama River, and Bear Trap
170 River mouth) and eight fresh reference samples collected from the Pokegama River, Douglas
171 County, Superior, Wisconsin (Table 1). *Z. palustris* anthers were soaked in 5% potassium
172 hydroxide, and the plant material sieved through 125 μm Nitrex mesh to remove extraneous
173 plant material. Cells were then dehydrated with glacial acetic acid and organic material removed
174 by heating for 3 minutes at 100^0 C in a 9:1 mixture of acetic anhydride and sulfuric acid. Samples
175 were washed in deionized water to remove chemical traces and then divided for scanning
176 electron microscopy (SEM) and light microscopy studies. SEM samples were stored in deionized
177 water. Light microscopy samples were dehydrated with 95% ethanol before addition of silicon
178 oil for long-term storage. Light microscopy samples were not stained. Prepared slides were
179 sealed with paraffin.

180 While SEM is an effective tool to definitively identify similar Poaceae pollen types (Lee
181 et al., 2004), the procedure is too expensive and time consuming for practical application in most
182 palaeoecological studies. We used SEM images in our preliminary pollen differentiation to

183 verify light microscopy morphology.

184 Pollen cells were examined at 400x magnification under light microscopy. One hundred
185 ninety *Z. palustris* grains from Pokegama River stands were photographed using SPOT Imaging
186 5.1 software; exine sculpture classified as psilate, scabrate, or rugulate; measurements made of
187 the annulus diameter and of polar and equatorial widths; and polar-to-equatorial ratio (P/E)
188 calculated. Twenty pollen cells from each of the three *Z. palustris* herbarium samples collected
189 outside Pokegama River were evaluated and compared to the Pokegama River reference samples
190 to evaluate regional consistency. Reference pollen cells for *C. canadensis* collected within the
191 SLRE watershed were processed as described above and photographed with both SEM and
192 SPOT imagery. Twenty cells of *C. canadensis* were evaluated for exine sculpture, annulus
193 diameter, and P/E ratio. *Z. palustris* polar width and exine sculpture were also compared with
194 aquatic grasses evaluated by Lee et al. (2004).

195 SEM imagery was carried out at the University of Maine School of Biology and Ecology
196 Electron Microscopy Laboratory. Pollen samples were wrapped in Whatman filter paper (Grade
197 50, hardened) and dehydrated in progressively concentrated ethanol solutions of 10%, 20%,
198 30%, 50%, 70%, 80%, 85%, and 90% increments. Pollen cells were then dried in a Tousimis
199 Samdri PVT-3 Critical-Point Dryer using carbon dioxide at critical pressure and temperature.
200 Samples were mounted on carbon tape and sputter coated in a Cressington 108 Auto/SE Sputter
201 Coater with gold at parameters of 0.08 mBar pressure, 30 mA current, 60 seconds, at 14
202 nm/140A thickness. Images were captured on an AMRay 1820 Digital Scanning Electron
203 Microscope using a 10 kV working distance at magnifications ranging from 126x to 252x. We
204 compared SEM images to cells examined at 400x light microscopy to clarify exine sculpture.

205 *3.4. Fossil pollen and phytolith analyses*

206 Sediment samples from five core sites within the SLRE (Fig. 1) were submitted to the
207 National Lacustrine Core Facility (LacCore), University of Minnesota, Minneapolis, Minnesota,
208 for pollen and phytolith processing. A total of 105 samples from varying core depths were
209 analyzed for this study: Billings Park (n=22), North Bay (n=23), Minnesota (MN) Point (n=20),
210 North of Clough Island (n=20), and Pokegama Bay (n=20).
211 LacCore followed procedures adapted from Faegri et al. (1989) for pollen extraction. For
212 each sample one cubic centimeter (cc) of wet sediment was disarticulated in 10% potassium
213 hydroxide (KOH) and 10% hydrochloric acid (HCl) and then sieved through 160 μm mesh.
214 Treatment with hydrofluoric acid removed fine silts from the supernatant. Samples were treated
215 with glacial acetic acid before and after acetolysis to dehydrate the cells. Acetolysis with a 1:9
216 mix of sulfuric acid and acetic anhydride removed humic acids and cleared the pollen cells.
217 Samples were then rinsed three times with deionized water to remove chemical traces.
218 Consecutive rinses in 95% ethanol (EtOH), 100% EtOH, and tertiary butyl alcohol dehydrated
219 the pollen concentrate. The pollen concentrate was suspended in silicon oil. A 2.5×10^4
220 microsphere spike (± 8% sd) developed by LacCore was added to each sample to allow
221 calculation of pollen concentrations. Pollen concentrates were shipped to the Climate Change
222 Institute, University of Maine, Orono, Maine, where pollen, fern and fern-ally spores, and
223 charcoal particles larger than 50 μm were evaluated using a Nikon Phase microscope at 400x
224 magnification. For each sample, pollen cells were counted until a total of 300 arboreal and shrub
225 pollen cells were reached (a number deemed suitable by Faegri et al. [1989]) or, in the case of
226 pollen-depauperate samples, until one entire slide was analyzed.
227 *3.5. Phytoliths*
228 Phytoliths were extracted from core sediment samples using methodology developed by

229 Yost et al. (2014). For each sample 1-2 cc of wet sediment was placed in a 400 mL beaker with
230 10 mL of 36% HCl. After the carbonate reaction subsided, 50 mL of 68% nitric acid (HNO_3) was
231 added to each beaker, and samples were heated to ~115^0 C until organic oxidation visibly
232 stopped as indicated by the coloration of the gasses (1-2 hours). The samples remained
233 uncovered on heat until the solution volume was reduced to ~20 mL. Samples were rinsed five
234 times to neutral pH using high purity distilled water (dH_2O). To remove humic acids, nine mL of
235 10% KOH was added to each sample and allowed to stand for five minutes. Samples were then
236 rinsed four times in dH_2O to neutral pH. Each sample was then passed through a 70 μm sieve
237 into a 100 mL graduated cylinder using a 5% solution of sodium hexametaphosphate (SHMP).
238 Each graduated cylinder was filled to the 100 mL mark with 5% SHMP, thoroughly mixed, and
239 allowed to settle for one hour. The top 10 cm of solution containing suspended clay-sized
240 particles, diatoms, and sponge spicules was aspirated and discarded. This sequence of mixing,
241 settling, and aspiration was repeated at least five times or until the top 10 cm of solution was
242 clear after one hour of gravity settling. Samples were then transferred to 15 ml centrifuge tubes.
243 Three ml of lithium sodium tungstate heavy liquid solution (density of 2.3 g/ml) was added to
244 the samples and thoroughly mixed. Samples were centrifuged for 10 min at 1500 rpm, after
245 which the light fraction with the phytoliths was decanted and retained. The heavy liquid step was
246 repeated to ensure the successful separation of the phytoliths from the remaining sediment. The
247 recovered phytolith fraction for each sample was rinsed five times using dH_2O. A 5×10^4
248 microsphere spike (± 8% sd) developed by LacCore was added to each sample for phytolith
249 concentration calculations. Samples then received two rinses with 95% EtOH and transferred to
250 1.5 ml vials for storage.

251 For microscopy and counting, a subsample of each sample was mounted in Type-A

252 immersion oil to facilitate phytolith rotation and three-dimensional observation. Samples were
253 viewed using bright field illumination from 400-630x magnification for counting, and diagnostic
254 phytolith morphotypes were imaged at 630x magnification using a Leica DM (2500P)
255 petrographic, visible light microscope with compound objectives, a photo-tube mount, and
256 differential interference contrast capabilities. Images were obtained using a Leica EC3 digital
257 camera mounted on the phototube and the Leica Application Suite software. Phytoliths were
258 counted and identified, as were charred plant cells and microcharcoal particles in the 5 to 150 μm
259 range. For each sample, cells were counted until a total of 200 phytoliths or 500 exotic
260 microspheres were counted or until the entire slide was analyzed.
261 Pollen and phytolith percentages and influx densities (number of cells deposited on a cm^2
262 surface each year) were graphed using Tilia version 1.7.16 (Grimm, 2011). Paleo-vegetative
263 zones were defined by the plant assemblages with zone clarification supported by Coniss total
264 sum of squares-derived cladograms.
265 In an effort to distinguish wildfire charcoal from industrial charcoal, we calculated the
266 percent of charred phytoliths from the total phytolith sum. Microcharcoal percentages were
267 calculated from the phytolith sums.

268 **4. Results**

269 *4.1. Reference pollen*

270 SEM imaging clarified exine surface and annulus morphology between *Z. palustris* and
271 *C. canadensis* (Fig. 4). SEM magnification of 2500x showed the densely spaced areola that give
272 *Z. palustris* a smooth, psilate appearance under light microscopy. In contrast, areolae on the
273 surface of *C. canadensis* were more widely spaced to create a scabrate appearance at 400x. Table
274 1 shows subtle size differences among the four sites. Because *Z. palustris* pollen collected in

1959 at the mouth of Bear Trap River (St. Louis County, 48°12.57'N; 91°56.15'W) was notably smaller, we did not include pollen from this site in our grass pollen evaluation for the SLRE.

Evaluation of multiple morphologic traits of *Z. palustris* pollen cells allowed separation of *Zizania* pollen from other grass pollens in the SLRE. *Phragmites* and *Calamagrostis* pollen have the same size, shape and exine sculpture in modern and fossil pollen assemblages, but vary from *Zizania* pollen in all three traits (Fig. 4; Table 2). Aside from grouping *C. canadensis* and *P. australis* (reported as *Calamagrostis*-type) and species with distinctive pollen morphology (e.g. *Andropogon gerardii* Vitman (big bluestem) and *Zea mays*), all other Poaceae were grouped as "Poaceae undifferentiated."

4.2. Lead-210 dates and sedimentation rates

Alexson et al. (unpublished data) / Alexson (2016) summarized ^{210}Pb dates and calculated sediment accumulation in centimeters per year (cm/yr) for each of the five core sites. Sediment accumulation rates increased at North Bay (Fig. 5) and Pokegama Bay (Fig. 6) core sites around the time of European settlement. Sediment accumulation rates of 0.5-1.0 cm/yr at Billings Park (Fig. 7) core site limited the paleo-record to the past 113 years. We adjusted ^{210}Pb dates in the Billings Park core to fit microcharcoal peaks associated with known fire events within or downwind of the catchment area. Dates and sediment accumulation values beyond the range of ^{210}Pb dating accuracy (approximately pre-1850) at North Bay, Pokegama Bay, and MN Point (Fig. 8) sites were extrapolated from oldest ^{210}Pb values.

In the North of Clough Island core (Fig. 9) unsupported (excess) ^{210}Pb data were relatively monotonous with depth, aside from an uppermost section with higher concentrations. Supplementary dating showed high concentrations of ^{137}Cs isotope around 1963 due to nuclear weapons testing (Krishnaswami and Lal, 1978). Based on the 1963 ^{137}Cs peak at 60 cm depth,

298 we assigned a rough, recent chronology acknowledging that dates since 1963 are highly
299 uncertain (Alexson et al., unpublished data) (Alexson, 2016), a probable result of a large
300 flooding and sediment deposition event in 2012. Stratigraphic information from pre-1960
301 sediments are deemed trustworthy.

302 *4.3. Pollen and phytolith results*

303 Phytoliths considered to be locally and regionally diagnostic of wild rice (Fig. 3; Yost
304 and Blinnikov, 2011) were observed in varying amounts in North Bay, Billings Park, and MN
305 Point cores. No *Zizania* phytolith morphotypes were found in North of Clough and Pokegama
306 Bay cores. *Zizania* phytoliths calculated as a percentage of the total phytolith count within these
307 samples ranged from a high of 6.4% to a low of 0%. For different phytolith types, multiple
308 *Zizania* plant-part morphotypes (Fig. 3) were aggregated to determine frequency of occurrence.

309 Cool season (C_3) grasses dominate the total phytolith assemblage in all samples in
310 relation to warm season (C_4) grass indicators. Rondel phytoliths (Fig. 3A-C) produced in the
311 inflorescence and typical of *Zizania* were observed in many of the samples and were included in
312 the C_3 grass functional type category. Trapeziform sinuate phytoliths (Fig. 3H) within the C_3
313 grass grouping were likely contributed by *Phalaris arundinacea, Calamagrostis canadensis*
314 (Mich.) Beauv. (Canada blue joint), and *Glyceria grandis* S. Wats. (tall manna grass). Keeled
315 rondel forms (Fig. 3T) were likely contributed by *Leersia oryzoides* (L.) Sw. (rice cutgrass) and
316 possibly *Poa palustris* L. (waterfowl meadow grass). The C_4 xerophytic grass phytolith
317 assemblage was composed primarily of saddle forms from Chloridoideae taxa (Fig. 3J). The C_4
318 mesophytic grass phytolith assemblage was composed primarily of cross and bilobate forms
319 from Panicoideae taxa (Fig. 3N). In this habitat the most likely contributors of Chloridoideae
320 phytoliths include *Spartina pectinata* Link (cordgrass) and *Muhlenbergia glomerata* (Willd.)

Trin. (spike muhly). Species of *Panicum* (switchgrass, millets) and *Andropogon* (big bluestem) most likely contributed the bilobate and cross phytoliths. Despite being a C_3 grass, it is possible that *Phragmites americanus* contributed some of the saddle morphotypes in the C_4 category, as *Phragmites* produces both saddle-like and keeled rondels that resemble C_4 and C_3 grasses, respectively.

Because we could not characterize "Poaceae pollen undifferentiated" as aquatic, emergent, or upland species, we excluded undifferentiated Poaceae pollen from pollen percent and density calculations.

4.4. North Bay (Figs. 5 and 10a).

The 76 cm of sediment in the North Bay core encompassed the time period between ~1757 through 2014. These samples contained the strongest phytolith signature of *Zizania* presence of all of the cores from the SLRE; a total of 127 *Zizania* forms were observed. Phytolith morphotypes diagnostic of *Zizania* appeared in the core at ~1875 and were present to the core surface. *Zizania* pollen was present throughout the core.

Phytoliths produced in the achene and other plant tissues of the Cyperaceae family and Cyperaceae pollen were observed in all samples. Based on the phytolith morphotypes, the most likely contributors were taxa belonging to the genera *Carex*, *Scirpus*, and *Schoenoplectus* (bulrush).

In zone 1 (prior to 1875) *Zizania* pollen percent was slightly higher while pollen of other emergent species was lower than in later zones. Low percentages of *Ambrosia* (ragweed) and higher percentages of *Pinus* in zone 1 indicated that this zone likely pre-dates the start of intensive farming and logging in the region.

Zone 2 (1875-1946) was characterized by increased *Ambrosia,* increased non-wild rice

344 grass phytolith influx density (PhID), increased microcharcoal (Fig. 10a), increased percent and
345 pollen influx density (PID) of emergent plants, and decreasing percent of *Pinus* relative to other
346 tree and shrub species. Percent of xeric C_4 phytoliths began to increase as microcharcoal
347 increased, but peak percent lagged behind the microcharcoal peak by as much as a decade.
348 Upland herbaceous flora increased in this zone. *Z. palustris* pollen reached its highest PID in
349 zone 2.

350 In zone 3 (1946-1980) microcharcoal exhibited a constant 10% throughout the zone,
351 while tree and shrub PID increased up the core. Xeric C_4 grasses increased as cool season C_3
352 grasses declined between 1946 and 1965 (Fig. 10a). *Zizania* phytolith PhID peaked at the start
353 and end of zone 3, with mid-zone decline corresponding with an increase in floating leaf aquatics
354 between 1952 and 1975.

355 The transition into zone 4 (1985-2014) was marked by a brief increase in sediment
356 accumulation, which corresponds with increased phytolith and pollen index densities. This brief
357 shift was followed by a stable conifer/deciduous forest mix extending to the top of the core.
358 Microcharcoal declined sharply and cool season C_3 grasses increased around 1900, with
359 microcharcoal remaining 5% or less through 2014 (Fig. 10a). Consistent presence of the
360 emergent flora *Typha* (cattail), *Sagittaria* (arrowhead), and Cyperaceae indicate stable
361 shorelines. Epiphytic diatoms increased in the fossil record during this time, corroborating the
362 likelihood of macrophyte increases inferred by an increase in epiphytic diatoms (Alexson et al.,
363 unpublished data) (Alexson, 2016). *Zizania* pollen and phytolith values remained stable except
364 for a brief decline 2009-2010 AD when *Typha* and *Calamagrostis*-type pollen increased.

365 *4.5. Pokegama Bay (Figs. 6 and 10b).*

366 Sixty-five cm of sediment from Pokegama Bay covered the time from ~1722 through

2014. No sample yielded phytolith morphotypes diagnostic of *Zizania* despite known stands of wild rice in the bay upstream from the site (Fig. 1). *Zizania* pollen was present as 15-20% of emergent and aquatic flora until 2003 when percent increased to 40%.

Phytoliths produced in the achene and other plant tissues of Cyperaceae were observed in all samples. Based on the phytolith morphotypes, the most likely contributors are taxa belonging to the genera *Carex*, *Scirpus*, and/or *Schoenoplectus*. Microcharcoal particles were observed in all phytolith samples although percentages were significantly lower prior to ~1853 AD.

Zone 1 (prior to 1853) contained low microcharcoal percentages (not shown on Fig. 10b), higher percentages of trees and shrubs, particularly *Pinus, Betula* (birch) and *Larix* (larch), and low percent of *Ambrosia.* High PID of tree and shrub pollen offset low PID of upland herbaceous flora.

Zone 2 (1853-1950) began with microcharcoal exceeding 20%, increased *Ambrosia,* and the decline of *Pinus* and *Larix*. Microcharcoal and xeric C_4 grasses gradually increased to peak during the Dust Bowl fire period (1929-1936) (Fig. 10b). *Typha* and Cyperaceae pollen percentages declined coincident with the appearance of floating leaf aquatics.

During zone 3 (1950-2014) the forest was a stable mix dominated by *Pinus,* Cupressaceae, *Betula*, and *Quercus* (oak). A brief increase in sediment accumulation coincided with peaks in Cyperaceae and non-wild rice grasses PhID ~1954. *Pinus* and *Betula* declined from 1950 to ~1976. Increased xeric C_4 grasses followed an increase in microcharcoal beginning around 1970. From 2003 to 2014 upland and emergent species increased, and *Zizania* pollen reached its highest percent and PID.

4.6. Billings Park (Fig. 7 and 10c).

Samples analyzed from the Billings Park core covered 110 years from 1904 to 2014. We

390 adjusted ^{210}Pb dates for the Billings Park core according to microcharcoal peaks associated with
391 wildfires within the SLR catchment or west (downwind) of the catchment area, e.g. 1918
392 Cloquet-Moose Lake fire burned 1.2 million acres within the SLR catchment (Minnesota
393 Department of Natural Resources, 2008). *Zizania* pollen was present as 10-25% of the emergent
394 and aquatic pollen throughout the core. Phytolith morphotypes diagnostic of *Zizania* only
395 appeared in the record prior to 1933. Only one sample (40-41 cm depth; ~1933) contained the
396 unequivocal Inflorescence Type 1 *Zizania* phytolith (Fig. 3A-C). Phytoliths produced in the
397 achene and other plant tissues of members of the Cyperaceae family were observed at all levels.
398 Microcharcoal was observed in all phytolith samples.
399 In zone 1 (1904-1920) the gradual increase in microcharcoal (Fig. 10c) was accompanied
400 by a decline in *Pinus* pollen. Microcharcoal and sedimentation rates peaked with the 1918
401 Cloquet-Moose Lake fire. The presence of floating leaf aquatics *Nuphar* (yellow pond lily) and
402 *Nymphaea* (water lily), combined with decreased percentages of emergent species *Typha* and
403 *Sagittaria*, suggest higher water levels (diminished shorelines) relative to the second half of the
404 20th century. *Zizania* PhID peaked ~1914 and then dropped to zero by 1918.
405 At the start of zone 2 (1920-1976) the peak in sediment accumulation coinciding with a
406 peak in microcharcoal probably accounts for concurrent peaks in other influx density values.
407 Increased xeric C_4 grasses and peaks in microcharcoal (Fig. 10c) delineated the Dust Bowl years
408 of 1929 to 1936. *Pinus* pollen continued to decline and *Ambrosia* pollen increased. *Zizania* PhID
409 peaked again ~1935, and then disappeared for the duration of the core.
410 In zone 3 (1976-2014) percentages of *Typha, Sagittaria* and *Alnus* (alder) pollen
411 increased and floating leaf aquatic flora declined, indicating an expansion of shoreline habitat.
412 An increase in both *Betula* and *Pinus* pollen followed the decline in microcharcoal between 1976

and ~1990. Around 1990 microcharcoal increased with a peak ~2007. This increase in fire indicator was accompanied by a decrease in cool season C_3 grasses and an increase in xeric C_4 grasses (Fig. 10c).

4.7. MN Point (Figs. 8 and 10d)

Low rates of sediment accumulation at the MN Point core site limited accurate ^{210}Pb dating to the top 17 cm of the core. Because calculation of sediment accumulation requires multiple dates at regular intervals along the core, sediment accumulation values are not listed below the limits of ^{210}Pb dating accuracy. However, if we assume a constant, pre-European settlement sediment accumulation rate of 0.019 cm/yr, 35 cm of sediment contains ~800 years of deposition (Alexson et al., unpublished data) (Alexson, 2016). MN Point is located at the estuary/Lake Superior interface (Fig. 1) and is subject to greater lake effects than the other core sites. MN Point is the only core to contain significant *Artemisia* and *Andropogon gerardii* pollen signals.

Phytolith morphotypes diagnostic of *Zizania* appeared at three discrete intervals in the core, the first interval appearing more than 750 years ago. *Zizania* pollen percent and PID were low throughout the core, with periods of no *Zizania* pollen deposition prior to 1900. Phytoliths indicative of Cyperaceae were observed at all levels. Microcharcoal did not appear in notable concentrations until the 1600s (not shown on Fig. 10d), but rose to 60% of the total phytolith sum by 1900 (Fig. 10d). The pollen assemblage indicated mixed coniferous/hardwood forests of *Pinus, Picea, Cupressaceae* (juniper), *Betula*, and *Quercus* (oak) throughout the core.

Zone 1 (est. prior to 1300 AD) was defined by the highest PID of trees and shrubs throughout the core and by the presence of floating leaf aquatics and *Zizania* pollen and phytoliths.

436 In zone 2 (est. 1300 to 1640) the absence of floating leaf aquatic flora and the strong
437 presence of *Typha* and Cyperaceae suggest lower water levels relative to zone 1. *Zizania*
438 phytoliths were present only in the lowest portion of the zone, and *Zizania* pollen percent varied
439 from 0-5%.
440 In zone 3 (~1640-1884) Cyperaceae PhID and *Typha* pollen were low relative to zone 2
441 and floating leaf aquatics appeared. Microcharcoal first appeared at the start of the zone (not
442 shown on Fig. 10d). *Zizania* pollen percent remained low, but constant, at <10%. Tree and shrub
443 PID declined throughout zone 3.
444 At the start of zone 4 (1884-2013) sediment accumulation rates began a gradual increase.
445 Microcharcoal increased to 62% by 1900, declined until ~1955, and then peaked around 1965
446 and again in 2007. *Pinus* pollen declined while *Fraxinus, Quercus, Ambrosia*, and *Andropogon*
447 pollen increased. *Zizania* PID attained the highest levels in the core by 1980, and *Zizania* PhID
448 were present from 1884 to ~1960 and again since 1995. Cyperaceae and non-wild rice grass
449 PhID increased dramatically in zone 4.
450 *4.8. North of Clough Island (Fig. 9).*
451 The North of Clough Island core site was unique for its high sediment accumulation rate
452 (85 cm in 72 years) and for the layer of fine sand present prior to 1958. ^{210}Pb data from the North
453 of Clough Island core suggested recent disturbance, likely due to increased sedimentation from a
454 500-year flood that affected the SLR in 2012 (Pelletier and Knight, 2014). Although none of the
455 sediment intervals yielded phytolith morphotypes diagnostic of *Zizania, Zizania* pollen is present
456 in all samples after 1958. Microcharcoal was observed in all phytolith samples after 1958.
457 Accumulation rates were uncertain since ~1960 at the North of Clough Island location, and it is
458 possible that much of zones 2 and 3 was deposited during the flood of 2012; a glut of

allochthonous material.

Zone 1 (prior to 1955) sediment consisted of fine sand not found in the other four cores. *Zizania* pollen was low and *Zizania* phytoliths were absent in zone 1. Cyperaceae, *Calamagrostis*, and *Typha* species dominated the emergent pollen record suggesting an expanded shoreline.

Zone 2 (1954-1987) starts at the top of the fine sand layer. *Zizania* pollen had a muted presence (<10% and PID averaging less than 600 cells/cc) throughout the core. Microcharcoal peaked between 1970 and 1980. Tree and shrub PID reached its highest level in zone 2.

In Zone 3 (1987-2014) *Pinus* declined and *Picea*, *Cupressaceae,* and *Quercus* increased. Upland herbaceous and emergent flora PID declined while floating leaf aquatic flora increased. *Zizania* pollen percent and PID increased after 2006.

5. Discussion

Analyses of plant phytoliths and pollen from sediment cores with deposition covering the past ~250 years show changes in vegetation within the estuary and surrounding landscape. By comparing the stratigraphic shifts in vegetation with the historical record, we can better understand human impacts on estuary habitats.

5.1. Differentiation of Zizania palustris *from other endemic grasses*

Identification of morphologic characters that distinguish *Zizania* pollen from other terrestrial and aquatic grasses allowed us to explore the response of *Zizania* to changes in estuary habitat. *Calamagrostis* and native *Phragmites americanus* pollens are similar to each other, but distinctive from other grass pollen and grouped as emergent species. Agricultural *Zea mays* and terrestrial *Andropogon gerardii* were distinguished by size and sculpture. Poaceae ≤ 20 μm

481 represent either *Poa annua* (upland annual bluegrass) or *Phragmites australis* (invasive emergent
482 species).

483 *5.2. Reconstruction of vegetative changes in the SLRE*

484 MN Point, North Bay, and Pokegama Bay cores captured the 200-year time frame that
485 allowed us to assess the effects of increased mining, logging, industry, and population growth on
486 the vegetation within and around the estuary. By the mid-1800s increasing logging and mining
487 within the SLR catchment (Baeten et al., 2016) was evident in the cores as increased sediment
488 accumulation, increased microcharcoal, and decreasing pine stands. Increase in *Ambrosia* pollen
489 further signaled land disturbance.

490 Forest cover across all core sites maintained its pre-European settlement mix of conifers
491 (*Pinus, Picea, Abies* (fir), Cupressaceae) and hardwoods (*Betula, Fraxinus, Quercus*). The
492 decline in *Pinus* from logging (mid-1800s to 1900s) and from fire opened the forest canopy for
493 *Betula, Fraxinus, and Quercus* expansion. Fire frequency in Minnesota increased in the 1840s
494 (Seeley, 2006) and peaked with the 1918 Cloquet-Moose Lake Forest Fire in the upper SLR
495 watershed and the drought-related dust bowl fires of the 1930s (Fig. 10) (Seeley, 2006;
496 Minnesota Department of Natural Resources, 2008). Increases in upland herbaceous pollen
497 coincident with declines in arboreal pollen at North Bay (Fig. 5) between 1875 and ~1970
498 probably reflect first logging and later urbanization around the upper estuary and lower SLR
499 catchment area. Increased microcharcoal, combined with increased xeric C_4 grasses, during the
500 1940s (Fig. 10) suggest a period of warm, dry summers. All cores except Pokegama Bay showed
501 a shift toward predominance of emergent species during this time, an indication of lower water
502 levels and expanding shorelines within the SLRE.

503 The consistent decline in arboreal pollen since the 14th century coupled with steady

herbaceous pollen concentrations at MN Point (Fig. 8) reflect inputs from the broader Lake Superior region. Significant percentages of *Artemisia* and *Andropogon* pollen, indicators of more open habitats not found at other core sites, support this premise.

While diatom fossil records in these cores provided strong evidence of a history of nutrient pollution (Alexson et al., unpublished data) (Alexson, 2016), these locations were less than ideal for assessment of wild rice history. *Zizania* pollen was present in varying concentrations in all cores and in general did not suggest post-settlement loss in wild rice abundance. Based on concentrations ranging from 5000-10,000 cells/cc throughout both cores, North Bay (Fig. 5) and Pokegama Bay (Fig. 6) sites supported *Zizania* stands for the time period represented in those cores, suggesting no apparent post-settlement loss of stands at these sites. The absence of *Zizania*-specific phytoliths is surprising for Pokegama Bay given the historic record of stands in that embayment (Fig. 1). However, Yost et al. (2013) found that wild rice phytolith abundance rapidly diminishes as distance from the stand increases, so our core site may be well outside of historic stand locations. Based on our findings, traditional paleolimnological methods for lacustrine systems (i.e. coring a deep region of an aquatic body) are not well suited to the study of *Zizania*, whose fossils do not tend to travel far from their origin.

Zizania pollen densities were low throughout Billings Park (Fig. 7) and MN Point (Fig. 8) cores. *Zizania* phytoliths were not observed after ~1947 for Billings Park, but were present periodically throughout the MN Point core. The paucity of *Zizania* inflorescence phytoliths at both of these locations is indicative of the distance of wild rice stands from the core sites. Another consideration is that changes in water level and/or wave action within the sheltered bays of North Bay, Pokegama Bay, and Billings Park (Fig. 1) effected the distribution of plant debris (including phytoliths) and the deposition and redistribution of pollen.

A number of studies cite shifts in the ratio of emergent flora (Cyperaceae and *Typha*) to floating leaf aquatic flora (*Nuphar* and *Nymphaea*) as an indicator of changes in water level (e.g. Hannon and Gaillard, 1997; Dieffenbacher-Krall and Halteman, 2000; Dieffenbacher-Krall and Nurse, 2005). In Pokegama Bay floating leaf aquatic *Nymphaea* increased and Cyperaceae and *Typha* decreased between 1885 and ~1945 (Fig. 6). This apparent increase in water level may be due to dam-induced water level stabilization that occurred in the late 1800s through the mid 1900s for log booming. The same emergent/aquatic plant sequence occurred at MN Point between ~1614 and 1850 (Fig. 8), Billings Park between 1904 and ~1930 (Fig. 7), and at North Bay from ~1943 to 1980 (Fig. 5). Groundwater levels typically increase following extensive deforestation of forested regions (Dieffenbacher-Krall and Nurse, 2005), but neither the water stabilization of the late 1800s nor the response to industrial deforestation is consistent across the cores.

Periodic increases in water levels could benefit wild rice stands. *Zizania palustris* is a pioneer species that requires periodic disturbance to remove competing aquatic and emergent aquatic plants such as *Typha* and *Sagittaria.* The *Zizania* pollen spike at the top of the Pokegama core (Fig. 6) may be evidence of just such a case.

Zones 3 of the Pokegama Bay (Fig. 6) and Billings Park (Fig. 7) cores show expanded shorelines and decreased floating leaf aquatic plants since 1960. This vegetative evidence of decreased water levels corresponds with declining water levels and decreasing percent ice cover in Lake Superior (Reavie et al., 2016). Lake Superior water levels declined in the 1960s, increased between 1970 and 1980, and declined steadily over the next 30 years.

The North of Clough Island core (Fig. 9) is problematic. Sediment accumulation rates were uncertain from ~1964 through 2012. The 2012 flood likely contributed several cm of

material to this site, and raised uncertain about the accuracy of ^{210}Pb dates. Total phytolith concentrations were low relative to other cores in the study; *Zizania* pollen was absent from the fine sand below 1958, and peaked around 1970 at the low value of ~700 cells/cc (Fig. 9). This location in the main stem of the estuary (not near known *Zizania* stands) likely accumulated allochthonous pollen and phytoliths from upstream sediments that had previously accumulated *Zizania* fossils.

5.3. Corroboration of pollen and phytolith data

In terms of total *Zizania* phytoliths identified per sample, the frequency of inflorescence rondel morphotypes relative to other phytolith morphotypes ranged from 0 to 33%. Following the work of Yost and Blinnikov (2011), a decay-in-place scenario for *Zizania* phytolith assemblages recovered from within-stand sediments should be comprised of at least 29% Inflorescence Type 1 rondels. However, cultural practices such as wild rice harvesting can remove large quantities of inflorescence phytoliths. Also, water currents and prevailing wind can cause a loss of large quantities of non-inflorescence material from a *Zizania* stand (Yost et al., 2013). This would result in Inflorescence Type 1 rondel percentages to be higher than 29%. A total of three samples (one from Billings Park ~1933 AD, and two from North Bay ~1999 AD and 1940 AD) had over 29% of the *Zizania* assemblage comprised of inflorescence rondels (Supplementary Table 1). Morphotype distribution and low abundance of *Zizania* phytoliths, in the presence of *Zizania* pollen (e.g. Pokegama Bay and Clough Island, Figs. 6 and 9) suggests that core locations are too far from *Zizania* stands to record their phytoliths (Yost et al., 2013). Based on good preservation of diatom remains in these cores (Alexson et al., unpublished data) (Alexson, 2016), recycling of sedimentary silica did not appear to contribute to phytolith loss.

6. Conclusions

573 This case study demonstrated the benefit of examining local pollen reference material to
574 refine pollen identification. Morphological analyses of *Zizania palustris* reference pollen
575 identified unique characters that differentiated *Zizania* from associated grasses within the SLRE.
576 Our ability to distinguish *Zizania* pollen from other endemic grasses, combined with the paired
577 study of pollen and phytolith deposition, allowed us to demonstrate *Zizania* population shifts
578 over time. The presence of *Zizania* pollen in all cores, and *Zizania*-specific phytoliths in three of
579 the five cores, indicates that *Zizania* stands occupied the SLRE since at least the early 1700s.
580 Significant *Zizania* pollen concentrations coupled with *Zizania*-specific phytoliths distinguished
581 active *Zizania* stands (Billings Park and North Bay) from areas of secondary pollen deposition
582 (e.g. North of Clough Island and Pokegama Bay), although we know of no observational data
583 indicating wild rice stands up-gradient from the Billings Park core (Schwartzkopf, 1999;
584 Minnesota Department of Natural Resources, 2014; Fig. 1). Interpretation of pollen and phytolith
585 data from MN Point is problematic due to its distance from known stands and lake effects on
586 sediment redistribution. The stratigraphic markers to track historical wild rice abundance were
587 probably muted in our cores due to sampling locations that were too far from areas that support
588 wild rice stands.
589 We combined pollen and phytolith data with estuary and upland vegetation changes with
590 historic records of settlement and industrialization. Pollen records from the SLRE suggest that
591 vitality of *Zizania* stands relates to site-specific shifts in water level. Shifts in water level may be
592 due to human activities (dams, dredging, and deforestation) or to natural occurrences (wildfire,
593 drought, floods). The relationship of stand vitality to water depth is a complicated but important
594 variable for wild rice. An increase or decrease in water level will expand wild rice populations, if
595 it increases the amount of lake bottom within the optimal depth range of 0.5 to 0.95 m (Pillsbury

596 and McGuire, 2009; Tucker et al., 2011).

597 This study demonstrated that focused taxonomic description of a similar pollen group can
598 enhance the record of plant community evolution. Although we recommend future studies use
599 sediment records from within or closer to known or previously existing wild rice stands, we
600 confirmed the complimentary nature of combined pollen and phytolith analyses. Analyses of
601 both of these plant microfossils, with their different taphonomies, better constrain local and
602 regional conditions.

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[3] See footnote 1

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727 Figure Captions

728 Figure 1. Core locations in the St. Louis River Estuary (green symbols). Historical, approximate
729 distributions of wild rice between 1920 and 1960 are based on anecdotal observations of areas
730 (red outline) and specific locations (red points) (Schwartzkopf, 1999). Anecdotal observations of
731 current distributions of wild rice stands during the period of 2007 to 2014 are indicated by areas
732 (light blue) and specific locations (dark blue points), based on multiple data sources (Minnesota
733 Department of Natural Resources, 2014).

734 Figure 2. *Zizania palustris* in bloom, Pokegama River tributary to the St. Louis River estuary,
735 August 6-12, 2014. *Zizania* stems, leaves, and flowers are held above the surface of the water,
736 except for a short period of time in the early summer when the shoot is still growing and the
737 leaves float on the surface. *Zizania* is monoecious with unisexual flowers. The pollen-bearing
738 staminate flowers are below and carpellate flowers above on the inflorescence. Photos by Carol
739 Reschke.

740 Figure 3. Micrographs of phytoliths recovered in sediment samples from the St. Louis River estuary,
741 Minnesota. All micrographs taken with light microscopy at 600x magnification. *Zizania* phytolith type
742 designations follow Yost and Blinnikov (2011).

743 A) *Zizania* spp. Inflorescence Type 1 rondel in oblique/side view.

744 B) *Zizania* spp. Inflorescence Type 1 rondel in oblique/side view.

745 C) *Zizania* spp. Inflorescence Type 1 rondel in oblique/side view.

746 D) *Zizania* spp. Leaf Type 1 obtuse-lobed cross.

747 E) *Zizania* spp. Floating Leaf Type 5 elongate sinuate/polylobate.

748 F) *Zizania* spp. Sheath Type 4 five-lobed cross, seen from multiple views.

749 G) Polygonal Cyperaceae cone cells in top (above) and side (below) views.

H) Trapeziform sinuate in top view, produced in leaf and sheath plant portions of the grass subfamily Pooideae.

I) Polygonal Cyperaceae achene phytolith.

J) Saddle phytolith morphotype in top view, diagnostic of grass subfamily Chloridoideae leaf and sheath material.

K) Oblong with projections phytolith produced in grass leaves and part of the interstomatal guard cell complex.

L) Irregular, semi-faceted phytolith fragment produced in *Commelina* spp.

M) Polygonal Cyperaceae achene phytolith.

N) Sequence of bilobate Poaceae phytoliths diagnostic of the Panicoideae.

O) Interstomatal guard cell Poaceae phytolith.

P) Blocky polyhedral-type phytolith produced in bark material of *Pinus* spp.

Q) A roughly ovoid Cyperaceae root/rhizome-type phytolith.

R) Bilobate phytolith with a microsphere (lower center) in the same field of view.

S) Cuneiform bulliform phytolith produced in the leaves of Poaceae.

T) *In-situ* keeled reniform rondels with elongate wavy cells commonly produced by species of *Poa* and other members of the Pooideae subfamily.

Figure 4. *Calamagrostis canadensis* (A) and *Zizania palustris* (B): SEM on left (scale bar = 10 μm), SPOT light microscopy 40x on right. A. *Calamagrostis canadensis*: Note presence of operculum, a structure not generally seen in fossil Poaceae pollen. Sculpture is scabrate. Measured values are annulus width, polar length, and equatorial width. P/E ratio for this cell is 1.00 (round). B. *Zizania palustris*: Sculpture is psilate. Measured values are annulus width, polar length, and equatorial width. P/E ratio for this cell is 1.2 (oval).

773 Figure 5. North Bay pollen and phytolith diagram of selected taxa. Zones derived from total sum
774 of squares of pollen and phytolith taxa values. Color code: Olive-Sediment accumulation; Green-
775 Pollen percent and influx density (PID) values (cells/cm^2/y); Blue-Phytolith percent and influx
776 density (PhID; cells/cm^2/y); Gray-Data exaggeration x 5.
777 Figure 6. Pokegama Bay pollen and phytolith diagram of selected taxa. Zones derived from total
778 sum of squares of pollen and phytolith taxa values. Color code: Olive-Sediment accumulation;
779 Green-Pollen percent and influx density (PID) values (cells/cm^2/y); Blue-Phytolith percent and
780 influx density (PhID; cells/cm^2/y); Gray-Data exaggeration x 5.
781 Figure 7. Billings Park pollen and phytolith diagram of selected taxa. Zones derived from total
782 sum of squares of pollen and phytolith taxa values. Color code: Olive-Sediment accumulation;
783 Green-Pollen percent and influx density (PID) values (cells/cm^2/y); Blue-Phytolith percent and
784 influx density (PhID; cells/cm^2/y); Gray-Data exaggeration x 5.
785 Figure 8. MN Point pollen and phytolith diagram of selected taxa. Sediment accumulation rates
786 not calculated beyond the limits of ^{210}Pb dating. Zones derived from total sum of squares of
787 pollen and phytolith taxa values. Color code: Olive-Sediment accumulation; Green-Pollen
788 percent and influx density (PID) values (cells/cm^2/y); Blue-Phytolith percent and influx density
789 (PhID; cells/cm^2/y); Gray-Data exaggeration x 5.
790 Figure 9. North of Clough Island pollen and phytolith diagram of selected taxa. Date of 1963 at
791 60 cm based on a peak in ^{137}Cs. Zones derived from total sum of squares of pollen and phytolith
792 taxa values. Color code: Olive-Sediment accumulation; Green-Pollen percent and influx density
793 (PID) values (cells/cm^2/y); Blue-Phytolith percent and influx density (PhID; cells/cm^2/y); Gray-
794 Data exaggeration x 5.

795 Figure 10. Percent of total sum of counted phytoliths for microcharcoal and functional-type grass
796 phytoliths: cool season C_3 grasses, e.g. *Festuca, Poa, Calamagrostis*; xeric C_4 grasses, e.g.
797 *Bouteloua, Sporobolus*; mesic C_4 grasses, e.g. *Andropogon, Schizachyrium, Sorghastrum.*
798

799 Table 1. Comparison of characters of fresh reference *Z. palustris* pollen collected at eight sites
800 along the Pokegama River with herbarium *Z. palustris* pollen from three additional sites in
801 Minnesota. Measured ranges are to 1 standard deviation. Sculpture is a numeric designation for
802 cell wall patterning: psilate (smooth) = 1; scabrate (scattered spicules smaller than 1 μm) = 2;
803 rugulate (irregular pattern of elements >1 μm) = 3.

Mean Character Value (Range)	*Z. palustris* Pokegama River n = 190	*Z. palusiris* Vermillion River DUL 26304 n = 20	*Z. palusiris* Rainey Lake DUL 37362 n = 20	*Z. palustris* Bear Trap River DUL 28555 n = 20
Site Location	46^0 40' 53" N 92^0 08' 57.5"W	48^0 14' 25" N 92^0 34' 54" W	48^0 35"14.5" N 93^0 10' 0.6" W	46^0 50' 13" N 92^0 00' 21.5" W
Collection date	08.21.2012[a] 08.06-12.2014	07.20.1955	08.12.1982	08.29.1956
Sculpture 1-3	1.01	1.1	1.1	1.1
Annulus width (μm)	8-10	7-9	9-10	7-9
Polar (μm)	31-35	32-35	32-35	30-33
Equatorial (μm)	27-31	29-33	28-31	27-30
P/E ratio	1.12	1.09	1.15	1.11

804 n = number of pollen cells evaluated.

805 [a]Herbarium number 2362, n = 20.

806

807 Table 2. Comparison of *Z. palustris* traits from Pokegama River with wetland and aquatic grass
808 species potentially present in the estuary. *Phalaris* annulus width estimated from SEM image in
809 Lee et al. (2004). Empty cells designate no data available.

Mean Character Value (Range)	*Zizania palustris*[a] n = 190	*Calamagrostis canadensis*[a] n= 20	*Phragmites australis*[b]	*Phalaris arundinacea*[b]	*Glyceria borealis*[b]	*Glyceria canadensis*[b]
Sculpture	psilate	scabrate	coarse	smooth	coarse	coarse
Annulus width (μm)	8-10	5-7		4-5		
Polar length (μm)	31-35	23-27	23-27	29-38	28-34	28-35
Equatorial width (μm)	27-31	23-26				
P/E ratio	1.12	1.02				

810 [a]Data from this study. [b]Data from Lee et al. (2004).

Fig. 1

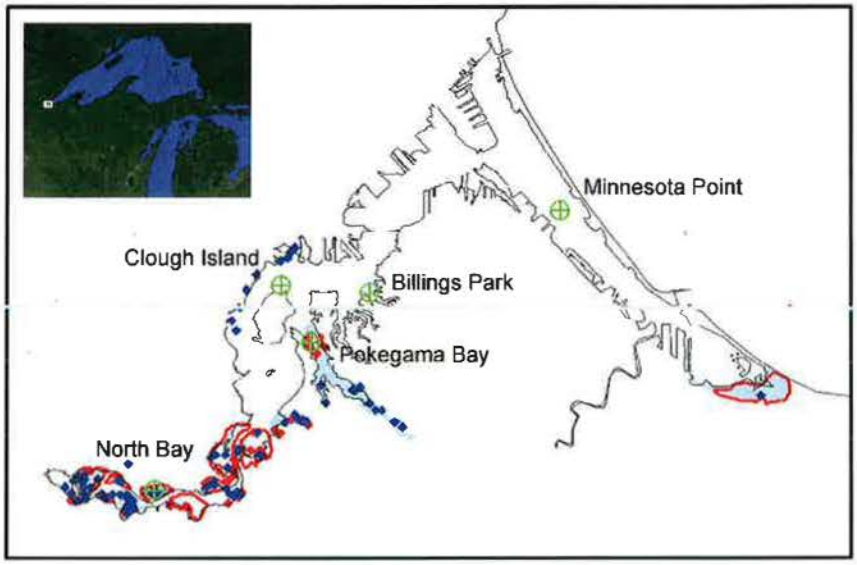

Minnesota Point
Clough Island
Billings Park
Pokegama Bay
North Bay

Fig. 2

Fig. 3

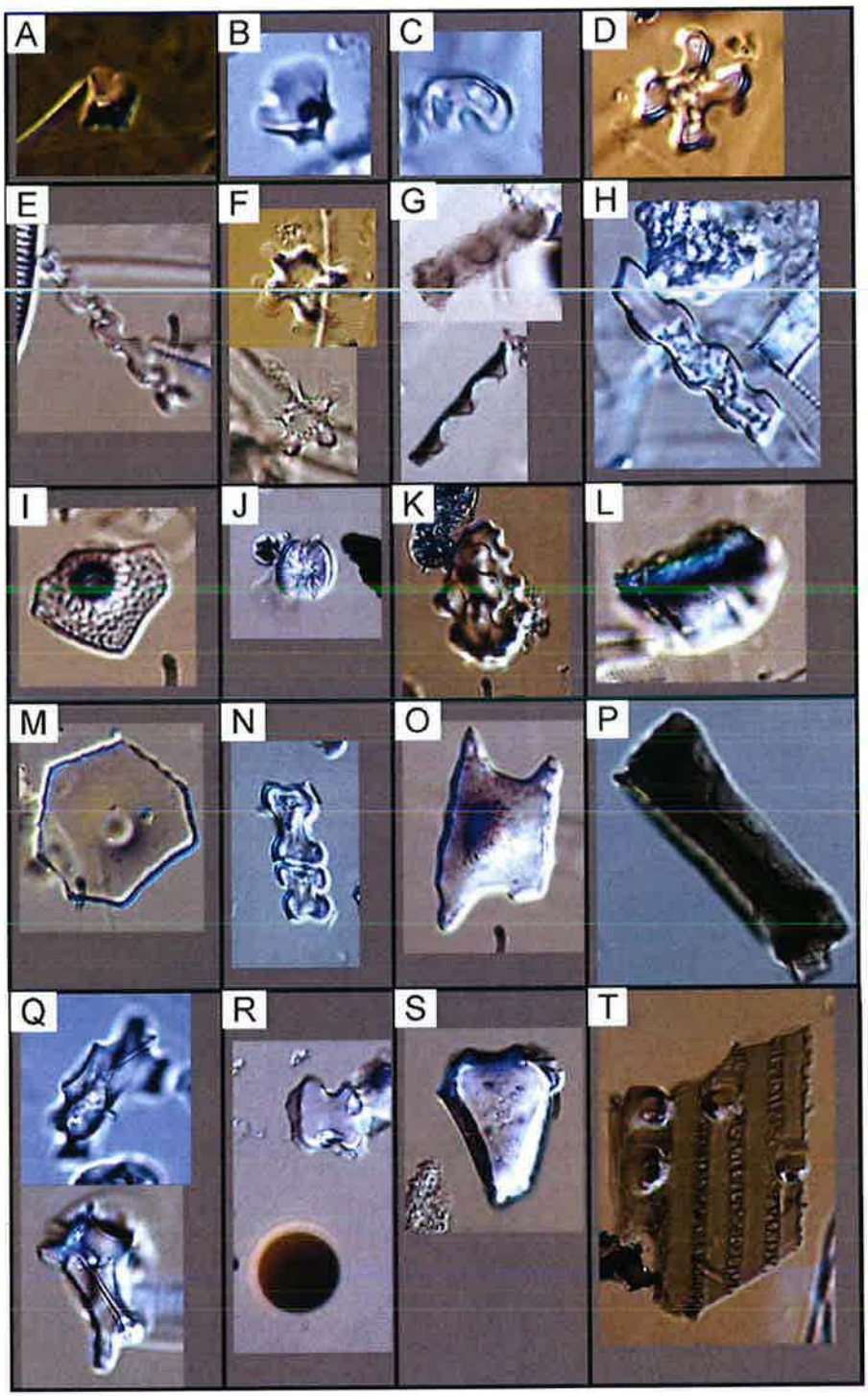


Fig. 4

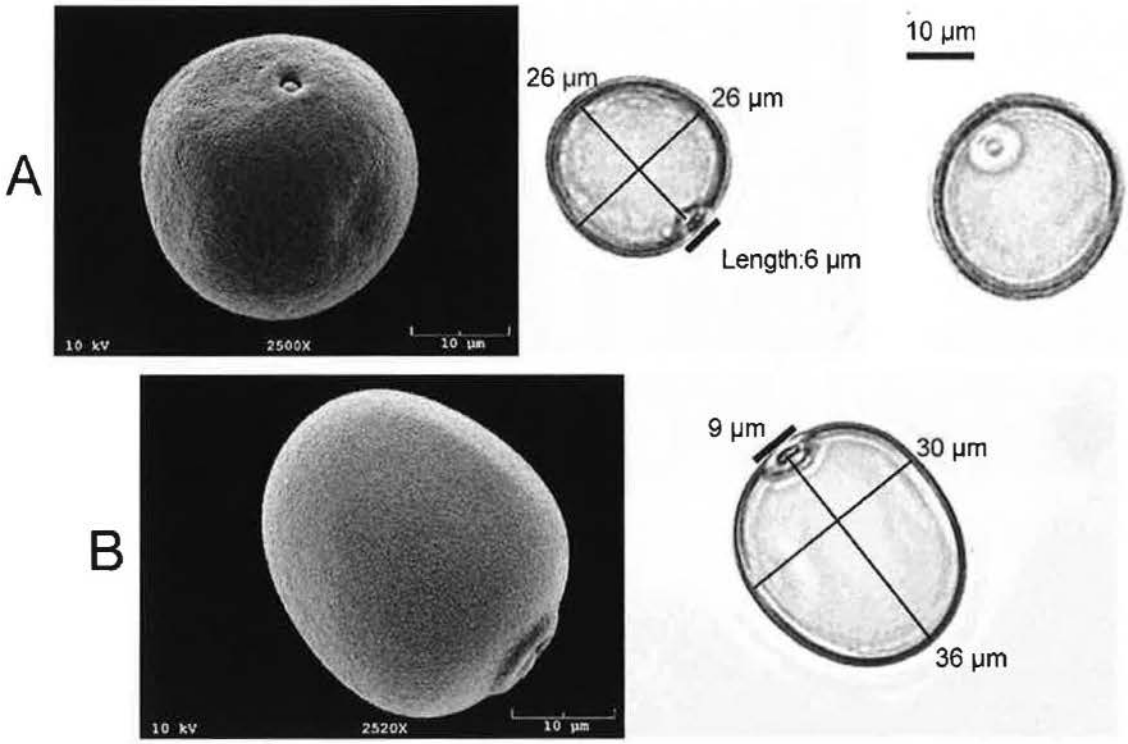

A
10 kV
2500X
10 µm
26 µm
26 µm
Length:6 µm
10 µm
B
10 kV
2520X
10 µm
9 µm
30 µm
36 µm

Fig. 5

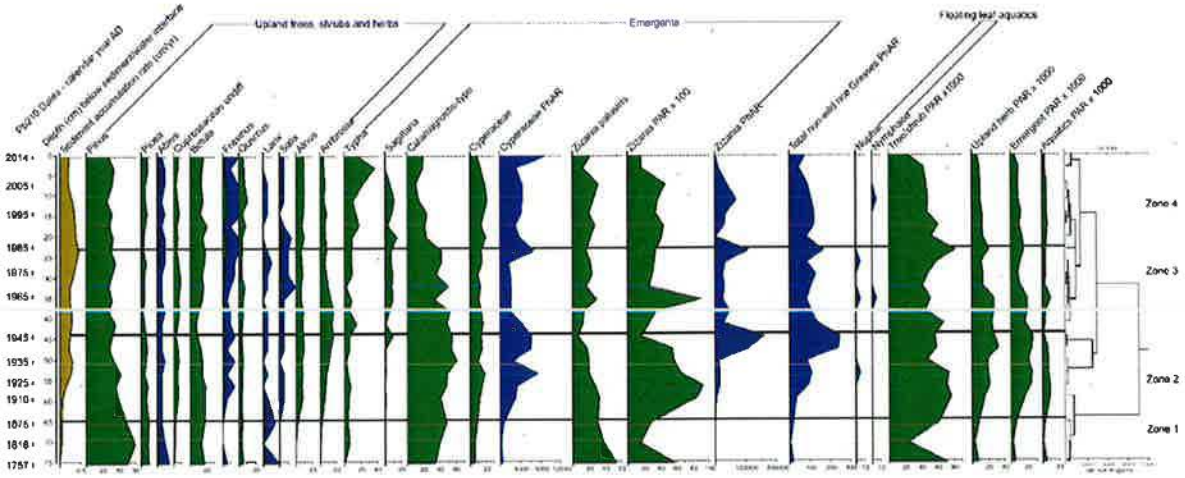

Upland trees, shrubs and herbs
Emergents
Floating leaf aquatics
Zone 4
Zone 3
Zone 2
Zone 1

Fig. 6

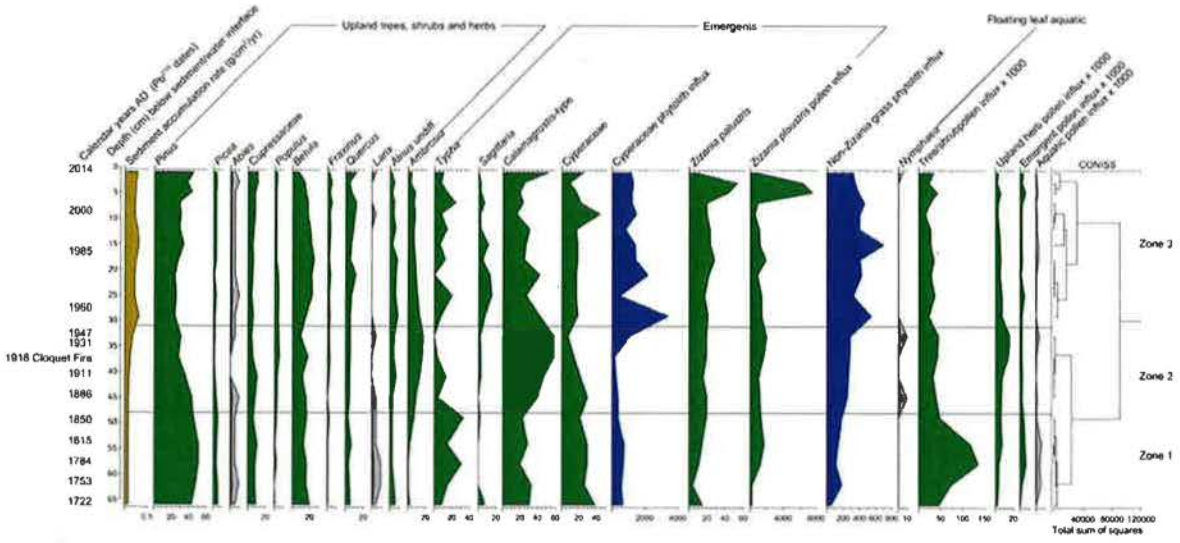

Upland trees, shrubs and herbs
Emergents
Floating leaf aquatic
Calendar years AD (Pb^210 dates)
Depth (cm) below sediment/water interface
Sediment accumulation rate (g/cm²/yr)
Pinus
Picea
Abies
Cupressaceae
Populus
Betula
Fraxinus
Quercus
Larix
Alnus sect/ss
Artemisia
Typha
Sagittaria
Calamagrostis-type
Cyperaceae
Cyperaceae phytolith influx
Zizania palustris
Zizania plaustris pollen influx
non-Zizania grass phytolith influx
Nymphaea
Tree/shrub/pteridium influx x 1000
Upland herb pollen influx x 1000
Emergent pollen influx x 1000
Aquatic pollen influx x 1000
CONISS
2014
2000
1985
1960
1947
1931
1918 Cloquet Fire
1911
1886
1850
1815
1784
1753
1722
Zone 3
Zone 2
Zone 1
40000 80000 120000
Total sum of squares

Fig. 7

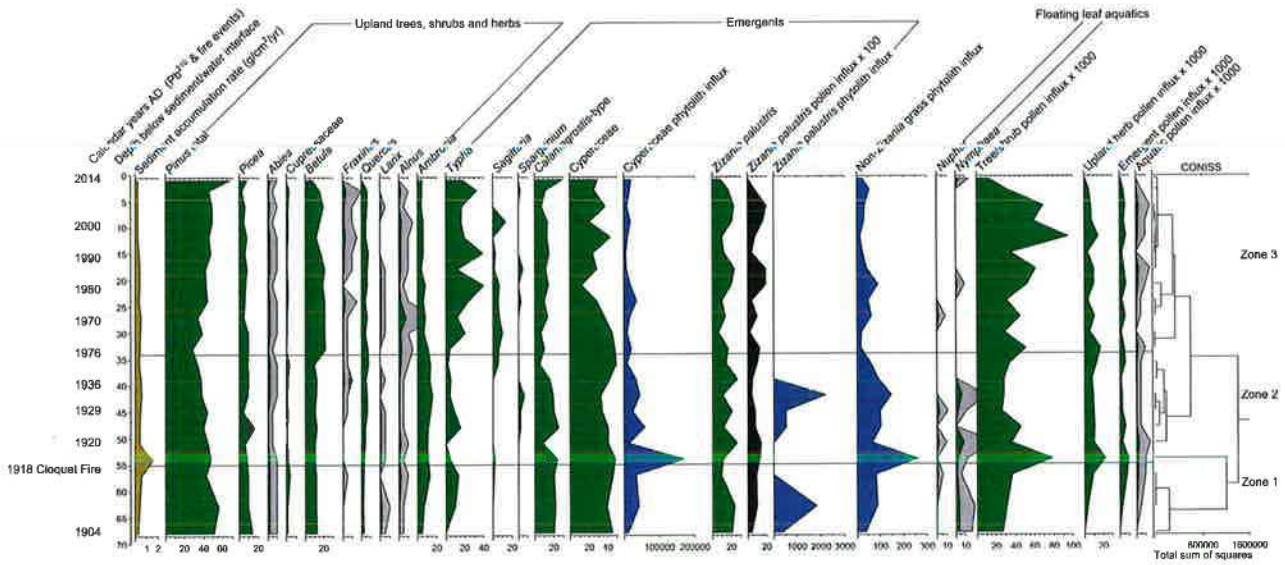

Upland trees, shrubs and herbs
Emergents
Floating leaf aquatics
Cal. calendar years AD (Pb-210 & fire events)
Depth below sediment/water interface
Sediment accumulation rate (g/cm2/yr)
Pinus
Picea
Abies
Cupressaceae
Betula
Fraxinus
Quercus
Larix
Alnus
Ambrosia
Typha
Sagittaria
Sparganium
Calamagrostis-type
Cyperaceae
Cyperaceae phytolith influx
Zizania palustris
Zizania palustris pollen influx x 100
Zizania palustris phytolith influx
Non-Zizania grass phytolith influx
Nuphar
Hydrocharis
Trees & shrub pollen influx x 1000
Upland herb pollen influx x 1000
Emergent pollen influx x 1000
Aquatic pollen influx x 1000
CONISS
2014
2000
1990
1980
1970
1976
1936
1929
1920
1918 Cloquet Fire
1904
Zone 3
Zone 2
Zone 1
Total sum of squares

Fig. 8.

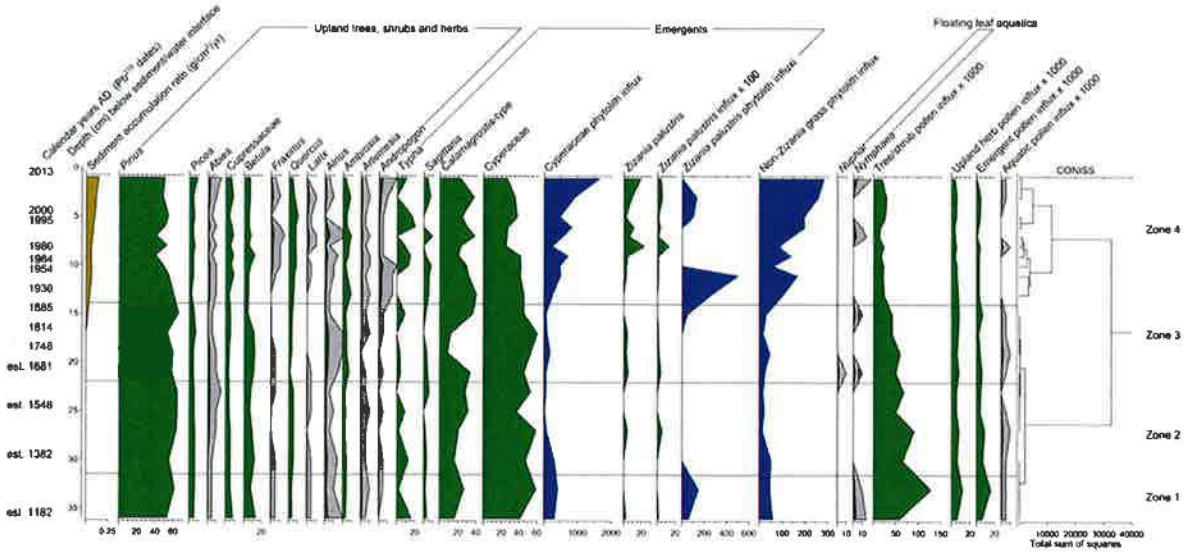

Upland trees, shrubs and herbs
Emergents
Floating leaf aquatics
Pinus
Picea
Abies
Cupressaceae
Betula
Fraxinus
Quercus
Ulmus
Alnus
Ambrosia
Artemisia
Andropogon
Typha
Sagittaria
Calamagrostis-type
Cyperaceae
Cyperaceae phytolith influx
Zizania palustris
Zizania palustris influx x 100
Zizania palustris phytolith influx
Non-Zizania grass phytolith influx
Nuphar
Nymphaea
Tree/shrub pollen influx x 1000
Upland herb pollen influx x 1000
Emergent pollen influx x 1000
Aquatic pollen influx x 1000
CONISS
2013
2000
1995
1980
1964
1954
1930
1885
1814
1748
est. 1681
est. 1548
est. 1382
est. 1182
Zone 4
Zone 3
Zone 2
Zone 1
Total sum of squares

Fig. 9

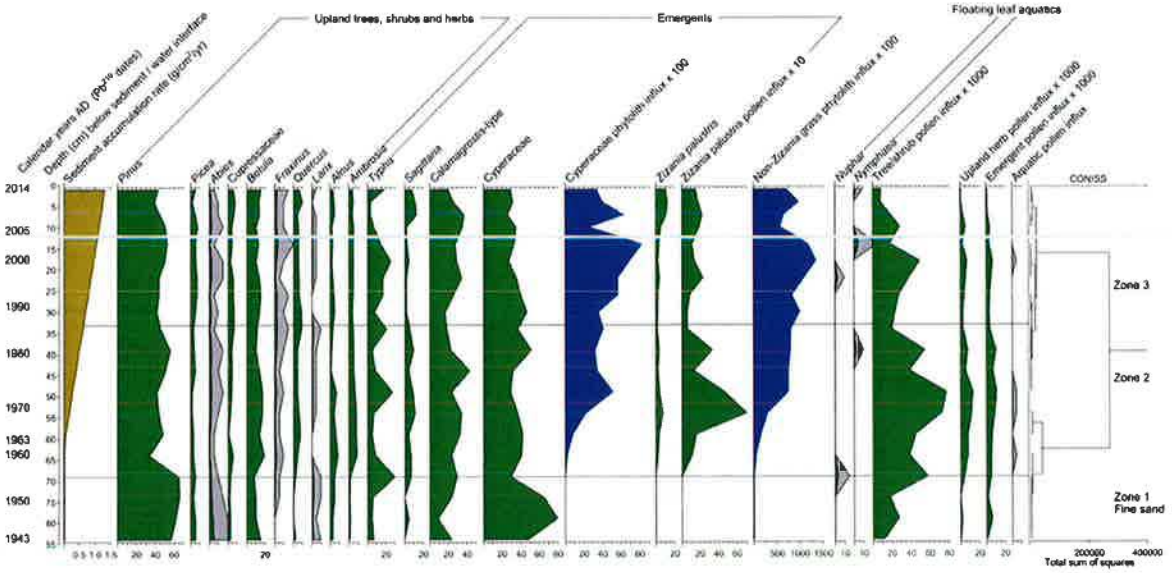
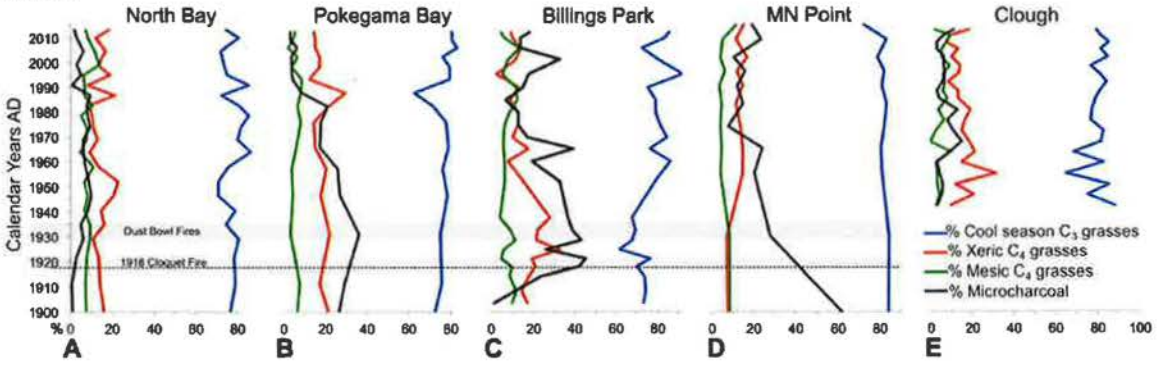

Upland trees, shrubs and herbs
Emergents
Floating leaf aquatics
Calendar years AD (Pb²¹⁰ dates)
Depth (cm) below sediment / water interface
Sediment accumulation rate (g/cm²/yr)
Pinus
Picea
Abies
Cupressaceae
Betula
Fraxinus
Quercus
Larix
Alnus
Ambrosia
Typha
Sagittaria
Calamagrostis-type
Cyperaceae
Cyperaceae phytolith influx x 100
Zizania palustris
Zizania palustris pollen influx x 10
Non-Zizania grass phytolith influx x 100
Nuphar
Nymphaea
Tree/shrub pollen influx x 1000
Upland herb pollen influx x 1000
Emergent pollen influx x 1000
Aquatic pollen influx
CONISS
2014
2005
2000
1990
1980
1970
1963
1960
1950
1943
Zone 3
Zone 2
Zone 1
Fine sand
Total sum of squares

Fig. 10


North Bay
Pokegama Bay
Billings Park
MN Point
Clough
Calendar Years AD
2010
2000
1990
1980
1970
1960
1950
1940
1930
1920
1910
1900
Dust Bowl Fires
1918 Cloquet Fire
% 0 20 40 60 80
0 20 40 60 80
0 20 40 60 80
0 20 40 60 80
0 20 40 60 80 100
A
B
C
D
E
% Cool season C3 grasses
% Xeric C4 grasses
% Mesic C4 grasses
% Microcharcoal

Supplementary Table 1. Wild rice (*Zizania palustris*) phytolith morphotype counts for Billings Park, North Bay and Minnesota Point cores

CORE	Depth (cm)	Inflor Type 1	cf. Inflor Type 1	Inflor Type 2	cf. Inflor Type 2	Leaf Type 1	Leaf Type 2	Leaf Type 3	Sheath Type 1	Sheath Type 2	Sheath Type 3	Sheath Type 4	Sheath Type 5	Culm Type 1	Culm Type 2	Culm Type 6	Culm Type 7	FL Type 2	FL Type 3	FL Type 4	FL Type 5	FL Type 6	TOTAL *Zizania*	TOTAL Inflor.	TOTAL non-Inflor.	Percent Inflor.
BP	.5																									
BP	2.5																									
BP	5.5																									
BP	8.5																									
BP	11.5																									
BP	14.5																									
BP	17.5																									
BP	2 .5																									
BP	23.5																									
BP	26.5																									
BP	29.5																									
BP	32.5																									
BP	35.5																									
BP	38.5																									
BP	41.5		1							1										1			3	1	2	33
BP	44.5									1													1		1	0

CORE	Depth (cm)	Inflor Type 1	cf. Inflor Type 1	Inflor Type 2	cf. Inflor Type 2	Leaf Type 1	Leaf Type 2	Leaf Type 3	Sheath Type 1	Sheath Type 2	Sheath Type 3	Sheath Type 4	Sheath Type 5	Culm Type 1	Culm Type 2	Culm Type 6	Culm Type 7	FL Type 2	FL Type 3	FL Type 4	FL Type 5	FL Type 6	TOTAL *Zizania*	TOTAL Inflor.	TOTAL non-Inflor.	Percent Inflor.
BP	47.5									1													1		1	0
BP	5 .5																									
BP	53.5																									
BP	56.5																									
BP	62.5						1	2							1								4		4	0
BP	67.5							1							1								2		2	0
NB	.5																1						1		1	0
NB	3.5		1					1						3									5	1	4	2
NB	7.5						1			2				1		5							9		9	0
NB	11.5		1	3			1	1		1						4		1			1		13	4	9	31
NB	14.5			1			2	1					1									1	6	1	5	17
NB	17.5		1				1			1			1		1								5	1	4	2
NB	2 .5																					1	1		1	0
NB	23.5			2	1		1						1		5	3							13	3	1	23
NB	26.5		2										1		2	1		1				1	8	2	6	25
NB	29.5			1											2							1	4	1	3	25
NB	32.5		1					1					2		1							1	6	1	5	17
NB	35.5											3			2							1	6		6	0

CORE	Depth (cm)	Inflor Type 1	cf. Inflor Type 1	Inflor Type 2	cf. Inflor Type 2	Leaf Type 1	Leaf Type 2	Leaf Type 3	Sheath Type 1	Sheath Type 2	Sheath Type 3	Sheath Type 4	Sheath Type 5	Culm Type 1	Culm Type 2	Culm Type 6	Culm Type 7	FL Type 2	FL Type 3	FL Type 4	FL Type 5	FL Type 6	TOTAL *Zizania*	TOTAL Inflor.	TOTAL non-Inflor.	Percent Inflor.
NB	38.5			2								1	1		2						3		9	2	7	22
NB	41.5			1										1	1						1		4	1	3	25
NB	44.5			2			2			1			2	1	3	1					1		13	2	11	15
NB	47.5		1	2						1			1		1							3	9	3	6	33
NB	5 .5													1	2								3		3	0
NB	53.5														2							1	3		3	0
NB	56.5														1							2	3		3	0
NB	59.5						1	1											1			1	4		4	0
NB	65.5														2								2		2	0
NB	7 .5																									
NB	75.5																									
MNP	.5																									
MNP	2.5									1													1		1	0
MNP	4.5					1																	1		1	0
MNP	5.5																									
MNP	6.5																									
MNP	7.5																									
MNP	8.5																									

CORE	Depth (cm)	Inflor Type 1	cf. Inflor Type 1	Inflor Type 2	cf. Inflor Type 2	Leaf Type 1	Leaf Type 2	Leaf Type 3	Sheath Type 1	Sheath Type 2	Sheath Type 3	Sheath Type 4	Sheath Type 5	Culm Type 1	Culm Type 2	Culm Type 6	Culm Type 7	FL Type 2	FL Type 3	FL Type 4	FL Type 5	FL Type 6	TOTAL *Zizania*	TOTAL Inflor.	TOTAL non-Inflor.	Percent Inflor.
MNP	9.5																									
MNP	10.5						1			1					1			1				2	6		6	0
MNP	12.5						1			2						1				1			5		5	0
MNP	14.5									1						1	1						3		3	0
MNP	16.5																									
MNP	18.5																									
MNP	2 .5																									
MNP	22.5																									
MNP	24.5																									
MNP	26.5																									
MNP	29.5																									
MNP	32.5														1	4						1	6		6	0
MNP	35.5															1			1				2		2	0

ATTACHMENT C

Attachment C. Geochemistry of the St. Louis River Area of Concern

Please refer to Attachment A for background and methods associated with sediment coring in the SLRE.

Metals methods

Metals and oxides. Analysis for trace metals was performed to provide stratigraphic surrogates for natural deposition due to erosion of soils and bedrock and human activities such as mining, tailings disposal and burning of fossil fuels. For each sample, sediment subsamples were freeze-dried and 0.25 ± 0.02 g of dry sediment were added to a 50-mL centrifuge tube. To this, 25 mL 0.5 N HCl was added and samples were heated at 80 - 85 °C in a hot-water bath for 30 minutes. Vials were transferred to an ice-water bath and allowed to cool for 5 minutes. Samples were centrifuged at 2000 rpm for 10 minutes, and then 10.0 mL of the supernatant was moved to 125-mL acid-washed poly-bottles. Each sample was diluted with 40 ± 0.5 g deionized water. Samples were assessed using inductively coupled plasma mass spectrometry (ICP-MS), which is capable of the determination of a range of metals and several non-metals (B'Hymer et al. 2000, Jarvis et al. 1992). These analyses were performed by personnel at the University of Minnesota, Department of Earth Sciences, Analytical Geochemistry Laboratory.

Due to the need for very low detection limits, mercury analysis was processed separately. This analysis followed EPA protocols (USEPA 2002; Appendix D1). Briefly, a subsample was preserved with 12 N hydrochloric acid (HCl) solution, and a 100-mL sample aliquot is oxidized to Hg(II) with bromine monochloride (BrCl). After oxidation, the sample is sequentially reduced with $NH_2OH \cdot HCl$ to destroy free halogens, then reduced with stannous chloride ($SnCl_2$) to convert Hg(II) to volatile Hg(0). The Hg is separated from solution by purging with nitrogen and the Hg is collected onto a gold trap. The Hg is thermally desorbed from the gold trap into an inert gas stream that carries the released Hg to a second gold (analytical) trap. The Hg is desorbed from the analytical trap into a gas stream that carries the Hg into the cell of a cold-vapor atomic fluorescence spectrometer (CVAFS) for detection.

Organic contaminants. We analyzed for a suite of 137 organic contaminants, polychlorinated biphenyls (PCBs, method 8082A, 9 analytes), volatile organic compounds (VOCs, method 8260B, 61 analytes) and semivolatile organic compounds (sVOCs, method 8270D, 67 analytes) in the single sediment core (Minnesota Point, in the harbor). We selected this core because we felt it would be the most representative of the harbor and estuary (upstream) simultaneously. Selecting a single core was necessary due to costs associated with these analyses, so that we could focus on details of that representative core. There were no detections of organic contaminants in any sediment intervals from that core, so we do not dwell on the details of those analyses or results, and instead refer the reader to the detailed list of analytes and methods which are provided in Appendix D1.

Multivariate analysis of geochemistry. We condensed the complex geochemical data from all cores simultaneously using nonmetric multidimensional scaling (NMDS) in order to track changes that may not be as easily visible via bulk trends, and to identify groups of variables that followed similar historical trajectories. NMDS compared sample assemblages based on distance metrics (Rabinowitz, 1975). NMDS is based on rank orders of variable data and so does not require meeting assumptions of data distributions. NMDS was performed using the R package

"vegan" (R Development Core Team, 2010; Oksanen et al., 2011). NMDS ordinated the geochemistry data using Bray-Curtis dissimilarity. Because of the contribution of Na to the uppermost intervals of each core by the Zorbitrol gel, Na values for those intervals were substituted with values from the adjacent interval.

Metals results

Based on stratigraphic plots (Fig. 1A-E), a subset of elements (Cd, Hg, Zn, Pb, Sn, Sb) had a clear mid-20th century peak in accumulation, followed by a decline to pre-impact levels by ~1990. The rise in these elements occurred earlier (late 1800s) in the MN Point core, but the timing of declines in that core conformed to that in other cores. Other elements increased in the upper portions of some or all cores, including Fe, Mn and P. Peaks in these elements occurred just below the sediment surface in the Lake Superior core, representing the known horizon formed by the migration of mobile Fe and Mn, and P adsorbed onto hydrated ferric oxides, resulting from redox activity (Li et al. 2012, Shaw Chraïbi et al. 2014). The surface peaks of Na (Fig. 1B) are from the Zorbitrol gel, but an increase in Lake Superior since the early 20th century may indicate increasing inputs from industrial activities and/or road salt applications.

As noted in other reports (Attachments A,B) the sediment core from North of Clough Island had a clear disturbance due to deposition from a 2012 flood event. This resulted in uncertainty in timing of geochemical stratigraphy from the late 1960s through ~2012. For readers examining stratigraphic details of that core, note that temporal and quantitative data from approximately 1965 through 2012 are unreliable. Uppermost and pre-1965 data are reliable.

For 60 sedimentary compounds and elements (Fig. 2; profiles of 59 shown in Figs. 1A-E), NMDS distinguished four groups:

(1) In the left-hand portion of the ordination a group of metals, including P, tended to be higher in concentration in the North Bay, Billings Park, Allouez Bay and Superior cores. Upper and lower portions of this cluster can be subdivided to distinguish North Bay and Billings Park from Allouez Bay and Superior, the latter pair of cores occupying the lower left quadrant by having higher Fe and Mn concentrations, well-known urban and industrial contaminants from iron and steel production.

(2) A tight cluster slightly to the right of the origin representing all of the analyzed lanthanides and actinides, and a few other metals (Sc, Y, Cs, Rb, Ga). These elements tended to be higher in North of Clough, MN Point and Pokegama Bay cores.

(3) Around the origin lies a cluster of oxides that extends to the positive end of the second axis, indicating higher concentrations of CaO and SrO in the North of Clough, MN Point, Billings Park and North Bay cores.

(4) Near the upper portion of Group 3 is the subset of elements (Zn [Fig. 1A], Pb [Fig. 1C], Sn [Fig. 1C], Sb [Fig. 1C]) marking the mid-century peak. Because Hg was not included in NMDS analysis due to lack of data in two cores, it is likely Hg also falls within this group due to very similar stratigraphic profiles.

Some metals were not so easily placed in comprehensive groups based on NMDS. Cu and Co occur together just below and to the left of the origin due to sporadic high concentrations in the Lake Superior core. Ta had periodic high concentrations in the Pokegama Bay core, and so

stands alone in the lower, right quadrant. The alkaline earth metals Ba and Sr are higher in the MN Point and Allouez Bay cores, and so occur in the upper, right quadrant.

Core trajectories in the NMDS ordination generally reveal a right-to-left migration to higher concentrations of elements associated with iron mining (Group 1) and likely accumulation of erosional materials from several activities associated with Euro-American development of the region. The MN Point and Pokegama Bay cores reveal the smallest geochemical change overall (i.e., the shortest distance between the oldest and most recent intervals). Billings Park had a strong migration in the direction of the Group 1 elements, and high mid-century concentrations of Ca and Mg (Fig. 1B) occurred as a temporary upward movement in the NMDS trajectory.

Metals discussion

Although the geochemical results contain a great deal of information, some general historical conditions are clear. (1) Unique soil and bedrock characteristics account for some of the NMDS grouping, such as the higher levels of lanthanides and actinides in Pokegama Bay and downstream cores (North of Clough Island, MN Point). These rare earth metals are relatively immobile (Smith and Huyck 1999) and so are probably naturally-occurring, detrital materials from Wisconsin catchments. They do not show historical changes related to known human activities. (2) Although varying among cores, some of the metals show clear historical increases associated with mining and other catchment developments. The mid-20th century peaks in Cd, Hg, Zn, Pb, Sn and Sb (Ca and Mg at Billings Park) are a likely result of catchment disruptions that exposed materials to erosion and runoff. Mercury is of special interest as it is an unmistakable marker of human activities such as mining, burning of fossil fuels and sewage disposal (Birks & Birks 1980). The recent decline in this heavy metal (among others) is indicative of recovery, likely resulting from better sewage treatment practices and reduced denudation of land surfaces.

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Figure captions

Fig. 1. Temporal, stratigraphic presentation of the geochemical data (ppm) from SLRE sediment cores. Each core location is indicated by a specific color.

Fig. 2. Two-dimensional Nonmetric multidimensional scaling (NMDS) analysis of the geochemical data from the SLRE sediment cores. Each core location is indicated by a specific color. Coordinates of the oldest and most recent samples analyzed in each core are indicated by dates.

Figure 1A

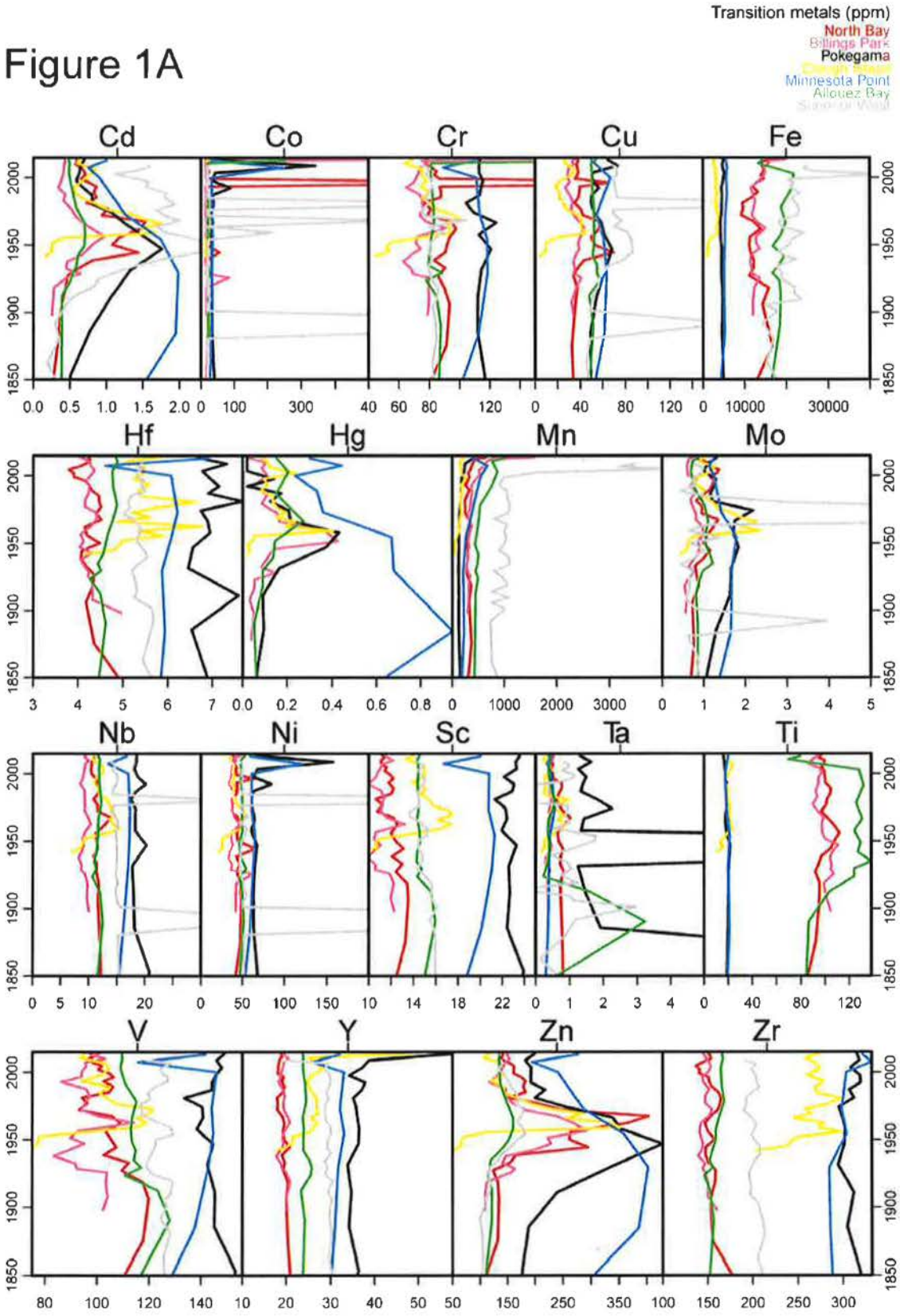

Transition metals (ppm)
North Bay
Billings Park
Pokegama
Minnesota Point
Alloquez Bay
Cd
Co
Cr
Cu
Fe
Hf
Hg
Mn
Mo
Nb
Ni
Sc
Ta
Ti
V
Y
Zn
Zr
2000
1950
1900
1850

Figure 1B

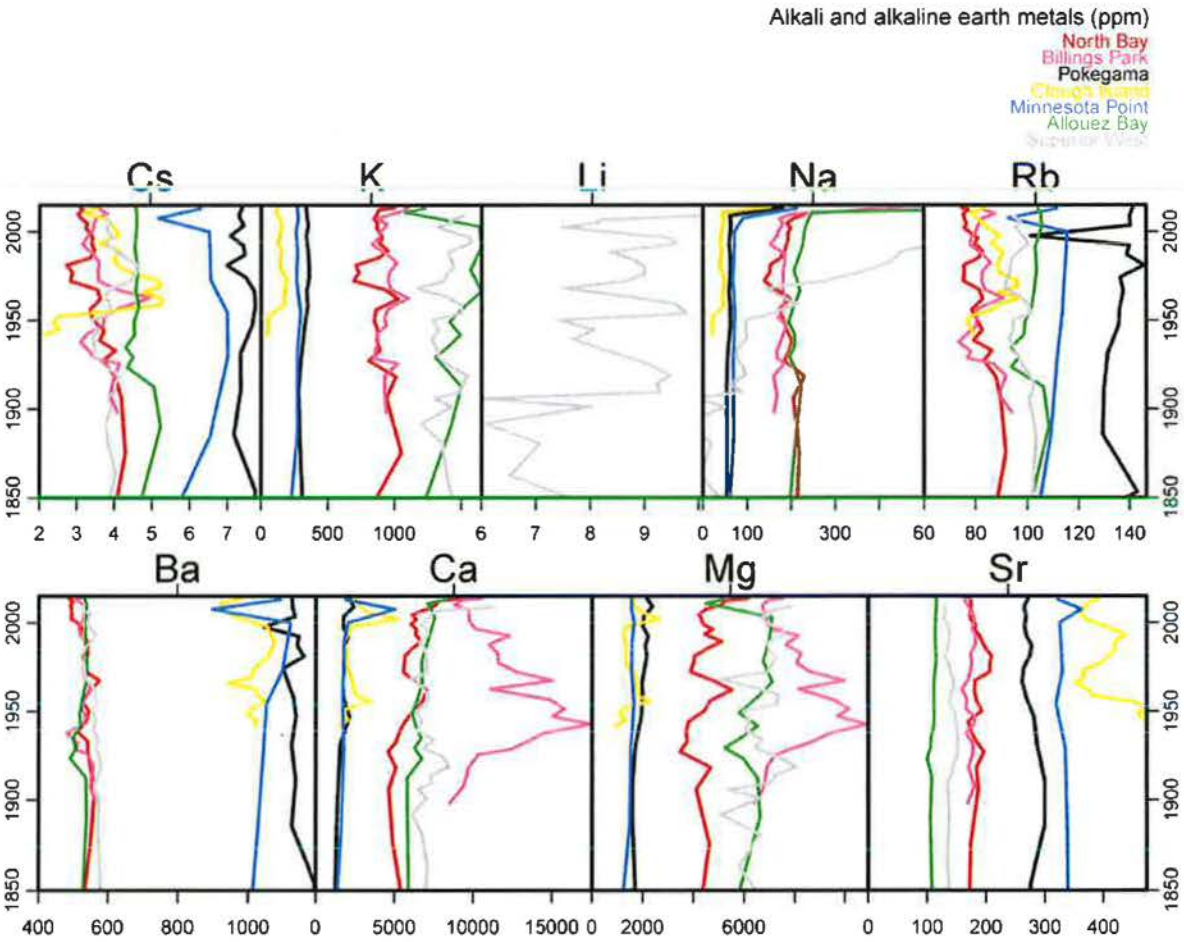

Alkali and alkaline earth metals (ppm)
North Bay
Billings Park
Pokegama
Minnesota Point
Allouez Bay
Cs
K
Li
Na
Rb
Ba
Ca
Mg
Sr
2000
1950
1900
1850

Figure 1C

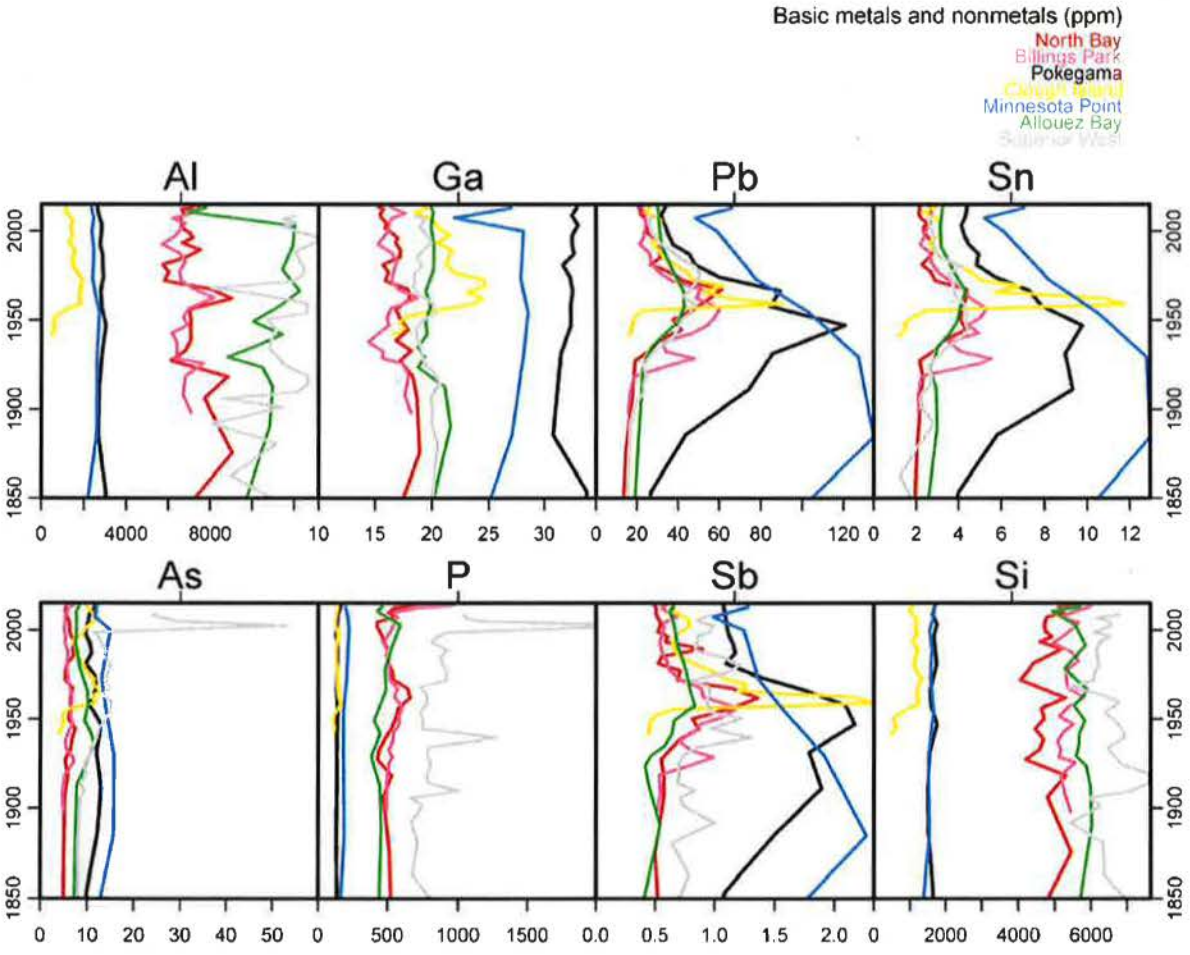

Basic metals and nonmetals (ppm)
North Bay
Billings Park
Pokegama
Minnesota Point
Allouez Bay
Al
Ga
Pb
Sn
As
P
Sb
Si
2000
1950
1900
1850
0
4000
8000
10
15
20
25
30
0
20
40
60
80
120
0
2
4
6
8
10
12
0
10
20
30
40
50
0
500
1000
1500
0.0
0.5
1.0
1.5
2.0
0
2000
4000
6000

Figure 1D

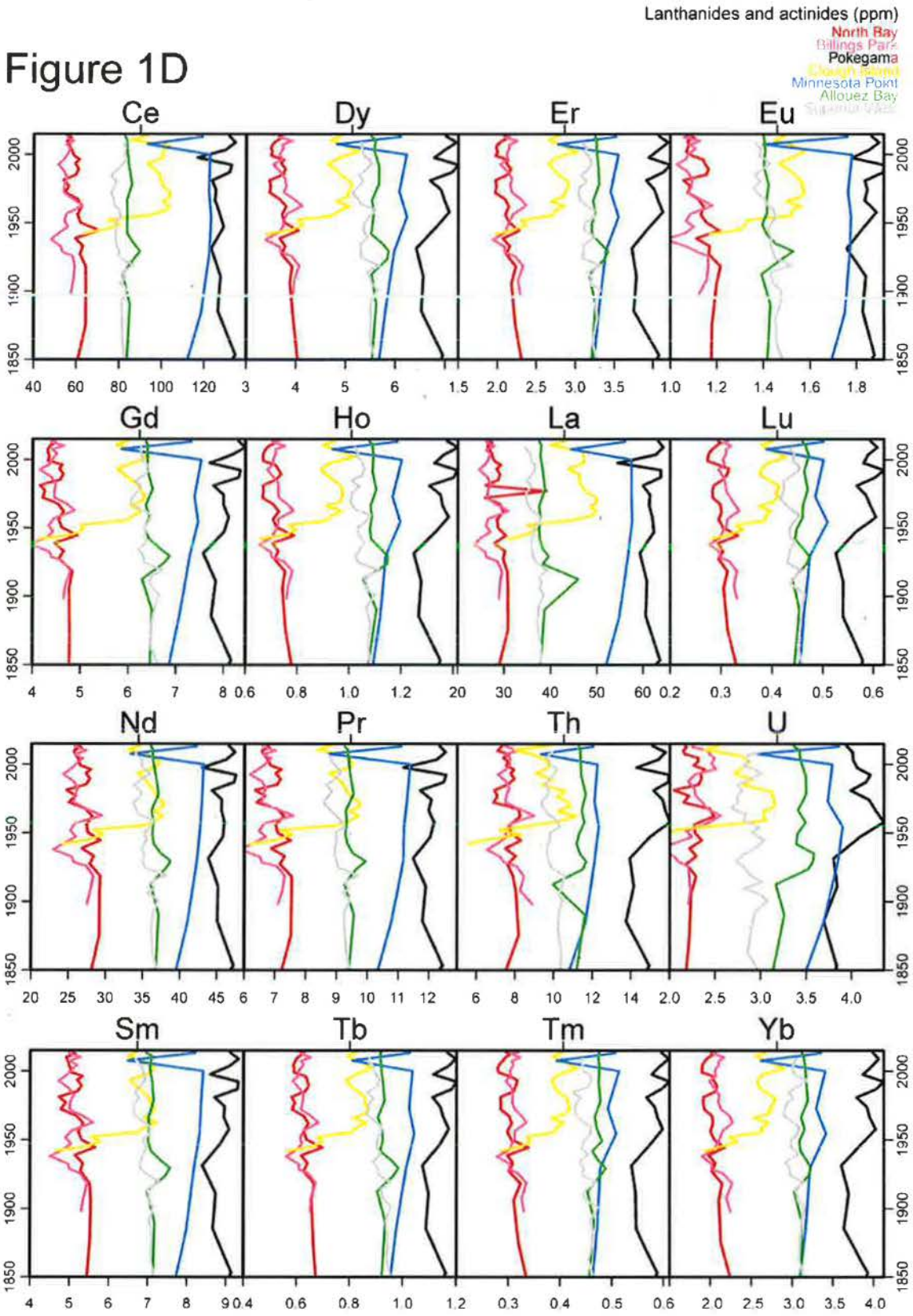

Lanthanides and actinides (ppm)
North Bay
Billings Parc
Pokegama
Clough Island
Minnesota Point
Allouez Bay
Ce
Dy
Er
Eu
Gd
Ho
La
Lu
Nd
Pr
Th
U
Sm
Tb
Tm
Yb
2000
1950
1900
1850

Figure 1E

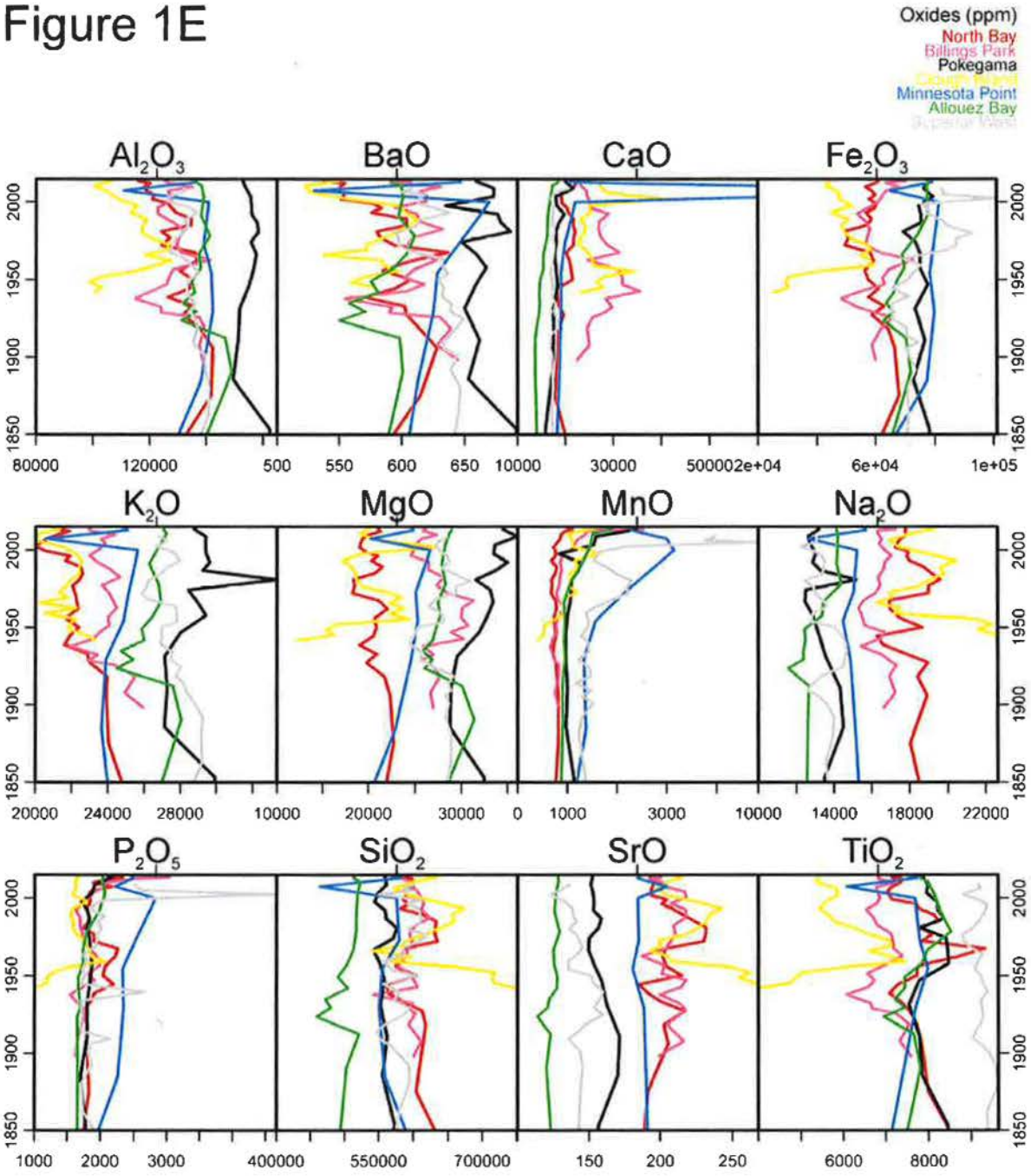
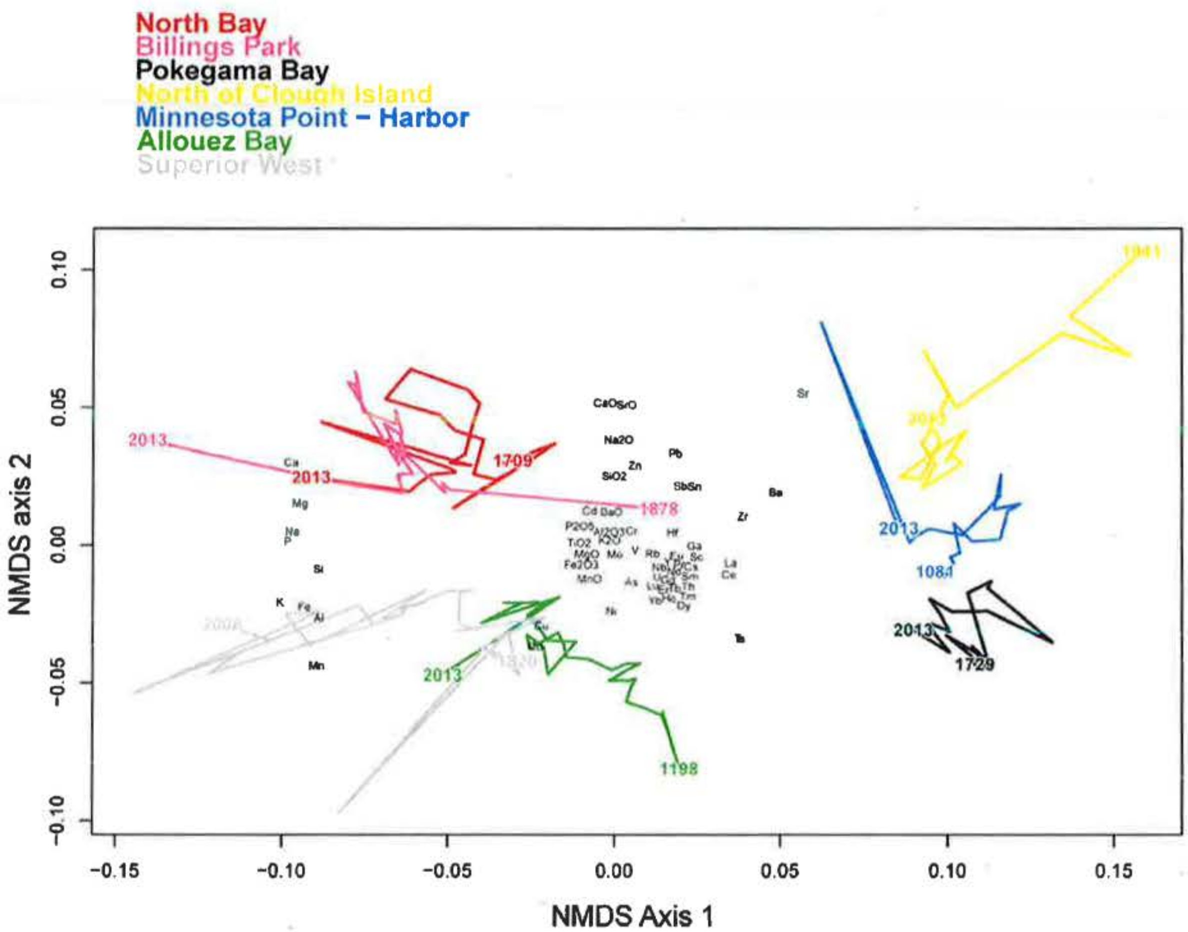

Oxides (ppm)
North Bay
Billings Park
Pokegama
Grand Island
Minnesota Point
Allouez Bay
Al2O3
BaO
CaO
Fe2O3
K2O
MgO
MnO
Na2O
P2O5
SiO2
SrO
TiO2
2000
1950
1900
1850
80000
120000
500
550
600
650
10000
30000
500002e+04
6e+04
1e+05
20000
24000
28000
10000
20000
30000
0
1000
3000
10000
14000
18000
22000
1000
2000
3000
400000
550000
700000
150
200
250
6000
8000

Figure 2


North Bay
Billings Park
Pokegama Bay
North of Clough Island
Minnesota Point – Harbor
Allouez Bay
Superior West
NMDS axis 2
NMDS Axis 1

METHOD 8082A

POLYCHLORINATED BIPHENYLS (PCBs) BY GAS CHROMATOGRAPHY

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be followed by individuals formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required method use for the analysis of method-defined parameters, are intended to be methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. The performance data included in this method are for guidance purposes only, and are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

1.0 SCOPE AND APPLICATION

1.1 This method may be used to determine the concentrations of polychlorinated biphenyls (PCBs) as Aroclors or as individual PCB congeners in extracts from solid, tissue, and aqueous matrices, using open-tubular, capillary columns with electron capture detectors (ECD) or electrolytic conductivity detectors (ELCD). The Aroclors and PCB congeners listed below have been determined by this method, using either a single- or dual column analysis system, and this method may be appropriate for additional congeners and Aroclors (see Sec. 1.4). The method also may be applied to other matrices such as oils and wipe samples, if appropriate sample extraction procedures are employed.

Compound	CAS Registry No.[a]	IUPAC #
Aroclor 1016	12674-11-2	-
Aroclor 1221	11104-28-2	-
Aroclor 1232	11141-16-5	-
Aroclor 1242	53469-21-9	-
Aroclor 1248	12672-29-6	-
Aroclor 1254	11097-69-1	-
Aroclor 1260	11096-82-5	-
2-Chlorobiphenyl	2051-60-7	1
2,3-Dichlorobiphenyl	16605-91-7	5
2,2',5-Trichlorobiphenyl	37680-65-2	18
2,4',5-Trichlorobiphenyl	16606-02-3	31
2,2',3,5'-Tetrachlorobiphenyl	41464-39-5	44
2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	52
2,3',4,4'-Tetrachlorobiphenyl	32598-10-0	66
2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8	87
2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	101
2,3,3',4',6-Pentachlorobiphenyl	38380-03-9	110
2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	138
2,2',3,4,5,5'-Hexachlorobiphenyl	52712-04-6	141

Compound	CAS Registry No.[a]	IUPAC #
2,2',3,5,5',6-Hexachlorobiphenyl	52663-63-5	151
2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	153
2,2',3,3',4,4',5-Heptachlorobiphenyl	35065-30-6	170
2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3	180
2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1	183
2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0	187
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	40186-72-9	206

[a]Chemical Abstract Service Registry No.

1.2 Aroclors are multi-component mixtures. When samples contain more than one Aroclor, a higher level of analyst expertise is required to attain acceptable levels of qualitative and quantitative analysis. The same is true of Aroclors that have been subjected to environmental degradation ("weathering") or degradation by treatment technologies. Such weathered multi-component mixtures may have significant differences in peak patterns compared to those of Aroclor standards.

1.3 The seven Aroclors listed in Sec. 1.1 are those that are commonly specified in EPA regulations. The quantitation of PCBs as Aroclors is appropriate for many regulatory compliance determinations, but is particularly difficult when the Aroclors have been weathered by long exposure in the environment. Therefore, this method provides procedures for the determination of a selected group of the 209 possible PCB congeners, as another means to measure the concentrations of weathered Aroclors. The 19 PCB congeners listed above have been tested by this method and were chosen for testing because many of them represent congeners specific to the common Aroclor formulations (see Table 6). These 19 PCB congeners do not represent the co-planar PCBs or the other PCBs of greatest toxicological significance. **The analytical procedures for these 19 congeners may be appropriate for the analysis of other congeners not specifically included in this method and may be used as a template for the development of such a procedure.** However, all 209 PCB congeners cannot be separated using the GC columns and procedures described in this method. If this procedure is expanded to encompass other congeners, then the analyst must either document the resolution of the congeners in question, or establish procedures for reporting the results of coeluting congeners that are appropriate for the intended application.

1.4 The PCB congener approach potentially affords greater quantitative accuracy when PCBs are known to be present. As a result, this method may be used to determine Aroclors, some PCB congeners, or "total PCBs," depending on regulatory requirements and project needs. The congener method is of particular value in determining weathered Aroclors. However, analysts should use caution when using the congener method when regulatory requirements are based on Aroclor concentrations. Also, this method is not appropriate as currently written for the determination of the co-planar PCB congeners at the very low (sub part per trillion) concentrations sometimes needed for risk assessment purposes.

1.5 Compound identification based on single-column analysis should be confirmed on a second column, or should be supported by at least one other qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm the measurements made with the primary column. GC/MS (e.g., Method 8270) is also recommended as a confirmation technique, if sensitivity permits (also see Sec. 11.11 of this method). GC/AED may also be used as a confirmation technique, if sensitivity permits (see Method 8085).

1.6 This method includes a dual-column option that describes a hardware configuration in which two GC columns are connected to a single injection port and to two separate detectors. The option allows one injection to be used for dual-column simultaneous analysis.

1.7 The analyst must select columns, detectors and calibration procedures most appropriate for the specific analytes of interest in a study. Matrix-specific performance data must be established and the stability of the analytical system and instrument calibration must be established for each analytical matrix (e.g., hexane solutions from sample extractions, diluted oil samples, etc.). Example chromatograms and GC conditions are provided as guidance.

1.8 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3500, 3600, and 8000) for additional information on quality control procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives for the intended application.

1.9 Use of this method is restricted to use by, or under the supervision of, personnel appropriately experienced and trained in the use of gas chromatographs (GCs) and skilled in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 A measured volume or weight of sample is extracted using the appropriate matrix-specific sample extraction technique.

2.1.1 Aqueous samples may be extracted at neutral pH with methylene chloride using either Method 3510 (separatory funnel), Method 3520 (continuous liquid-liquid extractor), Method 3535 (solid-phase extraction), or other appropriate technique or solvents.

2.1.2 Solid samples may be extracted with hexane-acetone (1:1) or methylene chloride-acetone (1:1) using Method 3540 (Soxhlet), Method 3541 (automated Soxhlet), Method 3545 (pressurized fluid extraction), Method 3546 (microwave extraction), Method 3550 (ultrasonic extraction), Method 3562 (supercritical fluid extraction), or other appropriate technique or solvents.

2.1.3 Tissue samples may be extracted using Method 3562 (supercritical fluid extraction), or other appropriate technique. The extraction techniques for other solid matrices (see Sec. 2.1.2) may be appropriate for tissue samples.

2.2 Extracts for PCB analysis may be subjected to a sequential sulfuric acid/potassium permanganate cleanup (Method 3665) designed specifically for these analytes. This cleanup technique will remove (destroy) many single component organochlorine or organophosphorus pesticides. Therefore, this method is not applicable to the analysis of those compounds. Instead, use Method 8081.

2.3 After cleanup, the extract is analyzed by injecting a measured aliquot into a gas chromatograph equipped with either a narrow- or wide-bore fused-silica capillary column and either an electron capture detector (GC/ECD) or an electrolytic conductivity detector (GC/ELCD).

2.4 The chromatographic data may be used to determine the seven Aroclors in Sec. 1.1, selected individual PCB congeners, or total PCBs (see Secs. 11.8 and 11.9).

3.0 DEFINITIONS

Refer to Chapter One and the manufacturer's instructions for definitions that may be relevant to this procedure.

4.0 INTERFERENCES

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be necessary. Refer to each method to be used for specific guidance on quality control procedures and to Chapter Four for general guidance on the cleaning of glassware. Also refer to Methods 3500, 3600, and 8000 for a discussion of interferences.

4.2 Interferences co-extracted from the samples will vary considerably from matrix to matrix. While general cleanup techniques are referenced or provided as part of this method, unique samples may require additional cleanup approaches to achieve desired degrees of discrimination and quantitation. Sources of interference in this method can be grouped into four broad categories, as follows:

4.2.1 Contaminated solvents, reagents, or sample processing hardware.

4.2.2 Contaminated GC carrier gas, parts, column surfaces, or detector surfaces.

4.2.3 Compounds extracted from the sample matrix to which the detector will respond, such as single-component chlorinated pesticides, including the DDT analogs (DDT, DDE, and DDD).

NOTE: A standard of the DDT analogs should be injected to determine which of the PCB or Aroclor peaks may be subject to interferences on the analytical columns used. There may be substantial DDT interference with the last major Aroclor 1254 peak in some soil and sediment samples.

4.2.4 Coelution of related analytes -- All 209 PCB congeners cannot be separated using the GC columns and procedures described in this method. If this procedure is expanded to encompass other congeners, then the analyst must either

document the resolution of the congeners in question or establish procedures for reporting the results of coeluting congeners that are appropriate for the intended application.

4.3 Interferences by phthalate esters introduced during sample preparation can pose a major problem in PCB determinations. Interferences from phthalate esters can best be minimized by avoiding contact with any plastic materials and checking all solvents and reagents for phthalate contamination.

4.3.1 Common flexible plastics contain varying amounts of phthalate esters which are easily extracted or leached from such materials during laboratory operations.

4.3.2 Exhaustive cleanup of solvents, reagents and glassware may be required to eliminate background phthalate ester contamination.

4.3.3 These materials can be removed prior to analysis using Method 3665 (sulfuric acid/permanganate cleanup).

4.4 Cross-contamination of clean glassware can routinely occur when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Glassware must be scrupulously cleaned.

4.4.1 Clean all glassware as soon as possible after use by rinsing with the last solvent used. This should be followed by detergent washing with hot water, and rinses with tap water and organic-free reagent water. Drain the glassware, and dry it in an oven at 130 °C for several hours, or rinse with methanol and drain. Store dry glassware in a clean environment.

CAUTION: Oven-drying of glassware used for PCB analysis can increase contamination because PCBs are readily volatilized in the oven and spread to other glassware. Therefore, exercise caution, and do not dry glassware from samples containing high concentrations of PCBs with glassware that may be used for trace analyses.

4.4.2 Other appropriate glassware cleaning procedures may be employed, such as using a muffle furnace at 430 °C for at least 30 min. However, analysts are advised not to place volumetric glassware in a muffle furnace, since the heat will burn off the markings on the glassware and may warp the glassware, changing its volume.

4.5 Sulfur (S_8) is readily extracted from soil samples and may cause chromatographic interferences in the determination of PCBs. Sulfur contamination should be expected with sediment samples. Sulfur can be removed through the use of Method 3660.

5.0 SAFETY

This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals listed in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this manual is for illustrative purposes only, and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those products and settings used during method development or subsequently evaluated by the Agency. Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented.

This section does not list common laboratory glassware (e.g., beakers and flasks).

6.1 Gas chromatograph -- An analytical system complete with gas chromatograph suitable for on-column and split-splitless injection and all necessary accessories including syringes, analytical columns, gases, electron capture detectors (ECD), and recorder/integrator or data system. Electrolytic conductivity detectors (ELCDs) may also be employed if appropriate for project needs. If the dual-column option is employed, the gas chromatograph must be equipped with two separate detectors.

6.2 GC columns

This method describes procedures for both single-column and dual-column analyses. The single-column approach involves one analysis to determine that a compound is present, followed by a second analysis to confirm the identity of the compound (Sec. 11.11 describes how GC/MS confirmation techniques may be employed). The single-column approach may employ either narrow-bore ($\leq$ 0.32-mm ID) columns or wide-bore (0.53-mm ID) columns. The dual-column approach generally employs a single injection that is split between two columns that are mounted in a single gas chromatograph. The dual-column approach generally employs wide-bore (0.53-mm ID) columns, but columns of other diameters may be employed if the analyst can demonstrate and document acceptable performance for the intended application. A third alternative is to employ dual columns mounted in a single GC, but with each column connected to a separate injector and a separate detector.

The columns listed in this section were the columns used in developing the method. The listing of these columns in this method is not intended to exclude the use of other columns that are available or that may be developed. Laboratories may use these columns or other columns provided that the laboratories document method performance data (e.g., chromatographic resolution, analyte breakdown, and sensitivity) that are appropriate for the intended application.

6.2.1 Narrow-bore columns for single-column analysis (use both columns to confirm compound identifications unless another confirmation technique such as GC/MS is employed). Narrow-bore columns should be installed in split/splitless (Grob-type) injectors.

6.2.1.1 30-m x 0.25-mm or 0.32-mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5 or equivalent), 1-µm film thickness.

6.2.1.2 30-m x 0.25-mm ID fused-silica capillary column chemically bonded with 35 percent phenyl methylpolysiloxane (DB-608, SPB-608, or equivalent), 2.5 µm coating thickness, 1-µm film thickness.

6.2.2 Wide-bore columns for single-column analysis (use two of the three columns listed to confirm compound identifications unless another confirmation technique

such as GC/MS is employed). Wide-bore columns should be installed in 1/4-inch injectors, with deactivated liners designed specifically for use with these columns.

6.2.2.1 30-m x 0.53-mm ID fused-silica capillary column chemically bonded with 35 percent phenyl methylpolysiloxane (DB-608, SPB-608, RTx-35, or equivalent), 0.5-µm or 0.83-µm film thickness.

6.2.2.2 30-m x 0.53-mm ID fused-silica capillary column chemically bonded with 14% cyanopropylmethylpolysiloxane (DB-1701, or equivalent), 1.0-µm film thickness.

6.2.2.3 30-m x 0.53-mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5, SPB-5, RTx-5, or equivalent), 1.5-µm film thickness.

6.2.3 Wide-bore columns for dual-column analysis -- The three pairs of recommended columns are listed below.

6.2.3.1 Column pair 1

30-m x 0.53-mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5, SPB-5, RTx-5, or equivalent), 1.5-µm film thickness.

30-m x 0.53-mm ID fused-silica capillary column chemically bonded with 14% cyanopropylmethylpolysiloxane (DB-1701, or equivalent), 1.0-µm film thickness.

Column pair 1 is mounted in a press-fit Y-shaped glass 3-way union splitter (J&W Scientific, Catalog No. 705-0733) or a Y-shaped fused-silica connector (Restek, Catalog No. 20405), or equivalent.

NOTE: When connecting columns to a press-fit Y-shaped connector, a better seal may be achieved by first soaking the ends of the capillary columns in alcohol for about 10 sec to soften the polyimide coating.

6.2.3.2 Column pair 2

30-m x 0.53-mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5, SPB-5, RTx-5, or equivalent), 0.83-µm film thickness.

30-m x 0.53-mm ID fused-silica capillary column chemically bonded with 14% cyanopropylmethylpolysiloxane (DB-1701, or equivalent), 1.0-µm film thickness.

Column pair 2 is mounted in an 8-in. deactivated glass injection tee (Supelco, Catalog No. 2-3665M), or equivalent.

6.2.3.3 Column pair 3

30-m x 0.53-mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5, SPB-5, RTx-5, or equivalent), 1.5-µm film thickness.

30-m x 0.53-mm ID fused-silica capillary column chemically bonded with 35 percent phenyl methylpolysiloxane (HP-608, DB-608, SPB-608, RTx-35, or equivalent), 0.5-µm film thickness.

Column pair 3 is mounted in separate injectors and separate detectors.

6.3 Column rinsing kit -- Bonded-phase column rinse kit (J&W Scientific, Catalog No. 430-3000), or equivalent.

6.4 Volumetric flasks -- 10-mL and 25-mL, for preparation of standards.

6.5 Analytical balance, capable of weighing to 0.0001 g.

7.0 REAGENTS AND STANDARDS.

7.1 Reagent-grade or pesticide-grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. Reagents should be stored in glass to prevent the leaching of contaminants from plastic containers.

NOTE: Store the standard solutions (stock, composite, calibration, internal, and surrogate) at ≤6 °C in polytetrafluoroethylene (PTFE)-sealed containers in the dark. When a lot of standards is prepared, aliquots of that lot should be stored in individual small vials. All stock standard solutions must be replaced after one year, or sooner if routine QC (see Sec. 9.0) indicates a problem. All other standard solutions must be replaced after six months, or sooner if routine QC (see Sec. 9.0) indicates a problem.

7.2 Solvents used in the extraction and cleanup procedures (appropriate 3500 and 3600 series methods) include *n*-hexane, diethyl ether, methylene chloride, acetone, ethyl acetate, and isooctane (2,2,4-trimethylpentane) and the solvents must be exchanged to *n*-hexane or isooctane prior to analysis. Therefore, *n*-hexane and isooctane will be required in this procedure. All solvents should be pesticide grade in quality or equivalent, and each lot of solvent should be determined to be free of phthalates.

7.3 The following solvents may be necessary for the preparation of standards. All solvent lots must be pesticide grade in quality or equivalent and should be determined to be free of phthalates.

7.3.1 Acetone, $(CH_3)_2CO$

7.3.2 Toluene, $C_6H_5CH_3$

7.4 Organic-free reagent water -- All references to water in this method refer to organic-free reagent water as defined in Chapter One.

7.5 Standard solutions

The following sections describe the preparation of stock, intermediate, and working standards for the compounds of interest. This discussion is provided as an example, and other approaches and concentrations of the target compounds may be used, as appropriate for the intended application. See Method 8000 for additional information on the preparation of calibration standards.

7.6 Stock standard solutions (1000 mg/L) -- May be prepared from pure standard materials or can be purchased as certified solutions.

7.6.1 Prepare stock standard solutions by accurately weighing 0.0100 g of pure compound. Dissolve the compound in isooctane or hexane and dilute to volume in a 10-mL volumetric flask. If compound purity is 96 percent or greater, the weight can be used without correction to calculate the concentration of the stock standard solution.

7.6.2 Commercially-prepared stock standard solutions may be used at any concentration if they are certified by the manufacturer or by an independent source.

7.7 Calibration standards for Aroclors

7.7.1 A standard containing a mixture of Aroclor 1016 and Aroclor 1260 will include many of the peaks represented in the other five Aroclor mixtures. As a result, a multi-point initial calibration employing a mixture of Aroclors 1016 and 1260 at five concentrations should be sufficient to demonstrate the linearity of the detector response without the necessity of performing multi-point initial calibrations for each of the seven Aroclors. In addition, such a mixture can be used as a standard to demonstrate that a sample does not contain peaks that represent any one of the Aroclors. This standard can also be used to determine the concentrations of either Aroclor 1016 or Aroclor 1260, should they be present in a sample.

Prepare a minimum of five calibration standards containing equal concentrations of both Aroclor 1016 and Aroclor 1260 by dilution of the stock standard with isooctane or hexane. The concentrations should correspond to the expected range of concentrations found in real samples and should bracket the linear range of the detector. See Method 8000 for additional information regarding the preparation of calibration standards.

7.7.2 Single standards of each of the other five Aroclors are required to aid the analyst in pattern recognition. Assuming that the Aroclor 1016/1260 standards described in Sec. 7.7.1 have been used to demonstrate the linearity of the detector, these single standards of the remaining five Aroclors also may be used to determine the calibration factor for each Aroclor when a linear calibration model through the origin is chosen (see Sec. 11.4). Prepare a standard for each of the other Aroclors. The concentrations should generally correspond to the mid-point of the linear range of the detector, but lower concentrations may be employed at the discretion of the analyst based on project requirements.

7.7.3 Other standards (e.g., other Aroclors) and other calibration approaches (e.g., non-linear calibration for individual Aroclors) may be employed to meet project needs. When the nature of the PCB contamination is already known, use standards of those particular Aroclors. See Method 8000 for information on non-linear calibration approaches.

7.8 Calibration standards for PCB congeners

7.8.1 If results are to be determined for individual PCB congeners, then standards for the pure congeners must be prepared. The table in Sec. 1.1 lists 19 PCB congeners that have been tested by this method along with the IUPAC numbers designating these congeners. This procedure may be appropriate for other congeners as well, but the analyst must either document the resolution of the congeners in question or establish procedures for reporting the results of coeluting congeners that are appropriate for the intended application.

7.8.2 Stock standards may be prepared in a fashion similar to that described for the Aroclor standards, or may be purchased as commercially-prepared solutions. Stock standards should be used to prepare a minimum of five concentrations by dilution of the stock standard with isooctane or hexane. The concentrations should correspond to the expected range of concentrations found in real samples and should bracket the linear range of the detector.

7.9 Internal standard

7.9.1 When PCB congeners are to be determined, the use of an internal standard is highly recommended. Decachlorobiphenyl may be used as an internal standard, added to each sample extract prior to analysis, and included in each of the initial calibration standards.

7.9.2 When PCBs are to be determined as Aroclors, an internal standard is typically not used, and decachlorobiphenyl is employed as a surrogate (see Sec. 7.10).

7.9.3 When decachlorobiphenyl is an analyte of interest, as in some PCB congener analyses, see Sec. 7.10.3.

7.10 Surrogate standards

The performance of the method should be monitored using surrogate compounds. Surrogate standards are added to all samples, method blanks, matrix spikes, and calibration standards. The choice of surrogate compounds will depend on analysis mode chosen, e.g., Aroclors or congeners. The following compounds are recommended as surrogates. Other surrogates may be used, provided that the analyst can demonstrate and document performance appropriate for the data quality needs of the particular application.

7.10.1 When PCBs are to be determined as Aroclors, decachlorobiphenyl may be used as a surrogate, and is added to each sample prior to extraction. Prepare a solution of decachlorobiphenyl in acetone. The recommended spiking solution concentration is 5 mg/L. Tetrachloro-*m*-xylene also may be used as a surrogate for Aroclor analysis. If used, the recommended spiking solution concentration is 5 mg/L in acetone. (Other surrogate concentrations may be used, as appropriate for the intended application.)

7.10.2 When PCB congeners are to be determined, decachlorobiphenyl is recommended for use as an internal standard, and therefore it cannot also be used as a surrogate. Tetrachloro-*m*-xylene may be used as a surrogate for PCB congener analysis. The recommended spiking solution concentration is 5 mg/L in acetone. (Other surrogate concentrations may be used, as appropriate for the intended application.)

7.10.3 If decachlorobiphenyl is a target congener for the analysis, 2,2',4,4',5,5'-hexabromobiphenyl may be used as an internal standard or a surrogate.

7.11 DDT analog standard -- Used to determine if the commonly found DDT analogs (DDT, DDE, and DDD) elute at the same retention times as any of the target analytes (congeners or Aroclors). A single standard containing all three compounds should be sufficient. The concentration of the standard is left to the judgement of the analyst.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 See the introductory material to Chapter Four, "Organic Analytes."

8.2 Extracts should be stored under refrigeration in the dark and should be analyzed within 40 days of extraction.

NOTE: The holding time above is a recommendation. PCBs are very stable in a variety of matrices, and holding times under the conditions listed above may be as long as a year.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for guidance on quality assurance (QA) and quality control (QC) protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and those criteria given in Chapter One, and technique-specific QC criteria take precedence over the criteria in Chapter One. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and quality control data should be maintained for reference or inspection.

9.2 Refer to Method 8000 for specific determinative method QC procedures. Refer to Method 3500 for QC procedures to ensure the proper operation of the various sample preparation techniques. If an extract cleanup procedure is performed, refer to Method 3600 for the appropriate QC procedures. Any more specific QC procedures provided in this method will supersede those noted in Methods 8000, 3500, or 3600.

9.3 Quality control procedures necessary to evaluate the GC system operation are found in Method 8000 and include evaluation of retention time windows, calibration verification and chromatographic analysis of samples.

9.3.1 Include a calibration standard after each group of 20 samples (it is *recommended* that a calibration standard be included after every 10 samples to minimize the number of repeat injections) in the analysis sequence as a calibration check. Thus, injections of method blank extracts, matrix spike samples, and other non-standards are counted in the total. Solvent blanks, injected as a check on cross-contamination, need not be counted in the total. The response factors for the calibration should be within ±20 percent of the initial calibration (see Sec. 11.6.2). When this continuing calibration is out of this acceptance window, the laboratory should stop analyses and take corrective action.

9.3.2 Whenever quantitation is accomplished using an internal standard, internal standards must be evaluated for acceptance. The measured area of the internal standard must be no more than 50 percent different from the average area calculated during initial calibration. When the internal standard peak area is outside the limit, all samples that fall outside the QC criteria must be reanalyzed. The retention times of the internal standards must also be evaluated. A retention time shift of >30 sec necessitates reanalysis of the affected sample.

9.4 Initial demonstration of proficiency

9.4.1 Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. If an autosampler is used to perform sample dilutions, before using the autosampler to dilute samples, the laboratory should satisfy itself that those dilutions are of equivalent or better accuracy than is achieved by an experienced analyst performing manual dilutions. The laboratory must also repeat the demonstration of proficiency whenever new staff members are trained or significant changes in instrumentation are made. See Method 8000 for information on how to accomplish a demonstration of proficiency.

9.4.2 It is suggested that the QC reference sample concentrate (as discussed in Methods 8000 and Method 3500) contain PCBs as Aroclors at 10-50 mg/L in the concentrate for water samples, or PCBs as congeners at the same concentrations. A 1-mL volume of this concentrate spiked into 1 L of reagent water will result in a sample concentration of 10-50 µg/L. If Aroclors are not expected in samples from a particular source, then prepare the QC reference samples with a mixture of Aroclors 1016 and 1260. However, when specific Aroclors are known to be present or expected in samples, the specific Aroclors should be used for the QC reference sample. See Method 8000 for additional information on how to accomplish this demonstration. Other concentrations may be used, as appropriate for the intended application.

9.4.3 Calculate the average recovery and the standard deviation of the recoveries of the analytes in each of the four QC reference samples. Refer to Method 8000 for procedures for evaluating method performance.

9.5 Initially, before processing any samples, the analyst should demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. As a continuing check, each time samples are extracted, cleaned up, and analyzed, and when there is a change in reagents, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. If a peak is observed within the retention time window of any analyte that would prevent the determination of that analyte, determine the source and eliminate it, if possible, before processing the samples. The blanks should be carried through all stages of sample preparation and analysis. When new reagents or chemicals are received, the laboratory should monitor the preparation and/or analysis blanks associated with samples for any signs of contamination. It is not necessary to test every new batch of reagents or chemicals prior to sample preparation if the source shows no prior problems. However, if reagents are changed during a preparation batch, separate blanks need to be prepared for each set of reagents.

9.6 Sample quality control for preparation and analysis

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, method sensitivity). At a minimum, this should include the analysis of QC samples including a method blank, a matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch and the addition of surrogates to each field sample and QC sample when surrogates are used. Any method blanks, matrix spike samples, and replicate samples should be subjected to the same analytical procedures (Sec. 11.0) as those used on actual samples.

9.6.1 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike

duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, the laboratories should use a matrix spike and matrix spike duplicate pair, spiked with the Aroclor 1016/1260 mixture. However, when specific Aroclors are known to be present or expected in samples, the specific Aroclors should be used for spiking. Consult Method 8000 for information on developing acceptance criteria for the MS/MSD.

9.6.2 A laboratory control sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike, when appropriate. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix. Consult Method 8000 for information on developing acceptance criteria for the LCS.

9.6.3 Also see Method 8000 for the details on carrying out sample quality control procedures for preparation and analysis. In-house acceptance criteria for evaluating method performance should be developed using the guidance found in Method 8000.

9.7 Surrogate recoveries

If surrogates are used, the laboratory should evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory. See Method 8000 for information on evaluating surrogate data and developing and updating surrogate limits. Procedures for evaluating the recoveries of multiple surrogates and the associated corrective actions should be defined in an approved project plan.

9.8 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

10.0 CALIBRATION AND STANDARDIZATION

See Sec. 11.0 for information on calibration and standardization.

11.0 PROCEDURE

11.1 Sample extraction

11.1.1 Refer to Chapter Two and Method 3500 for guidance in choosing the appropriate extraction procedure. In general, water samples are extracted at a neutral pH with methylene chloride using a separatory funnel (Method 3510), a continuous liquid-liquid extractor (Method 3520), solid-phase extraction (Method 3535), or other appropriate technique. Solid samples are extracted with hexane-acetone (1:1) or methylene chloride-acetone (1:1) using one of the Soxhlet extraction methods (Method 3540 or 3541), pressurized fluid extraction (Method 3545), microwave extraction (Method 3546),

ultrasonic extraction (Method 3550), supercritical fluid extraction (Method 3562), or other appropriate technique or solvents. Tissue samples are extracted using supercritical fluid extraction (Method 3562) or other appropriate technique.

NOTE: The use of hexane-acetone generally reduces the amount of interferences that are extracted and improves signal-to-noise.

The choice of extraction solvent and procedure will depend on the analytes of interest. No single solvent or extraction procedure is universally applicable to all analyte groups and sample matrices. The analyst *must* demonstrate adequate performance for the analytes of interest, at the levels of interest, for any solvent system and extraction procedure employed, *including* those specifically listed in this method. At a minimum, such a demonstration will encompass the initial demonstration of proficiency described in Method 3500, using a clean reference matrix. Each new sample type must be spiked with the compounds of interest to determine the percent recovery. Method 8000 describes procedures that may be used to develop performance criteria for such demonstrations as well as for matrix spike and laboratory control sample results.

11.1.2 Reference materials, field-contaminated samples, or spiked samples should be used to verify the applicability of the selected extraction technique to each new sample type. Such samples should contain or be spiked with the compounds of interest in order to determine the percent recovery and the limit of detection for that sample type (see Chapter One). When other materials are not available and spiked samples are used, they should be spiked with the analytes of interest, either specific Aroclors or PCB congeners. When the presence of specific Aroclors is not anticipated, the Aroclor 1016/1260 mixture may be an appropriate choice for spiking. See Methods 3500 and 8000 for guidance on demonstration of initial method proficiency as well as guidance on matrix spikes for routine sample analysis.

11.1.3 The extraction techniques for solids may be applicable to wipe samples and other sample matrices not addressed in Sec. 11.1.1. The analysis of oil samples may need special sample preparation procedures that are not described here. Analysts should follow the steps described in Sec. 11.1.2 to verify the applicability of the sample preparation and extraction techniques for matrices such as wipes and oils.

11.2 Extract cleanup

Cleanup procedures may not be necessary for a relatively clean sample matrix, but most extracts from environmental and waste samples will require additional preparation before analysis. The specific cleanup procedure used will depend on the nature of the sample to be analyzed and the data quality objectives for the measurements. Refer to Methods 3600, 3660 and 3665 for general guidance on extract cleanup.

11.3 GC conditions

This method allows the analyst to choose between a single-column or a dual-column configuration in the injector port. The columns listed in this section were the columns used to develop the method performance data. Listing these columns in this method is not intended to exclude the use of other columns that are available or that may be developed. Wide-bore or narrow-bore columns may be used with either option. Laboratories may use either the columns listed in this method or other capillary columns or columns of other dimensions, provided that the laboratories document method performance data (e.g., chromatographic resolution, analyte breakdown, and sensitivity) that are appropriate for the intended application.

11.3.1 Single-column analysis

This capillary GC/ECD method allows the analyst the option of using 0.25-mm or 0.32-mm ID capillary columns (narrow-bore) or 0.53-mm ID capillary columns (wide-bore). Narrow-bore columns generally provide greater chromatographic resolution than wide-bore columns, although narrow-bore columns have a lower sample capacity. As a result, narrow-bore columns may be more suitable for relatively clean samples or for extracts that have been prepared with one or more of the clean-up options referenced in the method. Wide-bore columns (0.53-mm ID) may be more suitable for more complex environmental and waste matrices. However, the choice of the appropriate column diameter is left to the professional judgement of the analyst.

11.3.2 Dual-column analysis

The dual-column/dual-detector approach recommends the use of two 30-m x 0.53-mm ID fused-silica open-tubular columns of different polarities, thus, different selectivities towards the target analytes. The columns may be connected to an injection tee and separate electron capture detectors, or to both separate injectors and separate detectors. However, the choice of the appropriate column dimensions is left to the professional judgement of the analyst.

11.3.3 GC temperature programs and flow rates

11.3.3.1 Table 1 lists suggested GC operating conditions for the analysis of PCBs as Aroclors for single-column analysis, using either narrow-bore or wide-bore capillary columns. Table 2 lists suggested GC operating conditions for the dual-column analysis. Use the conditions in these tables as guidance and establish the GC temperature program and flow rate necessary to separate the analytes of interest.

11.3.3.2 When determining PCBs as congeners, difficulties may be encountered with coelution of congener 153 and other sample components. When determining PCBs as Aroclors, chromatographic conditions should be adjusted to give adequate separation of the characteristic peaks in each Aroclor (see Sec. 11.4.6).

11.3.3.3 Tables 3 and 4 summarize example retention times of up to 73 Aroclor peaks determined during dual-column analysis using the operating conditions listed in Table 2. These retention times are provided as guidance as to what may be achieved using the GC columns, temperature programs, and flow rates described in this method. Each laboratory must determine retention times and retention time windows for their specific application of the method. Note that the peak numbers used in these tables are *not* the IUPAC congener numbers, but represent the elution order of the peaks on these GC columns.

11.3.3.4 Once established, the same operating conditions must be used for the analysis of samples and standards.

11.4 Calibration

11.4.1 Prepare calibration standards using the procedures in Sec. 7.0. Refer to Method 8000 and Sec. 9.3 for proper calibration techniques for both initial calibration and calibration verification. When PCBs are to be determined as congeners, the use of internal standard calibration is highly recommended. Therefore, the calibration standards

must contain the internal standard (see Sec. 7.9) at the same concentration as the sample extracts. When PCBs are to be determined as Aroclors, external standard calibration is generally used.

NOTE: Because of the sensitivity of the electron capture detector, always clean the injection port and column prior to performing the initial calibration.

11.4.2 When PCBs are to be quantitatively determined as congeners, an initial multi-point calibration must be performed that includes standards for all the target analytes (congeners). See Method 8000 for details on calibration options.

11.4.3 When PCBs are to be quantitatively determined as Aroclors, the initial calibration consists of two parts, described below.

11.4.3.1 As noted in Sec. 7.7.1, a standard containing a mixture of Aroclor 1016 and Aroclor 1260 will include many of the peaks represented in the other five Aroclor mixtures. Thus, such a standard may be used to demonstrate the linearity of the detector and that a sample does not contain peaks that represent any one of the Aroclors. This standard can also be used to determine the concentrations of either Aroclor 1016 or Aroclor 1260, should they be present in a sample. Therefore, an initial multi-point calibration is performed using the mixture of Aroclors 1016 and 1260 described in Sec. 7.7.1. See Method 8000 for guidance on the use of linear and non-linear calibrations.

11.4.3.2 Standards of the other five Aroclors are necessary for pattern recognition. When employing the traditional model of a linear calibration through the origin, these standards are also used to determine a single-point calibration factor for each Aroclor, assuming that the Aroclor 1016/1260 mixture in Sec. 11.4.3.1 has been used to describe the detector response. The standards for these five Aroclors should be analyzed before the analysis of any samples, and may be analyzed before or after the analysis of the five 1016/1260 standards in Sec. 11.4.3.1. For non-linear calibrations, see Sec. 11.4.3.3.

11.4.3.3 In situations where only a few Aroclors are of interest for a specific project, the analyst may employ a multi-point initial calibration of each of the Aroclors of interest (e.g., five standards of Aroclor 1232 if this Aroclor is of concern and linear calibration is employed) and not use the 1016/1260 mixture described in Sec. 11.4.3.1 or the pattern recognition standards described in 11.4.3.2. When non-linear calibration models are employed, more than five standards of each Aroclor of interest will be needed to adequately describe the detector response (see Method 8000).

11.4.4 Establish the GC operating conditions appropriate for the configuration (single-column or dual column, Sec. 11.3), using Tables 1 or 2 as guidance. Optimize the instrumental conditions for resolution of the target compounds and sensitivity. A final temperature of between 240 °C and 275 °C may be needed to elute decachlorobiphenyl. The use of injector pressure programming will improve the chromatography of late eluting peaks.

NOTE: Once established, the same operating conditions must be used for both calibrations and sample analyses.

11.4.5 A 2-µL injection of each calibration standard is recommended. Other injection volumes may be employed, provided that the analyst can demonstrate adequate sensitivity for the compounds of interest.

11.4.6 Record the peak area (or height) for each congener or each characteristic Aroclor peak to be used for quantitation.

11.4.6.1 A minimum of 3 peaks must be chosen for each Aroclor, and preferably 5 peaks. The peaks must be characteristic of the Aroclor in question. Choose peaks in the Aroclor standards that are at least 25% of the height of the largest Aroclor peak. For each Aroclor, the set of 3 to 5 peaks should include at least one peak that is unique to that Aroclor. Use at least five peaks for the Aroclor 1016/1260 mixture, none of which should be found in both of these Aroclors.

11.4.6.2 Late-eluting Aroclor peaks are generally the most stable in the environment. Table 5 lists diagnostic peaks in each Aroclor, along with example retention times on two GC columns suitable for single-column analysis. Table 6 lists 13 specific PCB congeners found in Aroclor mixtures. Table 7 lists PCB congeners with example retention times on a DB-5 wide-bore GC column. Use these tables as guidance in choosing the appropriate peaks. Each laboratory must determine retention times and retention time windows for their specific application of the method.

11.4.7 When determining PCB congeners by the internal standard procedure, calculate the response factor (RF) for each congener in the calibration standards relative to the internal standard, decachlorobiphenyl, using the equation that follows.

$$RF = \frac{A_s \times C_{is}}{A_{is} \times C_s}$$

where:

A_s = Peak area (or height) of the analyte or surrogate.
A_{is} = Peak area (or height) of the internal standard.
C_s = Concentration of the analyte or surrogate, in µg/L.
C_{is} = Concentration of the internal standard, in µg/L.

11.4.8 When determining PCBs as Aroclors by the external standard technique, calculate the calibration factor (CF) for each characteristic Aroclor peak in each of the initial calibration standards (from either Sec. 11.4.3.1 or 11.4.3.2) using the equation below.

$$CF = \frac{\text{Peak Area (or Height) in the Standard}}{\text{Total Mass of the Standard Injected (in nanograms)}}$$

Using the equation above, a calibration factor will be determined for each characteristic peak, using the total mass of the Aroclor injected. These individual calibration factors are used to quantitate sample results by applying the factor for each individual peak to the area of that peak, as described in Sec. 11.9.

For a five-point calibration, five sets of calibration factors will be generated for the Aroclor 1016/1260 mixture, each set consisting of the calibration factors for each of the five (or more) peaks chosen for this mixture, e.g., there will be at least 25 separate calibration factors for the mixture. The single standard for each of the other Aroclors (see Sec. 11.4.3.1) will generate at least three calibration factors, one for each selected peak.

If a non-linear calibration model is employed, as described in Method 8000, then additional standards containing each Aroclor of interest will be employed, with a corresponding increase in the total number of calibration factors.

11.4.9 The response factors or calibration factors from the initial calibration are used to evaluate the linearity of the initial calibration, if a linear calibration model is used. This involves the calculation of the mean response or calibration factor, the standard deviation, and the relative standard deviation (RSD) for each congener or Aroclor peak.

When the Aroclor 1016/1260 mixture is used to demonstrate the detector response, the linear calibration models must be applied to the other five Aroclors for which only single standards are analyzed. If multi-point calibration is performed for individual Aroclors (see Sec. 11.4.3.3), use the calibration factors from those standards to evaluate linearity.

See Method 8000 for the specifics of the evaluation of the linearity of the calibration and guidance on performing non-linear calibrations. In general, non-linear calibrations also will consider each characteristic Aroclor peak separately.

11.5 Retention time windows

Absolute retention times are generally used for compound identification. When absolute retention times are used, retention time windows are crucial to the identification of target compounds, and should be established by one of the approaches described in Method 8000. Retention time windows are established to compensate for minor shifts in absolute retention times as a result of sample loadings and normal chromatographic variability. The width of the retention time window should be carefully established to minimize the occurrence of both false positive and false negative results. Tight retention time windows may result in false negatives and/or may cause unnecessary reanalysis of samples when surrogates or spiked compounds are erroneously not identified. Overly wide retention time windows may result in false positive results that cannot be confirmed upon further analysis. Analysts should consult Method 8000 for the details of establishing retention time windows. Other approaches to compound identification may be employed, provided that the analyst can demonstrate and document that the approaches are appropriate for the intended application. When PCBs are determined as congeners by an internal standard technique, absolute retention times may be used in conjunction with relative retention times (relative to the internal standard).

When conducting either Aroclor or congener analysis, it is important to determine that common single-component pesticides such as DDT, DDD, and DDE do not elute at the same retention times as the target congeners. There may be substantial DDT interference with the last major Aroclor 1254 peak in some soil and sediment samples. Therefore, in conjunction with determining the retention time windows of the congeners, the analyst should analyze a standard containing the DDT analogs. This standard need only be analyzed when the retention time

windows are determined. It is not considered part of the routine initial calibration or calibration verification steps in the method, nor are there any performance criteria associated with the analysis of this standard.

If Aroclor analysis is performed and any of the DDT analogs elute at the same retention time as an Aroclor peak that was chosen for use in quantitation (see Sec. 11.4.6), then the analyst must either adjust the GC conditions to achieve better resolution, or choose another peak that is characteristic of that Aroclor and does not correspond to a peak from a DDT analog. If PCB congener analysis is performed and any of the DDT analogs elute at the same retention time as a PCB congener of interest, then the analyst must adjust the GC conditions to achieve better resolution.

11.6 Gas chromatographic analysis of sample extracts

11.6.1 The same GC operating conditions used for the initial calibration must be employed for the analysis of samples.

11.6.2 Verify calibration at least once each 12-hr shift by injecting calibration verification standards prior to conducting any sample analyses. A calibration standard must also be injected at intervals of not less than once every twenty samples (after every 10 samples is recommended to minimize the number of samples requiring reinjection when QC limits are exceeded) and at the end of the analysis sequence. For Aroclor analyses, the calibration verification standard should be a mixture of Aroclor 1016 and Aroclor 1260. The calibration verification process does not *require* analysis of the other Aroclor standards used for pattern recognition, but the analyst may wish to include a standard for one of these Aroclors after the 1016/1260 mixture used for calibration verification throughout the analytical sequence.

11.6.2.1 The calibration factor for each analyte calculated from the calibration verification standard (CF_v) should not exceed a difference of more than ±20 percent when compared to the mean calibration factor from the initial calibration curve. If a calibration approach other than the RSD method has been employed for the initial calibration (e.g., a linear model not through the origin, a non-linear calibration model, etc.), consult Method 8000 for the specifics of calibration verification.

$$\% \text{ Difference} = \frac{\overline{CF} - CF_v}{\overline{CF}} \times 100$$

11.6.2.2 When internal standard calibration is used for PCB congeners, the response factor calculated from the calibration verification standard (RF_v) should not exceed a ±20 percent difference when compared to the mean response factor from the initial calibration. If a calibration approach other than the RSD method has been employed for the initial calibration (e.g., a linear model not through the origin, a non-linear calibration model, etc.), consult Method 8000 for the specifics of calibration verification.

$$\% \text{ Difference} = \frac{\overline{RF} - RF_v}{\overline{RF}} \times 100$$

11.6.2.3 If the calibration does not meet the ±20% limit on the basis of each compound, check the instrument operating conditions, and if necessary, restore them to the original settings, and inject another aliquot of the calibration verification standard. If the response for the analyte is still not within ±20%, then a new initial calibration must be prepared. See Sec. 11.6.6 for a discussion on the effects of a failing calibration verification standard on sample results.

11.6.3 Inject a measured aliquot of the concentrated sample extract. A 2-µL aliquot is suggested, however, other injection volumes may be employed, provided that the analyst can demonstrate adequate sensitivity for the compounds of interest. The same injection volume should be used for both the calibration standards and the sample extracts, unless the analyst can demonstrate acceptable performance using different volumes or conditions. Record the volume injected and the resulting peak size in area units.

11.6.4 Qualitative identifications of target analytes are made by examination of the sample chromatograms, as described in Sec. 11.7.

11.6.5 Quantitative results are determined for each identified analyte (Aroclors or congeners), using the procedures described in Secs. 11.8 and 11.9 for either the internal or the external calibration procedure (Method 8000). If the responses in the sample chromatogram exceed the calibration range of the system, dilute the extract and reanalyze. Peak height measurements are recommended over peak area when overlapping peaks cause errors in area integration.

11.6.6 Each sample analysis employing external standard calibration must be bracketed with an acceptable initial calibration, calibration verification standard(s) (each 12-hr analytical shift), or calibration standards interspersed within the samples. The results from these bracketing standards must meet the calibration verification criteria in Sec. 11.6.2.

Multi-level standards (mixtures or multi-component analytes) are highly recommended to ensure that detector response remains stable for all analytes over the calibration range.

When a calibration verification standard fails to meet the QC criteria, all samples that were injected after the last standard that met the QC criteria must be evaluated to prevent misquantitations and possible false negative results, and reinjection of the sample extracts may be required. More frequent analyses of standards will minimize the number of sample extracts that would have to be reinjected if the QC limits are violated for the standard analysis.

However, if the standard analyzed after a group of samples exhibits a response for an analyte that is above the acceptance limit, i.e., >20%, and the analyte was not detected in the specific samples analyzed during the analytical shift, then the extracts for those samples do not need to be reanalyzed, since the verification standard has demonstrated that the analyte would have been detected if it were present. In contrast, if an analyte

above the QC limits was detected in a sample extract, then reinjection is necessary to ensure accurate quantitation.

If an analyte was not detected in the sample and the standard response is more than 20% below the initial calibration response, then reinjection is necessary. The purpose of this reinjection is to ensure that the analyte could be detected, if present, despite the change in the detector response, e.g., to protect against a false negative result.

11.6.7 Sample injections may continue for as long as the calibration verification standards and standards interspersed with the samples meet instrument QC requirements. It is *recommended* that standards be analyzed after every 10 samples (*required* after every 20 samples and at the end of a set) to minimize the number of samples that must be re-injected when the standards fail the QC limits. The sequence ends when the set of samples has been injected or when qualitative or quantitative QC criteria are exceeded.

11.6.8 The use of internal standard calibration techniques does not require that all sample results be bracketed with calibration verification standards. However, when internal standard calibration is used, the retention times of the internal standards and the area responses of the internal standards should be checked for each analysis. Retention time shifts of more than 30 sec from the retention time of the most recent calibration standard and/or changes in internal standard areas of more than -50 to +100% are cause for concern and must be investigated.

11.6.9 If the peak response is less than 2.5 times the baseline noise level, the validity of the quantitative result may be questionable. The analyst should consult with the source of the sample to determine whether further concentration of the sample is warranted.

11.6.10 Use the calibration standards analyzed during the sequence to evaluate retention time stability. If any of the standards fall outside their daily retention time windows, the system is out of control. Determine the cause of the problem and correct it.

11.6.11 If compound identification or quantitation is precluded due to interferences (e.g., broad, rounded peaks or ill-defined baselines are present), corrective action is warranted. Cleanup of the extract or replacement of the capillary column or detector may be necessary. The analyst may begin by rerunning the sample on another instrument to determine if the problem results from analytical hardware or the sample matrix. Refer to Method 3600 for the procedures to be followed in sample cleanup.

11.7 Qualitative identification

The identification of PCBs as either Aroclors or congeners using this method with an electron capture detector is based on agreement between the retention times of peaks in the sample chromatogram with the retention time windows established through the analysis of standards of the target analytes. See Method 8000 for information on the establishment of retention time windows.

Tentative identification of an analyte occurs when a peak from a sample extract falls within the established retention time window for a specific target analyte. Confirmation is necessary when the sample composition is not well characterized. See Method 8000 for information on confirmation of tentative identifications. See Sec. 11.11 of this procedure for information on the use of GC/MS as a confirmation technique.

When results are confirmed using a second GC column of dissimilar stationary phase, the analyst should check the agreement between the quantitative results on both columns once the identification has been confirmed. See Method 8000 for a discussion of such a comparison and appropriate data reporting approaches.

11.7.1 When simultaneous analyses are performed from a single injection (the dual-column GC configuration described in Sec. 11.3), it is not practical to designate one column as the analytical (primary) column and the other as the confirmation column. Since the calibration standards are analyzed on both columns, both columns must meet the calibration acceptance criteria. If the retention times of the peaks on both columns fall within the retention time windows on the respective columns, then the target analyte identification has been confirmed.

11.7.2 The results of a single column/single injection analysis may be confirmed, if necessary, on a second, dissimilar, GC column. In order to be used for confirmation, retention time windows must have been established for the second GC column. In addition, the analyst must demonstrate the sensitivity of the second column analysis. This demonstration must include the analysis of a standard of the target analyte at a concentration at least as low as the concentration estimated from the primary analysis. That standard may be either the individual congeners, individual Aroclor or the Aroclor 1016/1260 mixture.

11.7.3 When samples are analyzed from a source known to contain specific Aroclors, the results from a single-column analysis may be confirmed on the basis of a clearly recognizable Aroclor pattern. This approach should not be attempted for samples from unknown or unfamiliar sources or for samples that appear to contain mixtures of Aroclors. In order to employ this approach, the analyst must document:

- The peaks that were evaluated when comparing the sample chromatogram and the Aroclor standard.
- The absence of major peaks representing any other Aroclor.
- The source-specific information indicating that Aroclors are anticipated in the sample (e.g., historical data, generator knowledge, etc.).

This information should either be provided to the data user or maintained by the laboratory.

11.7.4 See Sec. 11.11 for information on GC/MS confirmation.

11.8 Quantitation of PCBs as congeners

11.8.1 The quantitation of PCB congeners is accomplished by the comparison of the sample chromatogram to those of the PCB congener standards, using the internal standard technique (see Method 8000). Calculate the concentration of each congener.

11.8.2 Depending on project requirements, the PCB congener results may be reported as congeners, or may be summed and reported as total PCBs. The analyst should use caution when using the congener method for quantitation when regulatory requirements are based on Aroclor concentrations. See Sec. 11.9.3.

11.8.3 The analytical procedures for these 19 congeners may be appropriate for the analysis of other congeners not specifically included in this method and may be used

as a template for the development of such a procedure. However, all 209 PCB congeners cannot be separated using the GC columns and procedures described in this method. If this procedure is expanded to encompass other congeners, then the analyst must either document the resolution of the congeners in question or establish procedures for reporting the results of coeluting congeners that are appropriate for the intended application.

11.9 Quantitation of PCBs as Aroclors

The quantitation of PCB residues as Aroclors is accomplished by comparison of the sample chromatogram to that of the most similar Aroclor standard. A choice must be made as to which Aroclor is most similar to that of the residue and whether that standard is truly representative of the PCBs in the sample.

11.9.1 Use the individual Aroclor standards (not the 1016/1260 mixtures) to determine the pattern of peaks on Aroclors 1221, 1232, 1242, 1248, and 1254. The patterns for Aroclors 1016 and 1260 will be evident in the mixed calibration standards.

11.9.2 Once the Aroclor pattern has been identified, compare the responses of 3 to 5 major peaks in the single-point calibration standard for that Aroclor with the peaks observed in the sample extract. The amount of Aroclor is calculated using the individual calibration factor for each of the 3 to 5 characteristic peaks chosen in Sec. 11.4.6.1. and the calibration model (linear or non-linear) established from the multi-point calibration of the 1016/1260 mixture. Non-linear calibration may result in different models for each selected peak. A concentration is determined using each of the characteristic peaks, using the individual calibration factor calculated for that peak in Sec. 11.4.8, and then those 3 to 5 concentrations are averaged to determine the concentration of that Aroclor.

11.9.3 Weathering of PCBs in the environment and changes resulting from waste treatment processes may alter the PCBs to the point that the pattern of a specific Aroclor is no longer recognizable. Samples containing more than one Aroclor present similar problems. If the purpose of the analysis is <u>not</u> regulatory compliance monitoring on the basis of Aroclor concentrations, then it may be more appropriate to perform the analyses using the PCB congener approach described in this method. If results in terms of Aroclors <u>are</u> required, then the quantitation as Aroclors may be performed by measuring the total area of the PCB pattern and quantitating on the basis of the Aroclor standard that is most similar to the sample. Any peaks that are not identifiable as PCBs on the basis of retention times should be subtracted from the total area. When quantitation is performed in this manner, the problems should be fully described for the data user and the specific procedures employed by the analyst should be thoroughly documented.

11.10 Confirmation

Tentative identification of an analyte occurs when a peak from a sample extract falls within the daily retention time window. Confirmation is necessary when the sample composition is not well characterized. Confirmatory techniques such as gas chromatography with a dissimilar column or a mass spectrometer should be used. See Method 8000 for information on confirmation of tentative identifications.

When results are confirmed using a second GC column of dissimilar stationary phase, the analyst should check the agreement between the quantitative results on both columns once the identification has been confirmed. See Method 8000 for a discussion of such a comparison and appropriate data reporting approaches.

When the dual-column approach is employed, the target phenols are identified and confirmed when they meet the identification criteria on both columns.

11.11 GC/MS confirmation

GC/MS confirmation may be used in conjunction with either single-or dual-column analysis if the concentration is sufficient for detection by GC/MS.

11.11.1 Full-scan quadrupole GC/MS will normally require a higher concentration of the analyte of interest than full-scan ion trap or selected ion monitoring techniques. The concentrations will be instrument-dependent, but values for full-scan quadrupole GC/MS may be as high as 10 ng/µL in the final extract, while ion trap or SIM may only be a concentration of 1 ng/µL.

11.11.2 The GC/MS must be calibrated for the target analytes when it is used for quantitative analysis. If GC/MS is used only for confirmation of the identification of the target analytes, then the analyst must demonstrate that those PCBs identified by GC/ECD can be confirmed by GC/MS. This demonstration may be accomplished by analyzing a single-point standard containing the analytes of interest at or below the concentrations reported in the GC/ECD analysis. When using SIM techniques, the ions and retention times should be characteristic of the Aroclors to be confirmed.

11.11.3 GC/MS confirmation should be accomplished by analyzing the same extract used for GC/ECD analysis and the extract of the associated blank.

11.12 GC/AED confirmation by Method 8085 may be used in conjunction with either single-column or dual-column analysis if the concentration is sufficient for detection by GC/AED.

11.13 Chromatographic system maintenance as corrective action

When system performance does not meet the established QC requirements, corrective action is required, and may include one or more of the following.

11.13.1 Splitter connections

For dual columns which are connected using a press-fit Y-shaped glass splitter or a Y-shaped fused-silica connector, clean and deactivate the splitter port insert or replace with a cleaned and deactivated splitter. Break off the first few centimeters (up to 30 cm) of the injection port side of the column. Remove the columns and solvent backflush according to the manufacturer's instructions. If these procedures fail to eliminate the degradation problem, it may be necessary to deactivate the metal injector body and/or replace the columns.

11.13.2 Metal injector body

Turn off the oven and remove the analytical columns when the oven has cooled. Remove the glass injection port insert (instruments with on-column injection). Lower the injection port temperature to room temperature. Inspect the injection port and remove any noticeable foreign material.

11.13.2.1 Place a beaker beneath the injector port inside the oven. Using a wash bottle, rinse the entire inside of the injector port with acetone and then rinse it with toluene, catching the rinsate in the beaker.

11.13.2.2 Consult the manufacturer's instructions regarding deactivating the injector port body. Glass injection port liners may need deactivation with a silanizing solution containing dimethyldichlorosilane. After all metal surfaces inside the injector body have been thoroughly coated with the deactivation solution, rinse the injector body with toluene, methanol, acetone, then hexane. Reassemble the injector and replace the columns.

11.13.3 Column rinsing

Rinse the column with several column volumes of an appropriate solvent. Both polar and nonpolar solvents are recommended. Depending on the nature of the sample residues expected, the first rinse might be water, followed by methanol and acetone. Methylene chloride is a good final rinse and in some cases may be the only solvent necessary. Fill the column with methylene chloride and allow it to stand flooded overnight to allow materials within the stationary phase to migrate into the solvent. Afterwards, flush the column with fresh methylene chloride, drain the column, and dry it at room temperature with a stream of ultrapure nitrogen.

12.0 DATA ANALYSIS AND CALCULATIONS

See Secs. 11.6 through 11.9 for information regarding data analysis and calculations.

13.0 METHOD PERFORMANCE

13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance goals for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method. These performance data are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

13.2 The accuracy and precision obtainable with this method depend on the sample matrix, sample preparation technique, optional cleanup techniques, and calibration procedures used. Table 8 provides single laboratory recovery data for Aroclors spiked into clay and soil and extracted with automated Soxhlet. Table 9 provides multiple laboratory data on the precision and accuracy for Aroclors spiked into soil and extracted by automated Soxhlet. These data are provided for guidance purposes only.

13.3 During method performance studies, the concentrations determined as Aroclors were higher than those obtained using the congener method for the limited set of congeners listed in Sec. 1.1. In certain soils, interference prevented the measurement of congener 66. Recoveries of congeners from environmental reference materials ranged from 51 - 66% of the certified Aroclor values, illustrating the potential difficulties in using congener analysis to demonstrate compliance with Aroclor-based regulatory limits. These data are provided for guidance purposes only.

13.4 Tables 10 and 11 contain laboratory performance data for several PCB congeners using supercritical fluid extraction (Method 3562) on an HP 7680 to extract solid samples, including soils, sewage sludge, and fish tissue. Seven replicate extractions were performed on each sample. The method was performed using a variable restrictor and solid trapping material (Florisil). These data are provided for guidance purposes only. Sample analysis was performed by GC/ECD. The following solid samples were used for this study:

13.4.1 Two field-contaminated certified reference materials were extracted by a single laboratory. One of the materials (EC-5) was a lake sediment from Environment Canada. The other material (EC-1) was soil from a dump site and was provided by the National Science and Engineering Research Council of Canada. The average recoveries for EC-5 are based on the certified value for that sample. The average recoveries for EC-1 are based on the certified value of the samples or a Soxhlet value, if a certified value was unavailable for a specific analyte. These data are provided for guidance purposes only.

13.4.2 Four certified reference materials were extracted by two independent laboratories. The materials included a marine sediment from NIST (SRM 1941), a fish tissue from NIST (SRM 2974), a sewage sludge from BCR European Union (CRM 392), and a soil sample from BCR European Union (CRM 481). The average recoveries were based on the certified value of the samples or a Soxhlet value, if a certified value was unavailable for a specific analyte. These data are provided for guidance purposes only.

13.4.3 A weathered sediment sample from Michigan (Saginaw Bay) was extracted by a single laboratory. Soxhlet extractions were carried out on this sample and the SFE recovery is relative to that for each congener. The average recoveries were based on the certified value of the samples. Additional data are shown in the tables for some congeners for which no certified values were available. These data are provided for guidance purposes only.

13.5 Tables 12 through 14 contain single laboratory recovery data for Aroclor 1254 using solid-phase extraction (Method 3535). Recovery data at 2, 10, and 100 µg/L are presented. Results represent three replicate solid-phase extractions of spiked wastewaters. Two different wastewaters from each wastewater type were spiked. All of the extractions were performed using 90-mm C_{18} disks. These data are provided for guidance purposes only.

13.6 Single-laboratory data were developed for PCBs extracted by pressurized fluid extraction (Method 3545) from sewage sludge, a river sediment standard reference material (SRM 1939), and a certified soil reference material (CRM911-050). Certified values were available for five PCB congeners for the sewage sludge and for four congeners in SRM 1939. The soil reference material was certified for Aroclor 1254. All pressurized fluid extractions were conducted using hexane:acetone (1:1), at 100 °C, 1300-1500 psi, and a 5-min static extraction. Extracts were analyzed by GC/ECD. The data are presented in Tables 15 through 17 and are reported in detail in Reference 13. These data are provided for guidance purposes only.

13.7 Single-laboratory accuracy data were obtained for PCBs extracted by microwave extraction (Method 3546) from three reference materials, EC-1, EC-2, and EC-3, from Environment Canada. Natural soils, glass fiber, and sand samples were also used as matrices that were spiked with PCBs. Concentrations varied between 0.2 and 10 µg/g (total PCBs). All samples were extracted using 1:1 hexane:acetone. Extracts were analyzed by GC/ECD. Method blanks, spikes and spike duplicates were included for the low concentration spikes; matrix spikes were included for all other concentrations. The data are presented in Tables 18 through 20 and are reported in detail in Reference 14. These data are provided for guidance purposes only.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of

environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical management for Waste Reduction* available from the American Chemical Society, Department of Government Relations and Science Policy, 1155 16th Street, NW, Washington, DC, 20036, http://www.acs.org.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available from the American Chemical Society at the address listed in Sec. 14.2.

16.0 REFERENCES

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17.0 TABLES, DIAGRAMS, FLOW CHARTS, AND VALIDATION DATA

The following pages contain the tables and figures referenced by this method.

TABLE 1

SUGGESTED GC OPERATING CONDITIONS FOR PCBs AS AROCLORS
SINGLE-COLUMN ANALYSIS

Narrow-bore columns

Narrow-bore Column 1 -- 30-m x 0.25 or 0.32-mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5 or equivalent), 1 µm film thickness.

Carrier gas (He)	16 psi
Injector temperature	225 °C
Detector temperature	300 °C
Initial temperature	100 °C, hold 2 min
Temperature program	100 °C to 160 °C at 15 °C/min, followed by 160 °C to 270 °C at 5 °C/min
Final temperature	270 °C

Narrow-bore Column 2 -- 30-m x 0.25-mm ID fused-silica capillary column chemically bonded with 35 percent phenyl methylpolysiloxane (DB-608, SPB-608, or equivalent) 25 µm coating thickness, 1 µm film thickness

Carrier gas (N_2)	20 psi
Injector temperature	225 °C
Detector temperature	300 °C
Initial temperature	160 °C, hold 2 min
Temperature program	160 °C to 290 °C at 5 °C/min
Final temperature	290 °C, hold 1 min

Wide-bore columns

Wide-bore Column 1 -- 30-m x 0.53-mm ID fused-silica capillary column chemically bonded with 35 percent phenyl methylpolysiloxane (DB-608, SPB-608, RTx-35, or equivalent), 0.5 µm or 0.83 µm film thickness.

Wide-bore Column 2 -- 30-m x 0.53-mm ID fused-silica capillary column chemically bonded with 14% cyanopropylmethylpolysiloxane (DB-1701, or equivalent), 1.0 µm film thickness.

Carrier gas (He)	5-7 mL/min
Makeup gas (argon/methane [P-5 or P-10] or N_2)	30 mL/min
Injector temperature	250 °C
Detector temperature	290 °C
Initial temperature	150 °C, hold 0.5 min
Temperature program	150 °C to 270 °C at 5 °C/min
Final temperature	270 °C, hold 10 min

TABLE 1
(continued)

SUGGESTED GC OPERATING CONDITIONS FOR PCBs AS AROCLORS
SINGLE-COLUMN ANALYSIS

Wide-bore Columns (continued)

Wide-bore Column 3 -- 30-m x 0.53-mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5, SPB-5, RTx-5, or equivalent), 1.5 µm film thickness.

Carrier gas (He)	6 mL/min
Makeup gas (argon/methane [P-5 or P-10] or N_2)	30 mL/min
Injector temperature	205 °C
Detector temperature	290 °C
Initial temperature	140 °C, hold 2 min
Temperature program	140 °C to 240 °C at 10 °C/min, hold 5 min at 240 °C, 240 °C to 265 °C at 5 °C/min
Final temperature	265 °C, hold 18 min

TABLE 2

SUGGESTED GC OPERATING CONDITIONS FOR PCBs AS AROCLORS
FOR THE DUAL-COLUMN METHOD OF ANALYSIS

Column 1 -- DB-1701 or equivalent, 30-m x 0.53-mm ID, 1.0 µm film thickness.

Column 2 -- DB-5 or equivalent, 30-m x 0.53-mm ID, 1.5 µm film thickness.

Carrier gas (He) flow rate	6 mL/min
Makeup gas (N_2) flow rate	20 mL/min
Temperature program	0.5 min hold 150 °C to 190 °C, at 12 °C/min, 2 min hold 190 °C to 275 °C, at 4 °C/min, 10 min hold
Injector temperature	250 °C
Detector temperature	320 °C
Injection volume	2 µL
Solvent	Hexane
Type of injector	Flash vaporization
Detector type	Dual ECD
Range	10
Attenuation	64 (DB-1701)/64 (DB-5)
Type of splitter	J&W Scientific press-fit Y-shaped inlet splitter

TABLE 3
(continued)

TABLE 3

EXAMPLE RETENTION TIMES OF AROCLORS
ON THE DB-5 COLUMN[a], DUAL-COLUMN ANALYSIS

Peak No.	Aroclor 1016	Aroclor 1221	Aroclor 1232	Aroclor 1242	Aroclor 1248	Aroclor 1254	Aroclor 1260
1		5.85	5.85				
2		7.63	7.64	7.57			
3	8.41	8.43	8.43	8.37			
4	8.77	8.77	8.78	8.73			
5	8.98	8.99	9.00	8.94	8.95		
6	9.71			9.66			
7	10.49	10.50	10.50	10.44	10.45		
8	10.58	10.59	10.59	10.53			
9	10.90		10.91	10.86	10.85		
10	11.23	11.24	11.24	11.18	11.18		
11	11.88		11.90	11.84	11.85		
12	11.99		12.00	11.95			
13	12.27	12.29	12.29	12.24	12.24		
14	12.66	12.68	12.69	12.64	12.64		
15	12.98	12.99	13.00	12.95	12.95		
16	13.18		13.19	13.14	13.15		
17	13.61		13.63	13.58	13.58	13.59	13.59
18	13.80		13.82	13.77	13.77	13.78	
19	13.96		13.97	13.93	13.93	13.90	
20	14.48		14.50	14.46	14.45	14.46	
21	14.63		14.64	14.60	14.60		
22	14.99		15.02	14.98	14.97	14.98	
23	15.35		15.36	15.32	15.31	15.32	
24	16.01			15.96			
25			16.14	16.08	16.08	16.10	
26	16.27		16.29	16.26	16.24	16.25	16.26
27						16.53	
28			17.04		16.99	16.96	16.97
29			17.22	17.19	17.19	17.19	17.21
30			17.46	17.43	17.43	17.44	
31					17.69	17.69	
32				17.92	17.91	17.91	
33				18.16	18.14	18.14	
34			18.41	18.37	18.36	18.36	18.37
35			18.58	18.56	18.55	18.55	
36							18.68
37			18.83	18.80	18.78	18.78	18.79
38			19.33	19.30	19.29	19.29	19.29

TABLE 3
(continued)

Peak No.	Aroclor 1016	Aroclor 1221	Aroclor 1232	Aroclor 1242	Aroclor 1248	Aroclor 1254	Aroclor 1260
39						19.48	19.48
40						19.81	19.80
41			20.03	19.97	19.92	19.92	
42						20.28	20.28
43					20.46	20.45	
44						20.57	20.57
45				20.85	20.83	20.83	20.83
46			21.18	21.14	21.12	20.98	
47					21.36	21.38	21.38
48						21.78	21.78
49				22.08	22.05	22.04	22.03
50						22.38	22.37
51						22.74	22.73
52						22.96	22.95
53						23.23	23.23
54							23.42
55						23.75	23.73
56						23.99	23.97
57							24.16
58						24.27	
59							24.45
60						24.61	24.62
61						24.93	24.91
62							25.44
63						26.22	26.19
64							26.52
65							26.75
66							27.41
67							28.07
68							28.35
69							29.00

[a] GC operating conditions are given in Table 2. All retention times in minutes and are provided for illustrative purposes only. Each laboratory must determine retention times and retention time windows for their specific application of the method.

[b] The peaks listed in this table are sequentially numbered in elution order for illustrative purposes only and are not isomer numbers.

TABLE 4

EXAMPLE RETENTION TIMES OF AROCLORS
ON THE DB-1701 COLUMN[a], DUAL-COLUMN ANALYSIS

Peak No.	Aroclor 1016	Aroclor 1221	Aroclor 1232	Aroclor 1242	Aroclor 1248	Aroclor 1254	Aroclor 1260
1		4.45	4.45				
2		5.38					
3		5.78					
4		5.86	5.86				
5	6.33	6.34	6.34	6.28			
6	6.78	6.78	6.79	6.72			
7	6.96	6.96	6.96	6.90	6.91		
8	7.64			7.59			
9	8.23	8.23	8.23	8.15	8.16		
10	8.62	8.63	8.63	8.57			
11	8.88		8.89	8.83	8.83		
12	9.05	9.06	9.06	8.99	8.99		
13	9.46		9.47	9.40	9.41		
14	9.77	9.79	9.78	9.71	9.71		
15	10.27	10.29	10.29	10.21	10.21		
16	10.64	10.65	10.66	10.59	10.59		
17				10.96	10.95	10.95	
18	11.01		11.02	11.02	11.03		
19	11.09		11.10				
20	11.98		11.99	11.94	11.93	11.93	
21	12.39		12.39	12.33	12.33	12.33	
22			12.77	12.71	12.69		
23	12.92			12.94	12.93		
24	12.99		13.00	13.09	13.09	13.10	
25	13.14		13.16				
26						13.24	
27	13.49		13.49	13.44	13.44		
28	13.58		13.61	13.54	13.54	13.51	13.52
29				13.67		13.68	
30			14.08	14.03	14.03	14.03	14.02
31			14.30	14.26	14.24	14.24	14.25
32					14.39	14.36	
33			14.49	14.46	14.46		
34						14.56	14.56
35					15.10	15.10	
36			15.38	15.33	15.32	15.32	
37			15.65	15.62	15.62	15.61	16.61
38			15.78	15.74	15.74	15.74	15.79
39			16.13	16.10	16.10	16.08	
40							16.19
41						16.34	16.34

TABLE 4
(continued)

Peak No.	Aroclor 1016	Aroclor 1221	Aroclor 1232	Aroclor 1242	Aroclor 1248	Aroclor 1254	Aroclor 1260
42						16.44	16.45
43						16.55	
44			16.77	16.73	16.74	16.77	16.77
45			17.13	17.09	17.07	17.07	17.08
46						17.29	17.31
47				17.46	17.44	17.43	17.43
48				17.69	17.69	17.68	17.68
49					18.19	18.17	18.18
50				18.48	18.49	18.42	18.40
51						18.59	
52						18.86	18.86
53				19.13	19.13	19.10	19.09
54						19.42	19.43
55						19.55	19.59
56						20.20	20.21
57						20.34	
58							20.43
59					20.57	20.55	
60						20.62	20.66
61						20.88	20.87
62							21.03
63						21.53	21.53
64						21.83	21.81
65						23.31	23.27
66							23.85
67							24.11
68							24.46
69							24.59
70							24.87
71							25.85
72							27.05
73							27.72

[a] GC operating conditions are given in Table 2. All retention times are in minutes and are provided for illustrative purposes only. Each laboratory must determine retention times and retention time windows for their specific application of the method.

[b] The peaks listed in this table are sequentially numbered in elution order for illustrative purposes only and are not isomer numbers.

TABLE 5

EXAMPLE RETENTION TIMES OF PEAKS DIAGNOSTIC OF PCBs
ON A 0.53-mm ID COLUMNS DURING SINGLE-COLUMN ANALYSIS

Peak No.[a]	RT on DB-608[b]	RT on DB-1701[b]	Aroclor[c]
I	4.90	4.66	1221
II	7.15	6.96	1221, 1232, 1248
III	7.89	7.65	1061, 1221, 1232, 1242
IV	9.38	9.00	1016, 1232, 1242, 1248
V	10.69	10.54	1016, 1232, 1242
VI	14.24	14.12	1248, 1254
VII	14.81	14.77	1254
VIII	16.71	16.38	1254
IX	19.27	18.95	1254, 1260
X	21.22	21.23	1260
XI	22.89	22.46	1260

[a]Peaks are sequentially numbered in elution order and are not isomer numbers

[b]Temperature program: T_i = 150 °C, hold 30 sec; 5 °C/min to 275 °C.

[c]Underline indicates the largest peak in the pattern for that Aroclor

All retention times are in minutes and are provided for illustrative purposes only. Each laboratory must determine retention times and retention time windows for their specific application of the method.

TABLE 6

SPECIFIC PCB CONGENERS THAT ARE MAJOR COMPONENTS IN COMMON AROCLORS

		Aroclor						
Congener	IUPAC Number	1016	1221	1232	1242	1248	1254	1260
Biphenyl	--		X					
2-CB	1	X	X	X	X			
2,3-DCB	5	X	X	X	X	X		
3,4-DCB	12	X		X	X	X		
2,4,4'-TCB	28*	X		X	X	X	X	
2,2',3,5'-TCB	44			X	X	X	X	X
2,3',4,4'-TCB	66*					X	X	X
2,3,3',4',6-PCB	110						X	
2,3',4,4',5-PCB	118*						X	X
2,2',4,4',5,5'-HCB	153							X
2,2',3,4,4',5'-HCB	138							X
2,2',3,4,4',5,5'-HpCB	180							X
2,2',3,3',4,4',5-HpCB	170							X

*Apparent co-elution of: 28 with 31 (2,4',5-trichlorobiphenyl)
66 with 95 (2,2',3,5',6-pentachlorobiphenyl)
118 with 149 (2,2',3,4',5',6-hexachlorobiphenyl)

This table is not intended to illustrate all of the congeners that may be present in a given Aroclor, but rather to illustrate the major congener components.

TABLE 7
EXAMPLE RETENTION TIMES OF PCB CONGENERS ON THE DB-5 WIDE-BORE COLUMN

IUPAC Number	Retention Time (min)
1	6.52
5	10.07
18	11.62
31	13.43
52	14.75
44	15.51
66	17.20
101	18.08
87	19.11
110	19.45
151	19.87
153	21.30
138	21.79
141	22.34
187	22.89
183	23.09
180	24.87
170	25.93
206	30.70
209 (internal standard)	32.63

All data are provided for illustrative purposes only. Each laboratory must determine retention times and retention time windows for their specific application of the method.

TABLE 8

EXAMPLE SINGLE-LABORATORY RECOVERY DATA FOR THE EXTRACTION OF PCBs FROM CLAY AND SOIL BY AUTOMATED SOXHLET (METHOD 3541)[a]

Matrix	Aroclor	Spike Level (ppm)	Trial	Percent Recovery[b]
Clay	1254	5	1	87
			2	93
			3	94
			4	99
			5	79
			6	28
Clay	1254	50	1	65
			2	72
			3	97
			4	80
			5	50
			6	59
Clay	1260	5	1	87
			2	75
			3	61
			4	94
			5	97
			6	113
Clay	1260	50	1	74
			2	70
			3	92
			4	89
			5	90
			6	67

TABLE 8
(continued)

Matrix	Aroclor	Spike Level (ppm)	Trial	Percent Recovery[b]
Soil	1254	5	1	70
			2	89
			3	92
			4	83
			5	63
Soil	1254	50	1	84
			2	78
			3	92
			4	67
			5	82
			6	62
Soil	1260	5	1	84
			2	83
			3	82
			4	96
			5	94
			6	94
			7	98
Soil	1260	50	1	77
			2	69
			3	93
			4	82
			5	83
			6	76

[a]The operating conditions for the automated Soxhlet
Immersion time: 60 min
Reflux time: 60 min

[b]Multiple results from two different extractors

Data are taken from Reference 9
These data are provided for guidance purposes only.

TABLE 9

EXAMPLE MULTIPLE-LABORATORY PRECISION AND ACCURACY DATA
FOR THE EXTRACTION OF PCBs FROM SPIKED SOIL
BY AUTOMATED SOXHLET (METHOD 3541)

		Percent Recovery at Aroclor 1254 Spike Concentration (µg/kg)			Percent Recovery at Aroclor 1260 Spike Concentration (µg/kg)			Mean Recovery
		5	50	500	5	50	500	All Levels
	n	3	3		3	3		12
Lab 1	Mean	101.2	74.0		83.9	78.5		84.4
	S. D.	34.9	41.8		7.4	7.4		26.0
	n		6	6		6	6	24
Lab 2	Mean		56.5	66.9		70.1	74.5	67.0
	S. D.		7.0	15.4		14.5	10.3	13.3
	n	3	3		3	3		12
Lab 3	Mean	72.8	63.3		70.6	57.2		66.0
	S. D.	10.8	8.3		2.5	5.6		9.1
	n	6	6		6	6		24
Lab 4	Mean	112.6	144.3		100.3	84.8		110.5
	S. D.	18.2	30.4		13.3	3.8		28.5
	n		3	3		3	3	12
Lab 5	Mean		97.1	80.1		79.5	77.0	83.5
	S. D.		8.7	5.1		3.1	9.4	10.3
	n	2	3		3	4		12
Lab 6	Mean	140.9	127.7		138.7	105.9		125.4
	S. D.	4.3	15.5		15.5	7.9		18.4
	n	3	3		3	3		12
Lab 7	Mean	100.1	123.4		82.1	94.1		99.9
	S. D.	17.9	14.6		7.9	5.2		19.0
	n	3	3		3	3		12
Lab 8	Mean	65.0	38.3		92.8	51.9		62.0
	S. D.	16.0	21.9		36.5	12.8		29.1
	n	20	30	9	21	31	9	120
All	Mean	98.8	92.5	71.3	95.5	78.6	75.3	87.6
Labs	S. D.	28.7	42.9	14.1	25.3	18.0	9.5	29.7

Data are taken from Reference 7
These data are provided for guidance purposes only.

TABLE 10

EXAMPLE PERCENT RECOVERY (BIAS) OF PCBs IN VARIOUS SOILS
USING SUPERCRITICAL FLUID EXTRACTION (METHOD 3562)

PCB No.[a]	EC-1 Dump Site Soil Low #1	SRM 1941 Marine Sediment Low #2	EC-5 Lake Sediment Low #3	CRM 481[b] European Soil High #1	Saginaw Bay Sediment High #2	CRM 392 Sewage Sludge High #3	SRM 2974 Fish Tissue Mussel Low #4	Congener Mean
28	148.4	63.3	147.7	67.3	114.7	89.2	101.7	104.6
52	88.5	106.6	115.8	84.5	111.1	96.2	131.4	104.9
101	93.3	91.2	100.2	84.5	111.5	93.9	133.2	101.1
149	92.6	105.1	101.5	73.2	111.2		69.4	92.2
118	89.9	66.1	108.9	82.1	110.8	73.5	82.7	87.7
153	90.8	65.1	95.1	82.8	118.6	97.3	107.5	94.0
105[b]	89.1	72.6	96.6	83.4	111.8		79.4	88.8
138	90.1	57.4	97.9	76.9	126.9		73.1	87.1
128	90.8	69.9	101.2	65.9	87.6		62.5	79.7
156[b]	90.6	88.9	94.3	85.2	101.1		59.3	86.6
180	92.4	142.4	93.3	82.2	109.2	100.5	65.7	98.0
170	91.3	101.1	95.2	80.5			33.0	81.8
Matrix Mean	95.7	85.8	104.0	79.0	108.7	91.8	83.2	92.2

[a] Congeners which are either certified or have had Soxhlet confirmation.
[b] Congener 105 was not resolved from congener 132 and congener 156 was not resolved from congener 171 by the GC method used for samples EC-1 and EC-5.

TABLE 11

PRECISION (AS %RSD) OF PCBs EXTRACTED USING SUPERCRITICAL FLUID EXTRACTION (METHOD 3562)

PCB No.[a]	EC-1 Dump Site Soil Low #1	SRM 1941 Marine Sediment Low #2	EC-5 Lake Sediment Low #3	CRM 481 European Soil High #1	Saginaw Bay Sediment High #2	CRM 392 Sewage Sludge High #3	SRM 2974 Fish Tissue Mussel Low #4	Congener Mean
28	11.5	1.5	3.8	5.6	2.4	1.9	2.7	4.2
52	9.1	3.3	3.9	5.4	2.2	2.9	3.1	4.3
101	9.1	2.9	2.8	4.9	1.4	5.2	2.9	4.2
149	7.1	0.7	3.8	3.9	3.4		2.2	3.0
118	9.8	1.9	4.5	5.4	2.0	3.3	2.4	4.2
153	8.4	1.5	3.0	4.3	4.3	9.5	3.0	4.9
105[b]	6.6	3.7	2.7	4.3	2.7		2.5	3.2
138	9.2	1.8	3.1	4.7	2.3		2.9	3.4
128	6.0	5.3	3.3	4.9	2.8		3.3	3.7
156[b]	8.3	0.0	5.1	4.5	1.9		3.8	3.4
180	8.0	1.3	3.6	4.3	3.1	9.6	2.7	4.7
170	5.7	2.3	3.6	3.9	2.3		4.0	3.1
Matrix Mean	8.2	2.2	3.6	4.7	2.6	2.7	3.0	3.8

[a] Congeners which are either certified or have had Soxhlet confirmation.
[b] Congener 105 was not resolved from congener 132 and congener 156 was not resolved from congener 171 by the GC method used for samples EC-1 and EC-5.
These data are provided for guidance purposes only.

TABLE 12

EXAMPLE SINGLE-LABORATORY RECOVERY DATA FOR SOLID-PHASE EXTRACTION (METHOD 3535) OF AROCLOR 1254 FROM WASTEWATER MATRICES SPIKED AT 2 µg/L

Wastewater Type	Mean Conc. (µg/L)	Percent Recovery	Std. Dev. (µg/L)	RSD (%)
Chemical Industry	2.4	120	0.41	17.2
Chemical Industry	0.6	28	0.03	5.4
Paper Industry	3.0	150	0.56	18.5
Paper Industry	2.3	115	0.08	3.7
Pharmaceutical Industry	1.5	76	0.03	1.7
Pharmaceutical Industry	1.0	51	0.03	2.9
Refuse	0.5	27	0.04	6.7
Refuse	0.6	31	0.10	16.0
POTW	1.9	96	0.15	7.8
POTW	2.1	105	0.04	1.8

Results represent three replicate solid-phase extractions of spiked wastewaters. Two different wastewaters from each wastewater type were spiked. All extractions were performed using 90-mm C_{18} extraction disks.
These data are provided for guidance purposes only.

TABLE 13

EXAMPLE SINGLE-LABORATORY RECOVERY DATA FOR SOLID-PHASE EXTRACTION (METHOD 3535) OF AROCLOR 1254 FROM WASTEWATER MATRICES SPIKED AT 10 µg/L

Wastewater Type	Mean Conc. (µg/L)	Percent Recovery	Std. Dev. (µg/L)	RSD (%)
Chemical Industry	8.8	88	1.07	12.2
Chemical Industry	8.1	81	0.06	0.7
Paper Industry	8.9	89	0.71	7.9
Paper Industry	10.1	101	0.15	1.4
Pharmaceutical Industry	9.2	92	0.24	2.6
Pharmaceutical Industry	8.4	84	0.17	2.0
Refuse	8.8	88	0.49	5.6
Refuse	8.0	80	1.44	18.0
POTW	9.5	82	0.17	2.1
POTW	8.2	82	0.17	2.1

Results represent three replicate solid-phase extractions of spiked wastewaters. Two different wastewaters from each wastewater type were spiked. All extractions were performed using 90-mm C_{18} extraction disks.
These data are provided for guidance purposes only.

TABLE 14

EXAMPLE SINGLE-LABORATORY RECOVERY DATA
FOR SOLID-PHASE EXTRACTION (METHOD 3535) OF AROCLOR 1254
FROM WASTEWATER MATRICES SPIKED AT 100 µg/L

Wastewater Type	Mean Conc. (µg/L)	Percent Recovery	Std. Dev. (µg/L)	RSD (%)
Chemical Industry	81.7	82	1.46	1.8
Chemical Industry	89.7	90	0.66	0.7
Paper Industry	73.7	74	3.94	5.3
Paper Industry	95.3	95	1.89	2.0
Pharmaceutical Industry	86.4	86	1.95	2.3
Pharmaceutical Industry	79.2	79	3.92	4.9
Refuse	85.7	86	1.59	1.9
Refuse	71.5	72	1.61	2.2
POTW	87.8	88	1.76	2.0
POTW	80.6	81	0.40	0.5

Results represent three replicate solid-phase extractions of spiked wastewaters. Two different wastewaters from each wastewater type were spiked. All extractions were performed using 90-mm C_{18} extraction disks.
These data are provided for guidance purposes only.

TABLE 15

EXAMPLE SINGLE-LABORATORY PCB CONGENER DATA
FROM A SEWAGE SLUDGE SAMPLE EXTRACTED BY
PRESSURIZED FLUID EXTRACTION (METHOD 3545)

PCB No.	Mean Recovery (%)	%RSD	Certified Value (µg/kg)
52	114	4.7	163
101	143	7.4	161
138	110	3.9	193
153	110	5.8	198
180	160	7.5	207

Percent recoveries are the mean of six replicate extractions.

Data are taken from Reference 13.
These data are provided for guidance purposes only.

TABLE 16

EXAMPLE SINGLE-LABORATORY PCB CONGENER DATA
FROM A RIVER SEDIMENT REFERENCE MATERIAL
EXTRACTED BY PRESSURIZED FLUID EXTRACTION (METHOD 3545)

PCB No.	Mean Recovery (%)	%RSD	Certified Value (µg/kg)
101	89	3.7	780
138	122	2.3	570
153	62	4.1	370
180	112	5.9	180

Percent recoveries are the mean of six replicate extractions.
The river sediment reference material was SRM 1939.

Data are taken from Reference 13.
These data are provided for guidance purposes only.

TABLE 17

EXAMPLE SINGLE-LABORATORY AROCLOR 1254 DATA
FROM A SOIL REFERENCE MATERIAL
EXTRACTED BY PRESSURIZED FLUID EXTRACTION (METHOD 3545)

Replicate Extraction	Aroclor 1254 Concentration (µg/kg)
1	1290
2	1370
3	1280
4	1370
Mean	1330
%RSD	3.5%
Certified value	1340
Mean recovery (%)	99%

Data are taken from Reference 13.
These data are provided for guidance purposes only.

TABLE 18

EXAMPLE SINGLE-LABORATORY PCB HOMOLOGUE DATA BY MICROWAVE EXTRACTION (METHOD 3546) FROM A CERTIFIED GREAT LAKE SEDIMENT MATERIAL (EC-2)

	Microwave Extraction			Soxhlet Extraction		
PCB homologue	µg/kg	Peaks[a]	% RSD	µg/kg	Peaks[a]	% RSD
Trichlorobiphenyl	130	4	21.8	100	4	14.6
Tetrachlorobiphenyl	400	10	13.2	390	20	10.2
Pentachlorobiphenyl	310	9	1.9	300	9	8.7
Hexachlorobiphenyl	120	3	0.0	110	3	9.1

[a] Number of PCB peaks detected
Cl_3 to Cl_{10} homologues analyzed
n=3
Data are taken from Reference 14. These data are provided for guidance purposes only.

TABLE 19

EXAMPLE SINGLE-LABORATORY PCB HOMOLOGUE DATA BY MICROWAVE EXTRACTION (METHOD 3546) FROM A CERTIFIED HARBOR SEDIMENT MATERIAL (SRM-1944)

	Microwave Extraction			Soxhlet Extraction		
PCB homologue	µg/kg	Peaks[a]	% RSD	µg/kg	Peaks[a]	% RSD
Trichlorobiphenyl	450	8	10.1	360	6	5.8
Tetrachlorobiphenyl	580	12	3.9	580	11	6.0
Pentachlorobiphenyl	330	9	6.1	330	9	7.9
Hexachlorobiphenyl	260	3	12.4	240	3	5.1
Heptachlorobiphenyl	60	2	43.8	80	2	27.3

[a] Number of PCB peaks detected
Cl_3 to Cl_{10} homologues analyzed
n=3
Data are taken from Reference 14. These data are provided for guidance purposes only.

TABLE 20

EXAMPLE SINGLE-LABORATORY PCB DATA BY MICROWAVE EXTRACTION (METHOD 3546) FROM CERTIFIED GREAT LAKE SEDIMENT MATERIALS

Sediment	Total Aroclor Concentration (µg/kg)	Standard Deviation (µg/kg)	RSD (%)	n	Certified Value (µg/kg)
EC-1	1850	0.07	3.78	3	2000 ± 54
EC-2	1430	0.09	6.60	4	1160 ± 70
EC-3	670	0.02	3.12	3	660 ± 54

Sample size = 2 g extracted into a final volume of 4 mL

EC-2 and EC-3 certified values were only provisional values at the time the work was conducted. The data presented herein were part of the validation data package used to confirm the certified values.

Data are taken from Reference 14.
These data are provided for guidance purposes only.

FIGURE 1. Example GC/ECD chromatogram of the Aroclor 1016/1260 mixture analyzed on a Rtx-5/HP-608 column pair connected to separate injectors. The top trace is the Rtx-5 column (30-m x 0.53-mm ID, 1.5-µm film thickness) and the bottom trace is the HP-608 column (30-m x 0.53-mm ID, 0.5-µm film thickness). Temperature program: 150 °C (1.0 min hold) to 280 °C (17 min hold) at 8 °C/min.

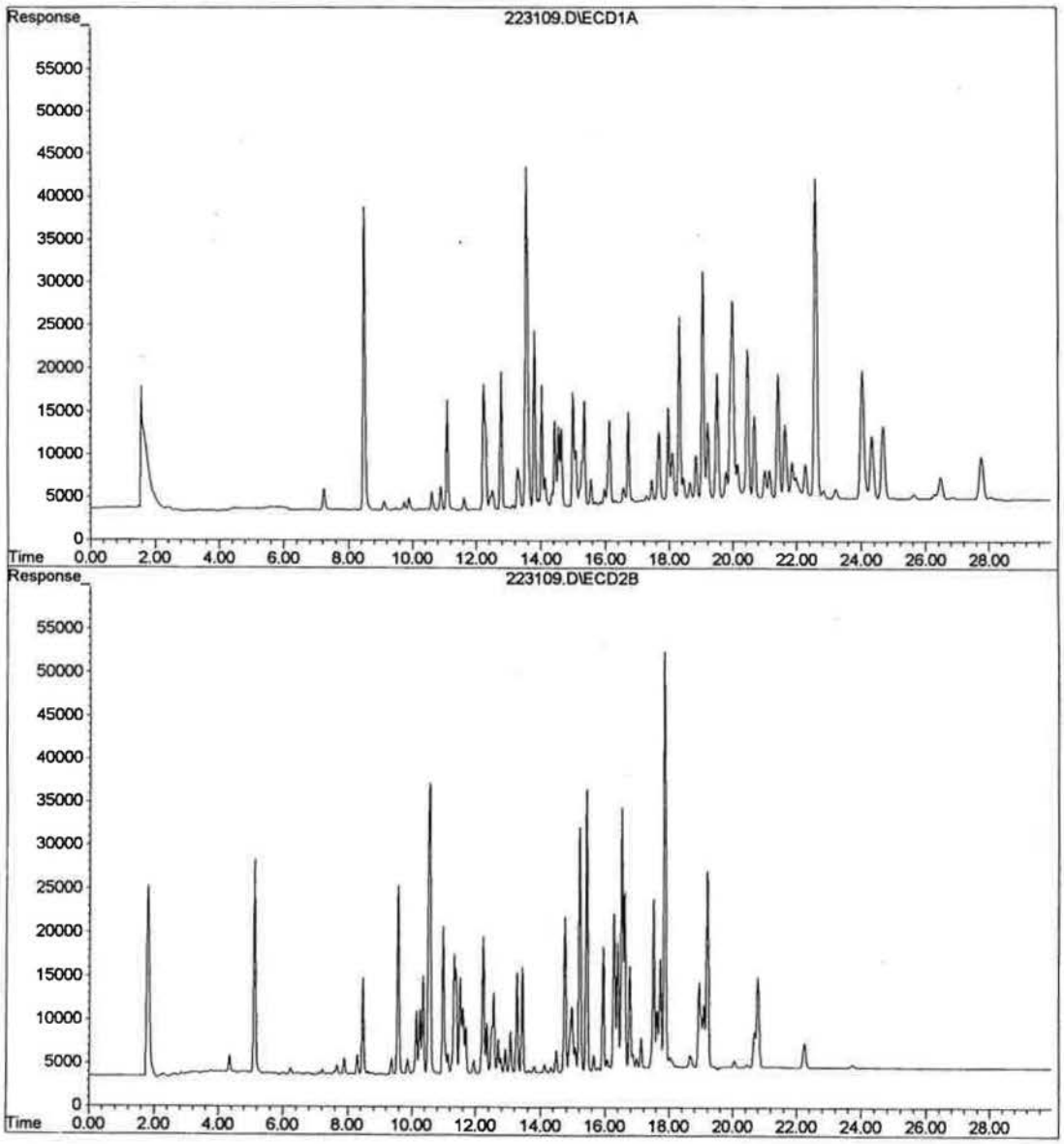


FIGURE 2. Example GC/ECD chromatogram of Aroclor 1221 analyzed on a Rtx-5/HP-608 column pair connected to separate injectors. The top trace is the Rtx-5 column (30-m x 0.53-mm ID, 1.5-µm film thickness) and the bottom trace is the HP-608 column (30-m x 0.53-mm ID, 0.5-µm film thickness). Temperature program: 150 °C (1.0 min hold) to 280 °C (17 min hold) at 8 °C/min.

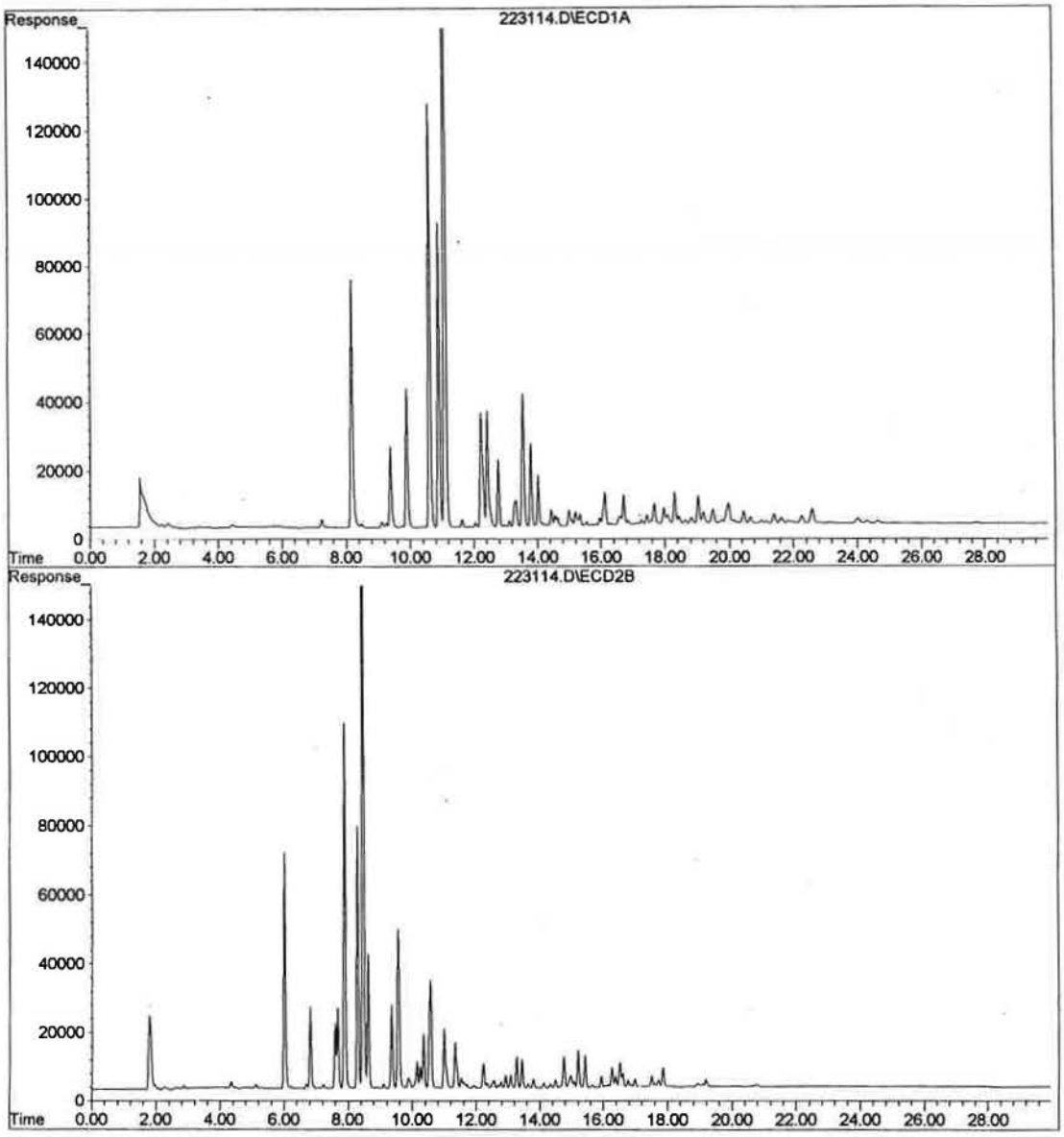


FIGURE 3. Example GC/ECD chromatogram of Aroclor 1232 analyzed on a Rtx-5/HP-608 column pair connected to separate injectors. The top trace is the Rtx-5 column (30-m x 0.53-mm ID, 1.5-µm film thickness) and the bottom trace is the HP-608 column (30-m x 0.53-mm ID, 0.5-µm film thickness). Temperature program: 150 °C (1.0 min hold) to 280 °C (17 min hold) at 8 °C/min.

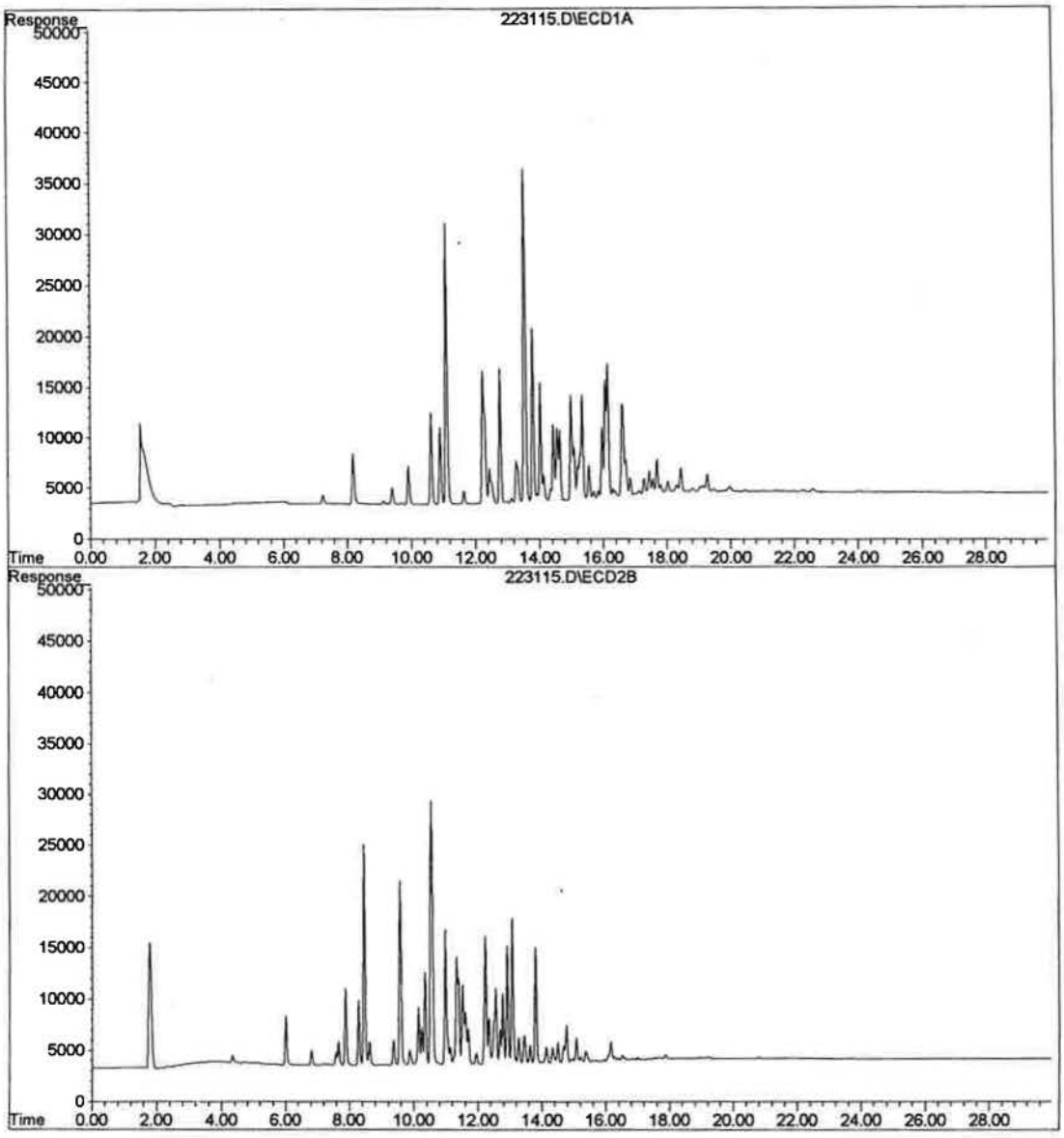


FIGURE 4. Example GC/ECD chromatogram of Aroclor 1242 analyzed on a Rtx-5/HP-608 column pair connected to separate injectors. The top trace is the Rtx-5 column (30-m x 0.53-mm ID, 1.5-µm film thickness) and the bottom trace is the HP-608 column (30-m x 0.53-mm ID, 0.5-µm film thickness). Temperature program: 150 °C (1.0 min hold) to 280 °C (17 min hold) at 8 °C/min.

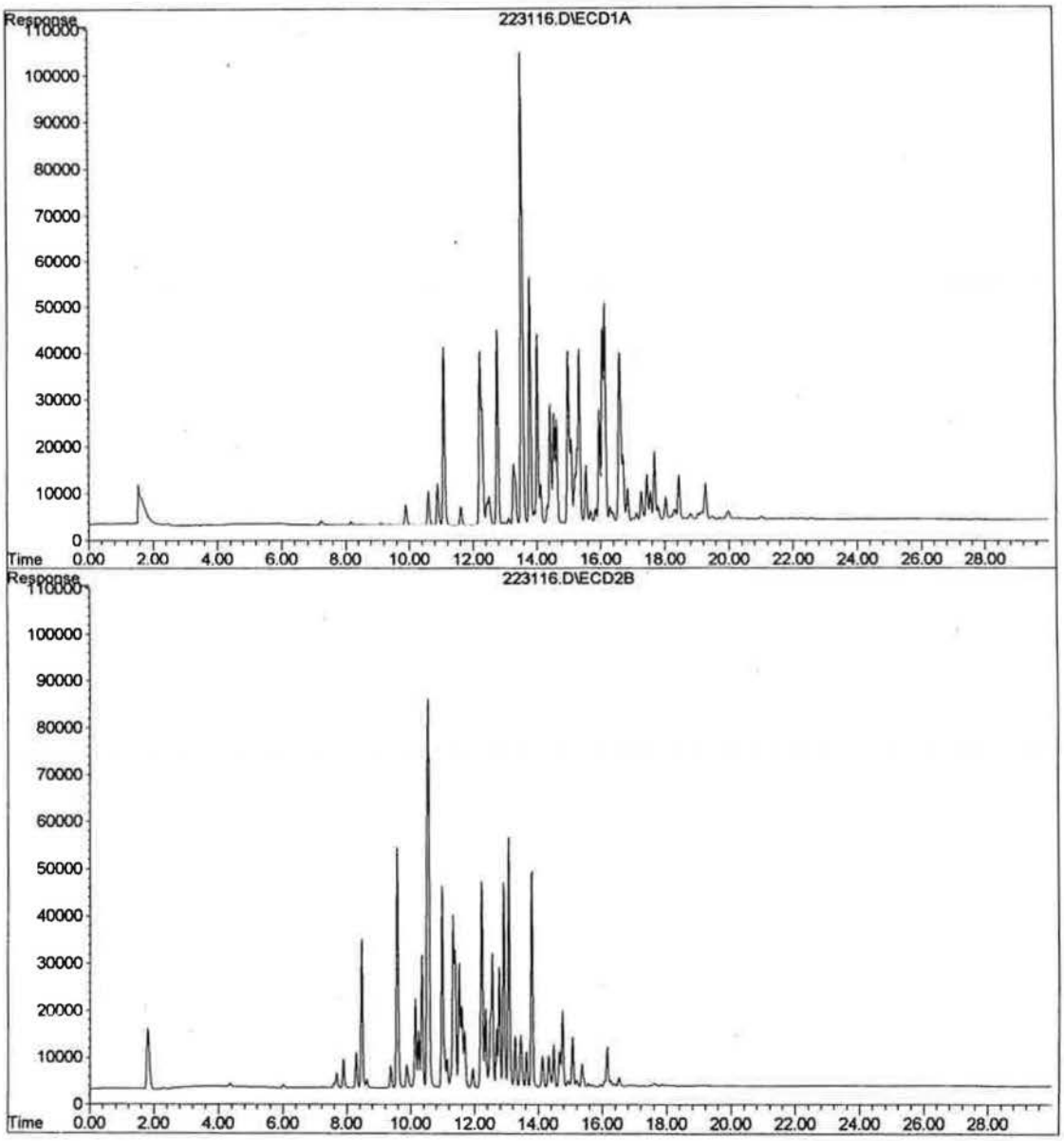


FIGURE 5. Example GC/ECD chromatogram of Aroclor 1248 analyzed on a Rtx-5/HP-608 column pair connected to separate injectors. The top trace is the Rtx-5 column (30-m x 0.53-mm ID, 1.5-µm film thickness) and the bottom trace is the HP-608 column (30-m x 0.53-mm ID, 0.5-µm film thickness). Temperature program: 150 °C (1.0 min hold) to 280 °C (17 min hold) at 8 °C/min.

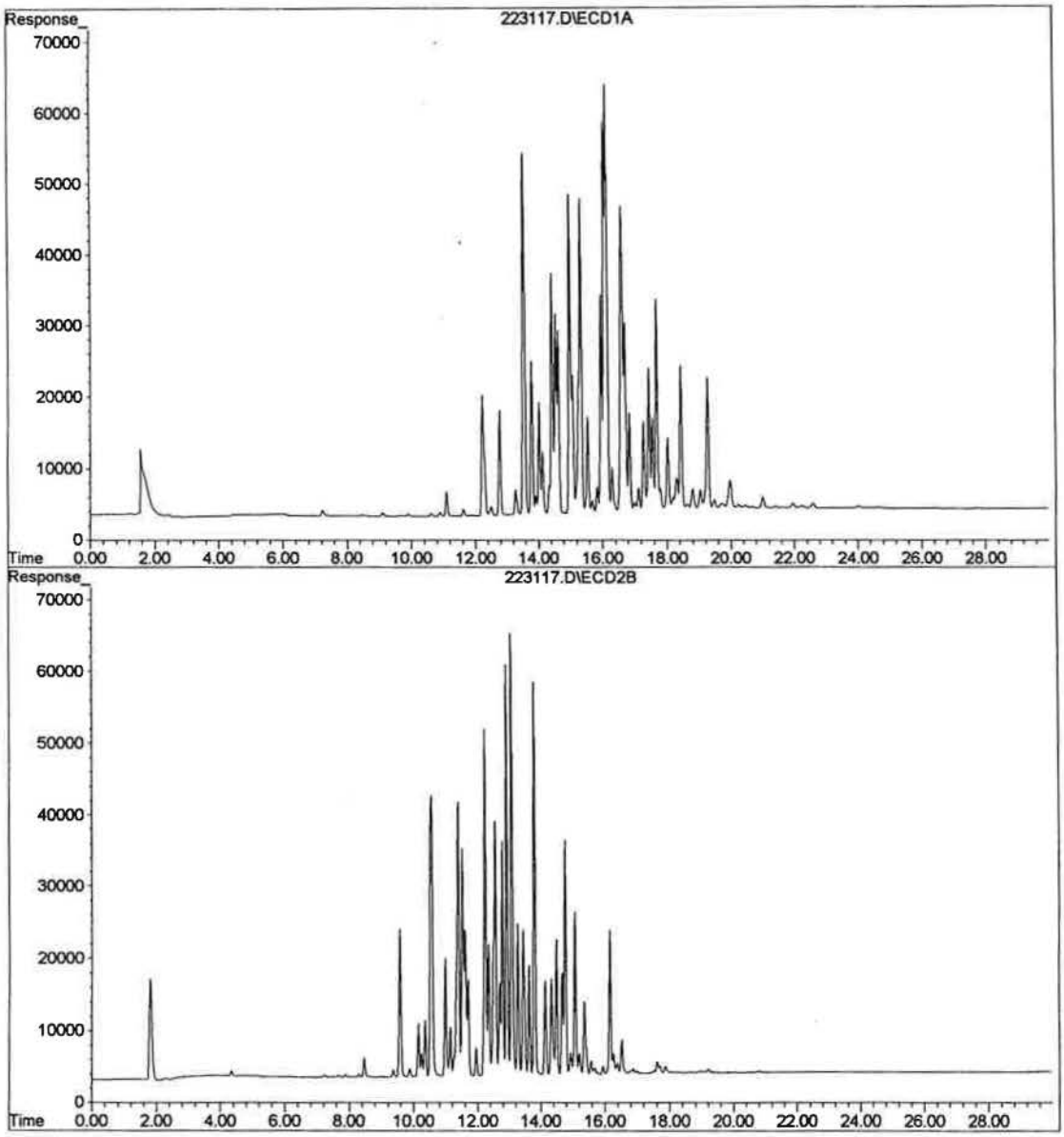
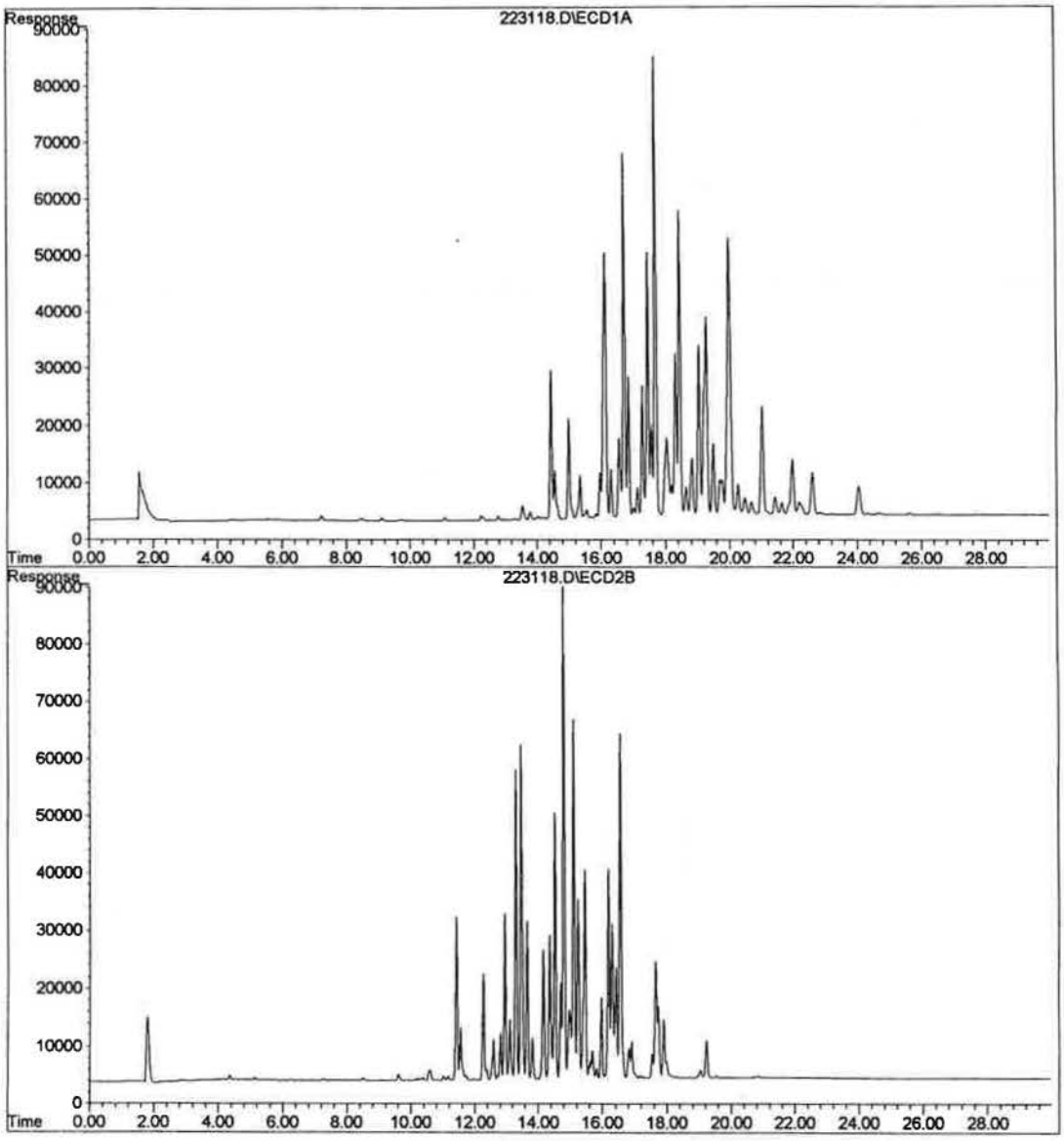


FIGURE 6. Example GC/ECD chromatogram of Aroclor 1254 analyzed on a Rtx-5/HP-608 column pair connected to separate injectors. The top trace is the Rtx-5 column (30-m x 0.53-mm ID, 1.5-µm film thickness) and the bottom trace is the HP-608 column (30-m x 0.53-mm ID, 0.5-µm film thickness). Temperature program: 150 °C (1.0 min hold) to 280 °C (17 min hold) at 8 °C/min.



METHOD 8260B
VOLATILE ORGANIC COMPOUNDS BY GAS CHROMATOGRAPHY/
MASS SPECTROMETRY (GC/MS)

1.0 SCOPE AND APPLICATION

1.1 Method 8260 is used to determine volatile organic compounds in a variety of solid waste matrices. This method is applicable to nearly all types of samples, regardless of water content, including various air sampling trapping media, ground and surface water, aqueous sludges, caustic liquors, acid liquors, waste solvents, oily wastes, mousses, tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils, and sediments. The following compounds can be determined by this method:

		Appropriate Preparation Technique[a]					
Compound	CAS No.[b]	5030/ 5035	5031	5032	5021	5041	Direct Inject.
Acetone	67-64-1	pp	c	c	nd	c	c
Acetonitrile	75-05-8	pp	c	nd	nd	nd	c
Acrolein (Propenal)	107-02-8	pp	c	c	nd	nd	c
Acrylonitrile	107-13-1	pp	c	c	nd	c	c
Allyl alcohol	107-18-6	ht	c	nd	nd	nd	c
Allyl chloride	107-05-1	c	nd	nd	nd	nd	c
Benzene	71-43-2	c	nd	c	c	c	c
Benzyl chloride	100-44-7	c	nd	nd	nd	nd	c
Bis(2-chloroethyl)sulfide	505-60-2	pp	nd	nd	nd	nd	c
Bromoacetone	598-31-2	pp	nd	nd	nd	nd	c
Bromochloromethane	74-97-5	c	nd	c	c	c	c
Bromodichloromethane	75-27-4	c	nd	c	c	c	c
4-Bromofluorobenzene (surr)	460-00-4	c	nd	c	c	c	c
Bromoform	75-25-2	c	nd	c	c	c	c
Bromomethane	74-83-9	c	nd	c	c	c	c
n-Butanol	71-36-3	ht	c	nd	nd	nd	c
2-Butanone (MEK)	78-93-3	pp	c	c	nd	nd	c
t-Butyl alcohol	75-65-0	pp	c	nd	nd	nd	c
Carbon disulfide	75-15-0	pp	nd	c	nd	c	c
Carbon tetrachloride	56-23-5	c	nd	c	c	c	c
Chloral hydrate	302-17-0	pp	nd	nd	nd	nd	c
Chlorobenzene	108-90-7	c	nd	c	c	c	c
Chlorobenzene-d_5 (IS)		c	nd	c	c	c	c
Chlorodibromomethane	124-48-1	c	nd	c	nd	c	c
Chloroethane	75-00-3	c	nd	c	c	c	c
2-Chloroethanol	107-07-3	pp	nd	nd	nd	nd	c
2-Chloroethyl vinyl ether	110-75-8	c	nd	c	nd	nd	c
Chloroform	67-66-3	c	nd	c	c	c	c
Chloromethane	74-87-3	c	nd	c	c	c	c
Chloroprene	126-99-8	c	nd	nd	nd	nd	c
3-Chloropropionitrile	542-76-7	l	nd	nd	nd	nd	pc

(continued)

Compound	CAS No.[b]	Appropriate Preparation Technique[a] 5030/ 5035	5031	5032	5021	5041	Direct Inject.
Crotonaldehyde	4170-30-3	pp	c	nd	nd	nd	c
1,2-Dibromo-3-chloropropane	96-12-8	pp	nd	nd	c	nd	c
1,2-Dibromoethane	106-93-4	c	nd	nd	c	nd	c
Dibromomethane	74-95-3	c	nd	c	c	c	c
1,2-Dichlorobenzene	95-50-1	c	nd	nd	c	nd	c
1,3-Dichlorobenzene	541-73-1	c	nd	nd	c	nd	c
1,4-Dichlorobenzene	106-46-7	c	nd	nd	c	nd	c
1,4-Dichlorobenzene-d_4 (IS)		c	nd	nd	c	nd	c
cis-1,4-Dichloro-2-butene	1476-11-5	c	nd	c	nd	nd	c
trans-1,4-Dichloro-2-butene	110-57-6	pp	nd	c	nd	nd	c
Dichlorodifluoromethane	75-71-8	c	nd	c	c	nd	c
1,1-Dichloroethane	75-34-3	c	nd	c	c	c	c
1,2-Dichloroethane	107-06-2	c	nd	c	c	c	c
1,2-Dichloroethane-d_4 (surr)		c	nd	c	c	c	c
1,1-Dichloroethene	75-35-4	c	nd	c	c	c	c
trans-1,2-Dichloroethene	156-60-5	c	nd	c	c	c	c
1,2-Dichloropropane	78-87-5	c	nd	c	c	c	c
1,3-Dichloro-2-propanol	96-23-1	pp	nd	nd	nd	nd	c
cis-1,3-Dichloropropene	10061-01-5	c	nd	c	nd	c	c
trans-1,3-Dichloropropene	10061-02-6	c	nd	c	nd	c	c
1,2,3,4-Diepoxybutane	1464-53-5	c	nd	nd	nd	nd	c
Diethyl ether	60-29-7	c	nd	nd	nd	nd	c
1,4-Difluorobenzene (IS)	540-36-3	nd	nd	nd	nd	c	nd
1,4-Dioxane	123-91-1	pp	c	c	nd	nd	c
Epichlorohydrin	106-89-8	l	nd	nd	nd	nd	c
Ethanol	64-17-5	l	c	c	nd	nd	c
Ethyl acetate	141-78-6	l	c	nd	nd	nd	c
Ethylbenzene	100-41-4	c	nd	c	c	c	c
Ethylene oxide	75-21-8	pp	c	nd	nd	nd	c
Ethyl methacrylate	97-63-2	c	nd	c	nd	nd	c
Fluorobenzene (IS)	462-06-6	c	nd	nd	nd	nd	nd
Hexachlorobutadiene	87-68-3	c	nd	nd	c	nd	c
Hexachloroethane	67-72-1	l	nd	nd	nd	nd	c
2-Hexanone	591-78-6	pp	nd	c	nd	nd	c
2-Hydroxypropionitrile	78-97-7	l	nd	nd	nd	nd	pc
Iodomethane	74-88-4	c	nd	c	nd	c	c
Isobutyl alcohol	78-83-1	pp	c	nd	nd	nd	c
Isopropylbenzene	98-82-8	c	nd	nd	c	nd	c
Malononitrile	109-77-3	pp	nd	nd	nd	nd	c
Methacrylonitrile	126-98-7	pp	l	nd	nd	nd	c
Methanol	67-56-1	l	c	nd	nd	nd	c
Methylene chloride	75-09-2	c	nd	c	c	c	c
Methyl methacrylate	80-62-6	c	nd	nd	nd	nd	c
4-Methyl-2-pentanone (MIBK)	108-10-1	pp	c	c	nd	nd	c
Naphthalene	91-20-3	c	nd	nd	c	nd	c

(continued)

Compound	CAS No.[b]	Appropriate Preparation Technique[a] 5030/5035	5031	5032	5021	5041	Direct Inject.
Nitrobenzene	98-95-3	c	nd	nd	nd	nd	c
2-Nitropropane	79-46-9	c	nd	nd	nd	nd	c
N-Nitroso-di-n-butylamine	924-16-3	pp	c	nd	nd	nd	c
Paraldehyde	123-63-7	pp	c	nd	nd	nd	c
Pentachloroethane	76-01-7	I	nd	nd	nd	nd	c
2-Pentanone	107-87-9	pp	c	nd	nd	nd	c
2-Picoline	109-06-8	pp	c	nd	nd	nd	c
1-Propanol	71-23-8	pp	c	nd	nd	nd	c
2-Propanol	67-63-0	pp	c	nd	nd	nd	c
Propargyl alcohol	107-19-7	pp	I	nd	nd	nd	c
β-Propiolactone	57-57-8	pp	nd	nd	nd	nd	c
Propionitrile (ethyl cyanide)	107-12-0	ht	c	nd	nd	nd	pc
n-Propylamine	107-10-8	c	nd	nd	nd	nd	c
Pyridine	110-86-1	I	c	nd	nd	nd	c
Styrene	100-42-5	c	nd	c	c	c	c
1,1,1,2-Tetrachloroethane	630-20-6	c	nd	nd	c	c	c
1,1,2,2-Tetrachloroethane	79-34-5	c	nd	c	c	c	c
Tetrachloroethene	127-18-4	c	nd	c	c	c	c
Toluene	108-88-3	c	nd	c	c	c	c
Toluene-d_8 (surr)	2037-26-5	c	nd	c	c	c	c
o-Toluidine	95-53-4	pp	c	nd	nd	nd	c
1,2,4-Trichlorobenzene	120-82-1	c	nd	nd	c	nd	c
1,1,1-Trichloroethane	71-55-6	c	nd	c	c	c	c
1,1,2-Trichloroethane	79-00-5	c	nd	c	c	c	c
Trichloroethene	79-01-6	c	nd	c	c	c	c
Trichlorofluoromethane	75-69-4	c	nd	c	c	c	c
1,2,3-Trichloropropane	96-18-4	c	nd	c	c	c	c
Vinyl acetate	108-05-4	c	nd	c	nd	nd	c
Vinyl chloride	75-01-4	c	nd	c	c	c	c
o-Xylene	95-47-6	c	nd	c	c	c	c
m-Xylene	108-38-3	c	nd	c	c	c	c
p-Xylene	106-42-3	c	nd	c	c	c	c

[a] See Sec. 1.2 for other appropriate sample preparation techniques
[b] Chemical Abstract Service Registry Number

c = Adequate response by this technique
ht = Method analyte only when purged at 80°C
nd = Not determined
I = Inappropriate technique for this analyte
pc = Poor chromatographic behavior
pp = Poor purging efficiency resulting in high Estimated Quantitation Limits
surr = Surrogate
IS = Internal Standard

1.2 There are various techniques by which these compounds may be introduced into the GC/MS system. The more common techniques are listed in the table above. Purge-and-trap, by Methods 5030 (aqueous samples) and 5035 (solid and waste oil samples), is the most commonly used technique for volatile organic analytes. However, other techniques are also appropriate and necessary for some analytes. These include direct injection following dilution with hexadecane (Method 3585) for waste oil samples; automated static headspace by Method 5021 for solid samples; direct injection of an aqueous sample (concentration permitting) or injection of a sample concentrated by azeotropic distillation (Method 5031); and closed system vacuum distillation (Method 5032) for aqueous, solid, oil and tissue samples. For air samples, Method 5041 provides methodology for desorbing volatile organics from trapping media (Methods 0010, 0030, and 0031). In addition, direct analysis utilizing a sample loop is used for sub-sampling from Tedlar® bags (Method 0040). Method 5000 provides more general information on the selection of the appropriate introduction method.

1.3 Method 8260 can be used to quantitate most volatile organic compounds that have boiling points below 200°C. Volatile, water soluble compounds can be included in this analytical technique by the use of azeotropic distillation or closed-system vacuum distillation. Such compounds include low molecular weight halogenated hydrocarbons, aromatics, ketones, nitriles, acetates, acrylates, ethers, and sulfides. See Tables 1 and 2 for analytes and retention times that have been evaluated on a purge-and-trap GC/MS system. Also, the method detection limits for 25-mL sample volumes are presented. The following compounds are also amenable to analysis by Method 8260:

Bromobenzene	1,3-Dichloropropane
n-Butylbenzene	2,2-Dichloropropane
sec-Butylbenzene	1,1-Dichloropropene
tert-Butylbenzene	p-Isopropyltoluene
Chloroacetonitrile	Methyl acrylate
1-Chlorobutane	Methyl-t-butyl ether
1-Chlorohexane	Pentafluorobenzene
2-Chlorotoluene	n-Propylbenzene
4-Chlorotoluene	1,2,3-Trichlorobenzene
Dibromofluoromethane	1,2,4-Trimethylbenzene
cis-1,2-Dichloroethene	1,3,5-Trimethylbenzene

1.4 The estimated quantitation limit (EQL) of Method 8260 for an individual compound is somewhat instrument dependent and also dependent on the choice of sample preparation/introduction method. Using standard quadrapole instrumentation and the purge-and-trap technique, limits should be approximately 5 µg/kg (wet weight) for soil/sediment samples, 0.5 mg/kg (wet weight) for wastes, and 5 µg/L for ground water (see Table 3). Somewhat lower limits may be achieved using an ion trap mass spectrometer or other instrumentation of improved design. No matter which instrument is used, EQLs will be proportionately higher for sample extracts and samples that require dilution or when a reduced sample size is used to avoid saturation of the detector.

1.5 This method is restricted to use by, or under the supervision of, analysts experienced in the use of gas chromatograph/mass spectrometers, and skilled in the interpretation of mass spectra and their use as a quantitative tool.

2.0 SUMMARY OF METHOD

2.1 The volatile compounds are introduced into the gas chromatograph by the purge-and-trap method or by other methods (see Sec. 1.2). The analytes are introduced directly to a wide-bore capillary column or cryofocussed on a capillary pre-column before being flash evaporated to a narrow-bore capillary for analysis. The column is temperature-programmed to separate the analytes, which are then detected with a mass spectrometer (MS) interfaced to the gas chromatograph (GC).

2.2 Analytes eluted from the capillary column are introduced into the mass spectrometer via a jet separator or a direct connection. (Wide-bore capillary columns normally require a jet separator, whereas narrow-bore capillary columns may be directly interfaced to the ion source). Identification of target analytes is accomplished by comparing their mass spectra with the electron impact (or electron impact-like) spectra of authentic standards. Quantitation is accomplished by comparing the response of a major (quantitation) ion relative to an internal standard using a five-point calibration curve.

2.3 The method includes specific calibration and quality control steps that supersede the general requirements provided in Method 8000.

3.0 INTERFERENCES

3.1 Major contaminant sources are volatile materials in the laboratory and impurities in the inert purging gas and in the sorbent trap. The use of non-polytetrafluoroethylene (PTFE) thread sealants, plastic tubing, or flow controllers with rubber components should be avoided, since such materials out-gas organic compounds which will be concentrated in the trap during the purge operation. Analyses of calibration and reagent blanks provide information about the presence of contaminants. When potential interfering peaks are noted in blanks, the analyst should change the purge gas source and regenerate the molecular sieve purge gas filter. Subtracting blank values from sample results is not permitted. If reporting values without correcting for the blank results in what the laboratory feels is a false positive result for a sample, the laboratory should fully explained this in text accompanying the uncorrected data.

3.2 Contamination may occur when a sample containing low concentrations of volatile organic compounds is analyzed immediately after a sample containing high concentrations of volatile organic compounds. A technique to prevent this problem is to rinse the purging apparatus and sample syringes with two portions of organic-free reagent water between samples. After the analysis of a sample containing high concentrations of volatile organic compounds, one or more blanks should be analyzed to check for cross-contamination. Alternatively, if the sample immediately following the high concentration sample does not contain the volatile organic compounds present in the high level sample, freedom from contamination has been established.

3.3 For samples containing large amounts of water-soluble materials, suspended solids, high boiling compounds, or high concentrations of compounds being determined, it may be necessary to wash the purging device with a soap solution, rinse it with organic-free reagent water, and then dry the purging device in an oven at 105°C. In extreme situations, the entire purge-and-trap device may require dismantling and cleaning. Screening of the samples prior to purge-and-trap GC/MS analysis is highly recommended to prevent contamination of the system. This is especially true for soil and waste samples. Screening may be accomplished with an automated headspace technique (Method 5021) or by Method 3820 (Hexadecane Extraction and Screening of Purgeable Organics).

3.4 Many analytes exhibit low purging efficiencies from a 25-mL sample. This often results in significant amounts of these analytes remaining in the sample purge vessel after analysis. After removal of the sample aliquot that was purged, and rinsing the purge vessel three times with organic-free water, the empty vessel should be subjected to a heated purge cycle prior to the analysis of another sample in the same purge vessel. This will reduce sample-to-sample carryover.

3.5 Special precautions must be taken to analyze for methylene chloride. The analytical and sample storage area should be isolated from all atmospheric sources of methylene chloride. Otherwise, random background levels will result. Since methylene chloride will permeate through PTFE tubing, all gas chromatography carrier gas lines and purge gas plumbing should be constructed from stainless steel or copper tubing. Laboratory clothing worn by the analyst should be clean, since clothing previously exposed to methylene chloride fumes during liquid/liquid extraction procedures can contribute to sample contamination.

3.6 Samples can be contaminated by diffusion of volatile organics (particularly methylene chloride and fluorocarbons) through the septum seal of the sample container into the sample during shipment and storage. A trip blank prepared from organic-free reagent water and carried through the sampling, handling, and storage protocols can serve as a check on such contamination.

3.7 Use of sensitive mass spectrometers to achieve lower detection level will increase the potential to detect laboratory contaminants as interferences.

3.8 Direct injection - Some contamination may be eliminated by baking out the column between analyses. Changing the injector liner will reduce the potential for cross-contamination. A portion of the analytical column may need to be removed in the case of extreme contamination. The use of direct injection will result in the need for more frequent instrument maintenance.

3.9 If hexadecane is added to waste samples or petroleum samples that are analyzed, some chromatographic peaks will elute after the target analytes. The oven temperature program must include a post-analysis bake out period to ensure that semivolatile hydrocarbons are volatilized.

4.0 APPARATUS AND MATERIALS

4.1 Purge-and-trap device for aqueous samples - Described in Method 5030.

4.2 Purge-and-trap device for solid samples - Described in Method 5035.

4.3 Automated static headspace device for solid samples - Described in Method 5021.

4.4 Azeotropic distillation apparatus for aqueous and solid samples - Described in Method 5031.

4.5 Vacuum distillation apparatus for aqueous, solid and tissue samples - Described in Method 5032.

4.6 Desorption device for air trapping media for air samples - Described in Method 5041.

4.7 Air sampling loop for sampling from Tedlar® bags for air samples - Described in Method 0040.

4.8 Injection port liners (HP Catalog #18740-80200, or equivalent) - modified for direct injection analysis by placing a 1-cm plug of glass wool approximately 50-60 mm down the length of the injection port towards the oven (see illustration below). A 0.53-mm ID column is mounted 1 cm into the liner from the oven side of the injection port, according to manufacturer's specifications.

4.9 Gas chromatography/mass spectrometer/data system

4.9.1 Gas chromatograph - An analytical system complete with a temperature-programmable gas chromatograph suitable for splitless injection with appropriate interface for sample introduction device. The system includes all required accessories, including syringes, analytical columns, and gases.

4.9.1.1 The GC should be equipped with variable constant differential flow controllers so that the column flow rate will remain constant throughout desorption and temperature program operation.

4.9.1.2 For some column configurations, the column oven must be cooled to less than 30°C, therefore, a subambient oven controller may be necessary.

4.9.1.3 The capillary column is either directly coupled to the source or interfaced through a jet separator, depending on the size of the capillary and the requirements of the GC/MS system.

4.9.1.4 Capillary pre-column interface - This device is the interface between the sample introduction device and the capillary gas chromatograph, and is necessary when using cryogenic cooling. The interface condenses the desorbed sample components and focuses them into a narrow band on an uncoated fused-silica capillary pre-column. When the interface is flash heated, the sample is transferred to the analytical capillary column.

4.9.1.5 During the cryofocussing step, the temperature of the fused-silica in the interface is maintained at -150°C under a stream of liquid nitrogen. After the desorption period, the interface must be capable of rapid heating to 250°C in 15 seconds or less to complete the transfer of analytes.

4.9.2 Gas chromatographic columns

4.9.2.1 Column 1 - 60 m x 0.75 mm ID capillary column coated with VOCOL (Supelco), 1.5-µm film thickness, or equivalent.

4.9.2.2 Column 2 - 30 - 75 m x 0.53 mm ID capillary column coated with DB-624 (J&W Scientific), Rt_x-502.2 (RESTEK), or VOCOL (Supelco), 3-µm film thickness, or equivalent.

4.9.2.3 Column 3 - 30 m x 0.25 - 0.32 mm ID capillary column coated with 95% dimethyl - 5% diphenyl polysiloxane (DB-5, Rt_x-5, SPB-5, or equivalent), 1-µm film thickness.

4.9.2.4 Column 4 - 60 m x 0.32 mm ID capillary column coated with DB-624 (J&W Scientific), 1.8-µm film thickness, or equivalent.

4.9.3 Mass spectrometer - Capable of scanning from 35 to 300 amu every 2 sec or less, using 70 volts (nominal) electron energy in the electron impact ionization mode. The mass spectrometer must be capable of producing a mass spectrum for 4-Bromofluorobenzene (BFB) which meets all of the criteria in Table 4 when 5-50 ng of the GC/MS tuning standard (BFB) are injected through the GC. To ensure sufficient precision of mass spectral data, the desirable MS scan rate allows acquisition of at least five spectra while a sample component elutes from the GC.

An ion trap mass spectrometer may be used if it is capable of axial modulation to reduce ion-molecule reactions and can produce electron impact-like spectra that match those in the EPA/NIST Library. Because ion-molecule reactions with water and methanol in an ion trap mass spectrometer may produce interferences that coelute with chloromethane and chloroethane, the base peak for both of these analytes will be at m/z 49. This ion should be used as the quantitation ion in this case. The mass spectrometer must be capable of producing a mass spectrum for BFB which meets all of the criteria in Table 3 when 5 or 50 ng are introduced.

4.9.4 GC/MS interface - Two alternatives may be used to interface the GC to the mass spectrometer.

4.9.4.1 Direct coupling, by inserting the column into the mass spectrometer, is generally used for 0.25 - 0.32 mm ID columns.

4.9.4.2 A jet separator, including an all-glass transfer line and glass enrichment device or split interface, is used with a 0.53 mm column.

4.9.4.3 Any enrichment device or transfer line may be used, if all of the performance specifications described in Sec. 8.0 (including acceptable calibration at 50 ng or less) can be achieved. GC/MS interfaces constructed entirely of glass or of glass-lined materials are recommended. Glass may be deactivated by silanizing with dichlorodimethylsilane.

4.9.5 Data system - A computer system that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program must be interfaced to the mass spectrometer. The computer must have software that allows searching any GC/MS data file for ions of a specified mass and plotting such ion abundances versus time or scan number. This type of plot is defined as an Extracted Ion Current Profile (EICP). Software must also be available that allows integrating the abundances in any EICP between specified time or scan-number limits. The most recent version of the EPA/NIST Mass Spectral Library should also be available.

4.10 Microsyringes - 10-, 25-, 100-, 250-, 500-, and 1,000-µL.

4.11 Syringe valve - Two-way, with Luer ends (three each), if applicable to the purging device.

4.12 Syringes - 5-, 10-, or 25-mL, gas-tight with shutoff valve.

4.13 Balance - Analytical, capable of weighing 0.0001 g, and top-loading, capable of weighing 0.1 g.

4.14 Glass scintillation vials - 20-mL, with PTFE-lined screw-caps or glass culture tubes with PTFE-lined screw-caps.

4.15 Vials - 2-mL, for GC autosampler.

4.16 Disposable pipets - Pasteur.

4.17 Volumetric flasks, Class A - 10-mL and 100-mL, with ground-glass stoppers.

4.18 Spatula - Stainless steel.

5.0 REAGENTS

5.1 Reagent grade inorganic chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all inorganic reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.3 Methanol, CH_3OH - Pesticide quality or equivalent, demonstrated to be free of analytes. Store apart from other solvents.

5.4 Reagent Hexadecane - Reagent hexadecane is defined as hexadecane in which interference is not observed at the method detection limit of compounds of interest. Hexadecane quality is demonstrated through the analysis of a solvent blank injected directly into the GC/MS. The results of such a blank analysis must demonstrate that all interfering volatiles have been removed from the hexadecane.

5.5 Polyethylene glycol, $H(OCH_2CH_2)_nOH$ - Free of interferences at the detection limit of the target analytes.

5.6 Hydrochloric acid (1:1 v/v), HCl - Carefully add a measured volume of concentrated HCl to an equal volume of organic-free reagent water.

5.7 Stock solutions - Stock solutions may be prepared from pure standard materials or purchased as certified solutions. Prepare stock standard solutions in methanol, using assayed liquids or gases, as appropriate.

5.7.1 Place about 9.8 mL of methanol in a 10-mL tared ground-glass-stoppered volumetric flask. Allow the flask to stand, unstoppered, for about 10 minutes or until all alcohol-wetted surfaces have dried. Weigh the flask to the nearest 0.0001 g.

5.7.2 Add the assayed reference material, as described below.

5.7.2.1 Liquids - Using a 100-µL syringe, immediately add two or more drops of assayed reference material to the flask; then reweigh. The liquid must fall directly into the alcohol without contacting the neck of the flask.

5.7.2.2 Gases - To prepare standards for any compounds that boil below 30°C (e.g., bromomethane, chloroethane, chloromethane, or vinyl chloride), fill a 5-mL valved gas-tight syringe with the reference standard to the 5.0 mL mark. Lower the needle to

5 mm above the methanol meniscus. Slowly introduce the reference standard above the surface of the liquid. The heavy gas will rapidly dissolve in the methanol. Standards may also be prepared by using a lecture bottle equipped with a septum. Attach PTFE tubing to the side arm relief valve and direct a gentle stream of gas into the methanol meniscus.

5.7.3 Reweigh, dilute to volume, stopper, and then mix by inverting the flask several times. Calculate the concentration in milligrams per liter (mg/L) from the net gain in weight. When compound purity is assayed to be 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially-prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

5.7.4 Transfer the stock standard solution into a bottle with a PTFE-lined screw-cap. Store, with minimal headspace and protected from light, at -10°C or less or as recommended by the standard manufacturer. Standards should be returned to the freezer as soon as the analyst has completed mixing or diluting the standards to prevent the evaporation of volatile target compounds.

5.7.5 Frequency of Standard Preparation

5.7.5.1 Standards for the permanent gases should be monitored frequently by comparison to the initial calibration curve. Fresh standards should be prepared if this check exceeds a 20% drift. Standards for gases usually need to be replaced after one week or as recommended by the standard manufacturer, unless the acceptability of the standard can be documented. Dichlorodifluoromethane and dichloromethane will usually be the first compounds to evaporate from the standard and should, therefore, be monitored very closely when standards are held beyond one week.

5.7.5.2 Standards for the non-gases should be monitored frequently by comparison to the initial calibration. Fresh standards should be prepared if this check exceeds a 20% drift. Standards for non-gases usually need to be replaced after six months or as recommended by the standard manufacturer, unless the acceptability of the standard can be documented. Standards of reactive compounds such as 2-chloroethyl vinyl ether and styrene may need to be prepared more frequently.

5.7.6 Preparation of Calibration Standards From a Gas Mixture

An optional calibration procedure involves using a certified gaseous mixture daily, utilizing a commercially-available gaseous analyte mixture of bromomethane, chloromethane, chloroethane, vinyl chloride, dichloro-difluoromethane and trichlorofluoromethane in nitrogen. Mixtures of documented quality are stable for as long as six months without refrigeration. (VOA-CYL III, RESTEK Corporation, Cat. #20194 or equivalent).

5.7.6.1 Before removing the cylinder shipping cap, be sure the valve is completely closed (turn clockwise). The contents are under pressure and should be used in a well-ventilated area.

5.7.6.2 Wrap the pipe thread end of the Luer fitting with PTFE tape. Remove the shipping cap from the cylinder and replace it with the Luer fitting.

5.7.6.3 Transfer half the working standard containing other analytes, internal standards, and surrogates to the purge apparatus.

5.7.6.4 Purge the Luer fitting and stem on the gas cylinder prior to sample removal using the following sequence:

a) Connect either the 100-µL or 500-µL Luer syringe to the inlet fitting of the cylinder.

b) Make sure the on/off valve on the syringe is in the open position.

c) Slowly open the valve on the cylinder and withdraw a full syringe volume.

d) Be sure to close the valve on the cylinder before you withdraw the syringe from the Luer fitting.

e) Expel the gas from the syringe into a well-ventilated area.

f) Repeat steps a through e one more time to fully purge the fitting.

5.7.6.5 Once the fitting and stem have been purged, quickly withdraw the volume of gas you require using steps 5.6.6.1.4(a) through (d). Be sure to close the valve on the cylinder and syringe before you withdraw the syringe from the Luer fitting.

5.7.6.6 Open the syringe on/off valve for 5 seconds to reduce the syringe pressure to atmospheric pressure. The pressure in the cylinder is ~30 psi.

5.7.6.7 The gas mixture should be quickly transferred into the reagent water through the female Luer fitting located above the purging vessel.

NOTE: Make sure the arrow on the 4-way valve is pointing toward the female Luer fitting when transferring the sample from the syringe. Be sure to switch the 4-way valve back to the closed position before removing the syringe from the Luer fitting.

5.7.6.8 Transfer the remaining half of the working standard into the purging vessel. This procedure insures that the total volume of gas mix is flushed into the purging vessel, with none remaining in the valve or lines.

5.7.6.9 The concentration of each compound in the cylinder is typically 0.0025 µg/µL.

5.7.6.10 The following are the recommended gas volumes spiked into 5 mL of water to produce a typical 5-point calibration:

Gas Volume	Calibration Concentration
40 µL	20 µg/L
100 µL	50 µg/L
200 µL	100 µg/L
300 µL	150 µg/L
400 µL	200 µg/L

5.7.6.11 The following are the recommended gas volumes spiked into 25 mL of water to produce a typical 5-point calibration:

Gas Volume	Calibration Concentration
10 µL	1 µg/L
20 µL	2 µg/L
50 µL	5 µg/L
100 µL	10 µg/L
250 µL	25 µg/L

5.8 Secondary dilution standards - Using stock standard solutions, prepare secondary dilution standards in methanol containing the compounds of interest, either singly or mixed together. Secondary dilution standards must be stored with minimal headspace and should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Store in a vial with no headspace. Replace after one week. Secondary standards for gases should be replaced after one week unless the acceptability of the standard can be documented. When using premixed certified solutions, store according to the manufacturer's documented holding time and storage temperature recommendations. The analyst should also handle and store standards as stated in Sec. 5.7.4 and return them to the freezer as soon as standard mixing or diluting is completed to prevent the evaporation of volatile target compounds.

5.9 Surrogate standards - The recommended surrogates are toluene-d_8, 4-bromofluorobenzene, 1,2-dichloroethane-d_4, and dibromofluoromethane. Other compounds may be used as surrogates, depending upon the analysis requirements. A stock surrogate solution in methanol should be prepared as described above, and a surrogate standard spiking solution should be prepared from the stock at a concentration of 50-250 µg/10 mL, in methanol. Each sample undergoing GC/MS analysis must be spiked with 10 µL of the surrogate spiking solution prior to analysis. If a more sensitive mass spectrometer is employed to achieve lower detection levels, then more dilute surrogate solutions may be required.

5.10 Internal standards - The recommended internal standards are fluorobenzene, chlorobenzene-d_5, and 1,4-dichlorobenzene-d_4. Other compounds may be used as internal standards as long as they have retention times similar to the compounds being detected by GC/MS. Prepare internal standard stock and secondary dilution standards in methanol using the procedures described in Secs. 5.7 and 5.8. It is recommended that the secondary dilution standard be prepared at a concentration of 25 mg/L of each internal standard compound. Addition of 10 µL of this standard to 5.0 mL of sample or calibration standard would be the equivalent of 50 µg/L. If a more sensitive mass spectrometer is employed to achieve lower detection levels, then more dilute internal standard solutions may be required. Area counts of the internal standard peaks should be between 50-200% of the areas of the target analytes in the mid-point calibration analysis.

5.11 4-Bromofluorobenzene (BFB) standard - A standard solution containing 25 ng/µL of BFB in methanol should be prepared. If a more sensitive mass spectrometer is employed to achieve lower detection levels, then a more dilute BFB standard solution may be required.

5.12 Calibration standards -There are two types of calibration standards used for this method: initial calibration standards and calibration verification standards. When using premixed certified solutions, store according to the manufacturer's documented holding time and storage temperature recommendations.

5.12.1 Initial calibration standards should be prepared at a minimum of five different concentrations from the secondary dilution of stock standards (see Secs. 5.7 and 5.8) or from a premixed certified solution. Prepare these solutions in organic-free reagent water. At least one of the calibration standards should correspond to a sample concentration at or below that necessary to meet the data quality objectives of the project. The remaining standards should correspond to the range of concentrations found in typical samples but should not exceed the working range of the GC/MS system. Initial calibration standards should be mixed from fresh stock standards and dilution standards when generating an initial calibration curve.

5.12.2 Calibration verification standards should be prepared at a concentration near the mid-point of the initial calibration range from the secondary dilution of stock standards (see Secs. 5.7 and 5.8) or from a premixed certified solution. Prepare these solutions in organic-free reagent water. See Sec. 7.4 for guidance on calibration verification.

5.12.3 It is the intent of EPA that all target analytes for a particular analysis be included in the initial calibration and calibration verification standard(s). These target analytes may not include the entire list of analytes (Sec. 1.1) for which the method has been demonstrated. However, the laboratory shall not report a quantitative result for a target analyte that was not included in the calibration standard(s).

5.12.4 The calibration standards must also contain the internal standards chosen for the analysis.

5.13 Matrix spiking and laboratory control sample (LCS) standards - Matrix spiking standards should be prepared from volatile organic compounds which are representative of the compounds being investigated. At a minimum, the matrix spike should include 1,1-dichloroethene, trichloroethene, chlorobenzene, toluene, and benzene. The matrix spiking solution should contain compounds that are expected to be found in the types of samples to be analyzed.

5.13.1 Some permits may require the spiking of specific compounds of interest, especially if polar compounds are a concern, since the spiking compounds listed above would not be representative of such compounds. The standard should be prepared in methanol, with each compound present at a concentration of 250 µg/10.0 mL.

5.13.2 The spiking solutions should not be prepared from the same standards as the calibration standards. However, the same spiking standard prepared for the matrix spike may be used for the LCS.

5.13.3 If a more sensitive mass spectrometer is employed to achieve lower detection levels, more dilute matrix spiking solutions may be required.

5.14 Great care must be taken to maintain the integrity of all standard solutions. It is recommended all standards in methanol be stored at -10°C or less, in amber bottles with PTFE-lined screw-caps.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

See the introductory material to this chapter, Organic Analytes, Sec. 4.1.

7.0 PROCEDURE

7.1 Various alternative methods are provided for sample introduction. All internal standards, surrogates, and matrix spiking compounds (when applicable) must be added to the samples before introduction into the GC/MS system. Consult the sample introduction method for the procedures by which to add such standards.

7.1.1 Direct injection - This includes: injection of an aqueous sample containing a very high concentration of analytes; injection of aqueous concentrates from Method 5031 (azeotropic distillation); and injection of a waste oil diluted 1:1 with hexadecane (Method 3585). Direct injection of aqueous samples (non-concentrated) has very limited applications. It is only used for the determination of volatiles at the toxicity characteristic (TC) regulatory limits or at concentrations in excess of 10,000 µg/L. It may also be used in conjunction with the test for ignitability in aqueous samples (along with Methods 1010 and 1020), to determine if alcohol is present at greater than 24%.

7.1.2 Purge-and-trap - This includes purge-and-trap for aqueous samples (Method 5030) and purge-and-trap for solid samples (Method 5035). Method 5035 also provides techniques for extraction of high concentration solid and oily waste samples by methanol (and other water-miscible solvents) with subsequent purge-and-trap from an aqueous matrix using Method 5030.

7.1.2.1 Traditionally, the purge-and-trap of aqueous samples is performed at ambient temperature, while purging of soil/solid samples is performed at 40°C, to improve purging efficiency.

7.1.2.2 Aqueous and soil/solid samples may also be purged at temperatures above those being recommended as long as all calibration standards, samples, and QC samples are purged at the same temperature, appropriate trapping material is used to handle the excess water, and the laboratory demonstrates acceptable method performance for the project. Purging of aqueous samples at elevated temperatures (e.g., 40°C) may improve the purging performance of many of the water soluble compounds which have poor purging efficiencies at ambient temperatures.

7.1.3 Vacuum distillation - this technique may be used for the introduction of volatile organics from aqueous, solid, or tissue samples (Method 5032) into the GC/MS system.

7.1.4 Automated static headspace - this technique may be used for the introduction of volatile organics from solid samples (Method 5021) into the GC/MS system.

7.1.5 Cartridge desorption - this technique may be for the introduction of volatile organics from sorbent cartridges (Method 5041) used in the sampling of air. The sorbent cartridges are from the volatile organics sampling train (VOST) or SMVOC (Method 0031).

7.2 Recommended chromatographic conditions

7.2.1 General conditions

Injector temperature:	200 - 225°C
Transfer line temperature:	250 - 300°C

7.2.2 Column 1 and Column 2 with cryogenic cooling (example chromatograms are presented in Figures 1 and 2)

Carrier gas (He) flow rate:	15 mL/min
Initial temperature:	10°C, hold for 5 minutes
Temperature program:	6°C/min to 70°C, then 15°C/min to 145°C
Final temperature:	145°C, hold until all expected compounds have eluted.

7.2.5 Direct injection - Column 2

Carrier gas (He) flow rate:	4 mL/min
Column:	J&W DB-624, 70m x 0.53 mm
Initial temperature:	40°C, hold for 3 minutes
Temperature program:	8°C/min
Final temperature:	260°C, hold until all expected compounds have eluted.
Column Bake out:	75 minutes
Injector temperature:	200-225°C
Transfer line temperature:	250-300°C

7.2.6 Direct split interface - Column 4

Carrier gas (He) flow rate:	1.5 mL/min
Initial temperature:	35°C, hold for 2 minutes
Temperature program:	4°C/min to 50°C 10°C/min to 220°C
Final temperature:	220°C, hold until all expected compounds have eluted
Split ratio:	100:1
Injector temperature:	125°C

7.3 Initial calibration

Establish the GC/MS operating conditions, using the following as guidance:

Mass range:	35 - 260 amu
Scan time:	0.6 - 2 sec/scan
Source temperature:	According to manufacturer's specifications
Ion trap only:	Set axial modulation, manifold temperature, and emission current to manufacturer's recommendations

7.3.1 Each GC/MS system must be hardware-tuned to meet the criteria in Table 4 for a 5-50 ng injection or purging of 4-bromofluorobenzene (2-µL injection of the BFB standard). Analyses must not begin until these criteria are met.

7.3.1.1 In the absence of specific recommendations on how to acquire the mass spectrum of BFB from the instrument manufacturer, the following approach has been shown to be useful: The mass spectrum of BFB may be acquired in the following manner. Three scans (the peak apex scan and the scans immediately preceding and following the apex) are acquired and averaged. Background subtraction is required, and must be accomplished using a single scan no more than 20 scans prior to the elution of

BFB. Do not background subtract part of the BFB peak. Alternatively, the analyst may use other documented approaches suggested by the instrument manufacturer.

7.3.1.2 Use the BFB mass intensity criteria in Table 4 as tuning acceptance criteria. Alternatively, other documented tuning criteria may be used (e.g., CLP, Method 524.2, or manufacturer's instructions), provided that method performance is not adversely affected.

NOTE: All subsequent standards, samples, MS/MSDs, LCSs, and blanks associated with a BFB analysis must use identical mass spectrometer instrument conditions.

7.3.2 Set up the sample introduction system as outlined in the method of choice (see Sec. 7.1). A different calibration curve is necessary for each method because of the differences in conditions and equipment. A set of at least five different calibration standards is necessary (see Sec. 5.12 and Method 8000). Calibration must be performed using the sample introduction technique that will be used for samples. For Method 5030, the purging efficiency for 5 mL of water is greater than for 25 mL. Therefore, develop the standard curve with whichever volume of sample that will be analyzed.

7.3.2.1 To prepare a calibration standard, add an appropriate volume of a secondary dilution standard solution to an aliquot of organic-free reagent water in a volumetric flask. Use a microsyringe and rapidly inject the alcoholic standard into the expanded area of the filled volumetric flask. Remove the needle as quickly as possible after injection. Mix by inverting the flask three times only. Discard the contents contained in the neck of the flask. Aqueous standards are not stable and should be prepared daily. Transfer 5.0 mL (or 25 mL if lower detection limits are required) of each standard to a gas tight syringe along with 10 µL of internal standard. Then transfer the contents to the appropriate device or syringe. Some of the introduction methods may have specific guidance on the volume of calibration standard and the way the standards are transferred to the device.

7.3.2.2 The internal standards selected in Sec. 5.10 should permit most of the components of interest in a chromatogram to have retention times of 0.80 - 1.20, relative to one of the internal standards. Use the base peak ion from the specific internal standard as the primary ion for quantitation (see Table 1). If interferences are noted, use the next most intense ion as the quantitation ion.

7.3.2.3 To prepare a calibration standard for direct injection analysis of waste oil, dilute standards in hexadecane.

7.3.3 Proceed with the analysis of the calibration standards following the procedure in the introduction method of choice. For direct injection, inject 1 - 2 µL into the GC/MS system. The injection volume will depend upon the chromatographic column chosen and the tolerance of the specific GC/MS system to water.

7.3.4 Tabulate the area response of the characteristic ions (see Table 5) against the concentration for each target analyte and each internal standard. Calculate response factors (RF) for each target analyte relative to one of the internal standards. The internal standard selected for the calculation of the RF for a target analyte should be the internal standard that has a retention time closest to the analyte being measured (Sec. 7.6.2).

The RF is calculated as follows:

$$RF = \frac{A_s \times C_{is}}{A_{is} \times C_s}$$

where:

A_s = Peak area (or height) of the analyte or surrogate.
A_{is} = Peak area (or height) of the internal standard.
C_s = Concentration of the analyte or surrogate.
C_{is} = Concentration of the internal standard.

7.3.5 System performance check compounds (SPCCs) - Calculate the mean RF for each target analyte using the five RF values calculated from the initial (5-point) calibration curve. A system performance check should be made before this calibration curve is used. Five compounds (the System Performance Check Compounds, or SPCCs) are checked for a minimum average response factor. These compounds are chloromethane; 1,1-dichloroethane; bromoform; chlorobenzene; and 1,1,2,2-tetrachloroethane. These compounds are used to check compound instability and to check for degradation caused by contaminated lines or active sites in the system. Example problems include:

7.3.5.1 Chloromethane is the most likely compound to be lost if the purge flow is too fast.

7.3.5.2 Bromoform is one of the compounds most likely to be purged very poorly if the purge flow is too slow. Cold spots and/or active sites in the transfer lines may adversely affect response. Response of the quantitation ion (m/z 173) is directly affected by the tuning of BFB at ions m/z 174/176. Increasing the m/z 174/176 ratio relative to m/z 95 may improve bromoform response.

7.3.5.3 Tetrachloroethane and 1,1-dichloroethane are degraded by contaminated transfer lines in purge-and-trap systems and/or active sites in trapping materials.

7.3.5.4 The minimum mean response factors for the volatile SPCCs are as follows:

Chloromethane	0.10
1,1-Dichloroethane	0.10
Bromoform	0.10
Chlorobenzene	0.30
1,1,2,2-Tetrachloroethane	0.30

7.3.6 Calibration check compounds (CCCs)

7.3.6.1 The purpose of the CCCs are to evaluate the calibration from the standpoint of the integrity of the system. High variability for these compounds may be indicative of system leaks or reactive sites on the column. Meeting the CCC criteria is not a substitute for successful calibration of the target analytes using one of the approaches described in Sec. 7.0 of Method 8000.

7.3.6.2 Calculate the standard deviation (SD) and relative standard deviation (RSD) of the response factors for all target analytes from the initial calibration, as follows:

$$SD = \sqrt{\frac{\sum_{i=1}^{n} (RF_i - \overline{RF})^2}{n-1}} \qquad RSD = \frac{SD}{\overline{RF}} \times 100$$

where:

RF_i = RF for each of the calibration standards
$\overline{RF}$ = mean RF for each compound from the initial calibration
n = Number of calibration standards, e.g., 5

7.3.6.3 The RSD should be less than or equal to 15% for each target analyte. However, the RSD for each individual Calibration Check Compound (CCC) must be equal or less than 30%. If the CCCs are not included in the list of analytes for a project, and therefore not included in the calibration standards, refer to Sec. 7.0 of Method 8000. The CCCs are:

1,1-Dichloroethene	Toluene
Chloroform	Ethylbenzene
1,2-Dichloropropane	Vinyl chloride

7.3.6.4 If an RSD of greater than 30% is measured for any CCC, then corrective action to eliminate a system leak and/or column reactive sites is necessary before reattempting calibration.

7.3.7 Evaluation of retention times - The relative retention times of each target analyte in each calibration standard should agree within 0.06 relative retention time units. Late-eluting compounds usually have much better agreement.

7.3.8 Linearity of target analytes

7.3.8.1 If the RSD of any target analyte is 15% or less, then the response factor is assumed to be constant over the calibration range, and the average response factor may be used for quantitation (Sec. 7.7.2).

7.3.8.2 If the RSD of any target analyte is greater than 15%, refer to Sec. 7.0 of Method 8000 for additional calibration options. One of the options must be applied to GC/MS calibration in this situation, or a new initial calibration must be performed.

NOTE: Method 8000 specifies a linearity criterion of 20% RSD. That criterion pertains to GC and HPLC methods other than GC/MS. Method 8260 requires 15% RSD as evidence of sufficient linearity to employ an average response factor.

7.3.8.3 When the RSD exceeds 15%, the plotting and visual inspection of a calibration curve can be a useful diagnostic tool. The inspection may indicate analytical problems, including errors in standard preparation, the presence of active sites in the chromatographic system, analytes that exhibit poor chromatographic behavior, etc.

NOTE: The 20% RSD criteria in Method 8000 pertains to GC and HPLC methods other than GC/MS. Method 8260 requires 15% RSD.

7.4 GC/MS calibration verification - Calibration verification consists of three steps that are performed at the beginning of each 12-hour analytical shift.

7.4.1 Prior to the analysis of samples or calibration standards, inject or introduce 5-50 ng of the 4-bromofluorobenzene standard into the GC/MS system. The resultant mass spectra for the BFB must meet the criteria given in Table 4 before sample analysis begins. These criteria must be demonstrated each 12-hour shift during which samples are analyzed.

7.4.2 The initial calibration curve (Sec. 7.3) for each compound of interest should be verified once every 12 hours prior to sample analysis, using the introduction technique used for samples. This is accomplished by analyzing a calibration standard at a concentration near the midpoint concentration for the calibrating range of the GC/MS. The results from the calibration standard analysis should meet the verification acceptance criteria provided in Secs. 7.4.4 through 7.4.7.

NOTE: The BFB and calibration verification standard may be combined into a single standard as long as both tuning and calibration verification acceptance criteria for the project can be met without interferences.

7.4.3 A method blank should be analyzed after the calibration standard, or at any other time during the analytical shift, to ensure that the total system (introduction device, transfer lines and GC/MS system) is free of contaminants. If the method blank indicates contamination, then it may be appropriate to analyze a solvent blank to demonstrate that the contamination is not a result of carryover from standards or samples. See Sec. 8.0 of Method 8000 for method blank performance criteria.

7.4.4 System Performance Check Compounds (SPCCs)

7.4.4.1 A system performance check must be made during every 12-hour analytical shift. Each SPCC compound in the calibration verification standard must meet its minimum response factor (see Sec. 7.3.5.4). This is the same check that is applied during the initial calibration.

7.4.4.2 If the minimum response factors are not met, the system must be evaluated, and corrective action must be taken before sample analysis begins. Possible problems include standard mixture degradation, injection port inlet contamination, contamination at the front end of the analytical column, and active sites in the column or chromatographic system. This check must be met before sample analysis begins.

7.4.5 Calibration Check Compounds (CCCs)

7.4.5.1 After the system performance check is met, the CCCs listed in Sec. 7.3.6 are used to check the validity of the initial calibration. Use percent difference when performing the average response factor model calibration. Use percent drift when calibrating using a regression fit model. Refer to Sec. 7.0 of Method 8000 for guidance on calculating percent difference and drift.

7.4.5.2 If the percent difference or drift for each CCC is less than or equal to 20%, the initial calibration is assumed to be valid. If the criterion is not met (i.e., greater

than 20% difference or drift), for any one CCC, then corrective action must be taken prior to the analysis of samples. If the CCC's are not included in the list of analytes for a project, and therefore not included in the calibration standards, then all analytes must meet the 20% difference or drift criterion.

7.4.5.3 Problems similar to those listed under SPCCs could affect the CCCs. If the problem cannot be corrected by other measures, a new five-point initial calibration must be generated. The CCC criteria must be met before sample analysis begins.

7.4.6 Internal standard retention time - The retention times of the internal standards in the calibration verification standard must be evaluated immediately after or during data acquisition. If the retention time for any internal standard changes by more than 30 seconds from the that in the mid-point standard level of the most recent initial calibration sequence, then the chromatographic system must be inspected for malfunctions and corrections must be made, as required. When corrections are made, reanalysis of samples analyzed while the system was malfunctioning is required.

7.4.7 Internal standard response - If the EICP area for any of the internal standards in the calibration verification standard changes by a factor of two (-50% to + 100%) from that in the mid-point standard level of the most recent initial calibration sequence, the mass spectrometer must be inspected for malfunctions and corrections must be made, as appropriate. When corrections are made, reanalysis of samples analyzed while the system was malfunctioning is required.

7.5 GC/MS analysis of samples

7.5.1 It is highly recommended that the sample be screened to minimize contamination of the GC/MS system from unexpectedly high concentrations of organic compounds. Some of the screening options available utilizing SW-846 methods are automated headspace-GC/FID (Methods 5021/8015), automated headspace-GC/PID/ELCD (Methods 5021/8021), or waste dilution-GC/PID/ELCD (Methods 3585/8021) using the same type of capillary column. When used only for screening purposes, the quality control requirements in the methods above may be reduced as appropriate. Sample screening is particularly important when Method 8260 is used to achieve low detection levels.

7.5.2 BFB tuning criteria and GC/MS calibration verification criteria must be met before analyzing samples.

7.5.3 All samples and standard solutions must be allowed to warm to ambient temperature before analysis. Set up the introduction device as outlined in the method of choice.

7.5.4 The process of taking an aliquot destroys the validity of remaining volume of an aqueous sample for future analysis. Therefore, if only one VOA vial is provided to the laboratory, the analyst should prepare two aliquots for analysis at this time, to protect against possible loss of sample integrity. This second sample is maintained only until such time when the analyst has determined that the first sample has been analyzed properly. For aqueous samples, one 20-mL syringe could be used to hold two 5-mL aliquots. If the second aliquot is to be taken from the syringe, it must be analyzed within 24 hours. Care must be taken to prevent air from leaking into the syringe.

7.5.5 Remove the plunger from a 5-mL syringe and attach a closed syringe valve. Open the sample or standard bottle, which has been allowed to come to ambient temperature, and carefully pour the sample into the syringe barrel to just short of overflowing. Replace the syringe plunger and compress the sample. Open the syringe valve and vent any residual air while adjusting the sample volume to 5.0 mL. If lower detection limits are required, use a 25-mL syringe, and adjust the final volume to 25.0 mL.

7.5.6 The following procedure may be used to dilute aqueous samples for analysis of volatiles. All steps must be performed without delays, until the diluted sample is in a gas-tight syringe.

7.5.6.1 Dilutions may be made in volumetric flasks (10- to 100-mL). Select the volumetric flask that will allow for the necessary dilution. Intermediate dilution steps may be necessary for extremely large dilutions.

7.5.6.2 Calculate the approximate volume of organic-free reagent water to be added to the volumetric flask, and add slightly less than this quantity of organic-free reagent water to the flask.

7.5.6.3 Inject the appropriate volume of the original sample from the syringe into the flask. Aliquots of less than 1 mL are not recommended. Dilute the sample to the mark with organic-free reagent water. Cap the flask, invert, and shake three times. Repeat above procedure for additional dilutions.

7.5.6.4 Fill a 5-mL syringe with the diluted sample, as described in Sec. 7.5.5.

7.5.7 Compositing aqueous samples prior to GC/MS analysis

7.5.7.1 Add 5 mL of each sample (up to 5 samples are allowed) to a 25-mL glass syringe. Special precautions must be made to maintain zero headspace in the syringe. Larger volumes of a smaller number of samples may be used, provided that equal volumes of each sample are composited.

7.5.7.2 The samples must be cooled to 4°C or less during this step to minimize volatilization losses. Sample vials may be placed in a tray of ice during the processing.

7.5.7.3 Mix each vial well and draw out a 5-mL aliquot with the 25-mL syringe.

7.5.7.4 Once all the aliquots have been combined on the syringe, invert the syringe several times to mix the aliquots. Introduce the composited sample into the instrument, using the method of choice (see Sec. 7.1).

7.5.7.5 If less than five samples are used for compositing, a proportionately smaller syringe may be used, unless a 25-mL sample is to be purged.

7.5.8 Add 10 µL of the surrogate spiking solution and 10 µL of the internal standard spiking solution to each sample either manually or by autosampler. The surrogate and internal standards may be mixed and added as a single spiking solution. The addition of 10 µL of the surrogate spiking solution to 5 mL of aqueous sample will yield a concentration of 50 µg/L of each surrogate standard. The addition of 10 µL of the surrogate spiking solution to 5 g of a non-aqueous sample will yield a concentration of 50 µg/kg of each standard.

If a more sensitive mass spectrometer is employed to achieve lower detection levels, more dilute surrogate and internal standard solutions may be required.

7.5.9 Add 10 µL of the matrix spike solution (Sec. 5.13) to a 5-mL aliquot of the sample chosen for spiking. Disregarding any dilutions, this is equivalent to a concentration of 50 µg/L of each matrix spike standard.

7.5.9.1 Follow the same procedure in preparing the laboratory control sample (LCS), except the spike is added to a clean matrix. See Sec. 8.4 and Method 5000 for more guidance on the selection and preparation of the matrix spike and the LCS.

7.5.9.2 If a more sensitive mass spectrometer is employed to achieve lower detection levels, more dilute matrix spiking and LCS solutions may be required.

7.5.10 Analyze the sample following the procedure in the introduction method of choice.

7.5.10.1 For direct injection, inject 1 to 2 µL into the GC/MS system. The volume limitation will depend upon the chromatographic column chosen and the tolerance of the specific GC/MS system to water (if an aqueous sample is being analyzed).

7.5.10.2 The concentration of the internal standards, surrogates, and matrix spiking standards (if any) added to the injection aliquot must be adjusted to provide the same concentration in the 1-2 µL injection as would be introduced into the GC/MS by purging a 5-mL aliquot.

NOTE: It may be a useful diagnostic tool to monitor internal standard retention times and responses (area counts) in all samples, spikes, blanks, and standards to effectively check drifting method performance, poor injection execution, and anticipate the need for system inspection and/or maintenance.

7.5.11 If the initial analysis of the sample or a dilution of the sample has a concentration of any analyte that exceeds the initial calibration range, the sample must be reanalyzed at a higher dilution. Secondary ion quantitation is allowed only when there are sample interferences with the primary ion.

7.5.11.1 When ions from a compound in the sample saturate the detector, this analysis must be followed by the analysis of an organic-free reagent water blank. If the blank analysis is not free of interferences, then the system must be decontaminated. Sample analysis may not resume until the blank analysis is demonstrated to be free of interferences.

7.5.11.2 All dilutions should keep the response of the major constituents (previously saturated peaks) in the upper half of the linear range of the curve.

7.5.12 The use of selected ion monitoring (SIM) is acceptable in situations requiring detection limits below the normal range of full EI spectra. However, SIM may provide a lesser degree of confidence in the compound identification unless multiple ions are monitored for each compound.

7.6 Qualitative analysis

7.6.1 The qualitative identification of each compound determined by this method is based on retention time, and on comparison of the sample mass spectrum, after background correction, with characteristic ions in a reference mass spectrum. The reference mass spectrum must be generated by the laboratory using the conditions of this method. The characteristic ions from the reference mass spectrum are defined to be the three ions of greatest relative intensity, or any ions over 30% relative intensity if less than three such ions occur in the reference spectrum. Compounds are identified as present when the following criteria are met.

7.6.1.1 The intensities of the characteristic ions of a compound maximize in the same scan or within one scan of each other. Selection of a peak by a data system target compound search routine where the search is based on the presence of a target chromatographic peak containing ions specific for the target compound at a compound-specific retention time will be accepted as meeting this criterion.

7.6.1.2 The relative retention time (RRT) of the sample component is within ± 0.06 RRT units of the RRT of the standard component.

7.6.1.3 The relative intensities of the characteristic ions agree within 30% of the relative intensities of these ions in the reference spectrum. (Example: For an ion with an abundance of 50% in the reference spectrum, the corresponding abundance in a sample spectrum can range between 20% and 80%.)

7.6.1.4 Structural isomers that produce very similar mass spectra should be identified as individual isomers if they have sufficiently different GC retention times. Sufficient GC resolution is achieved if the height of the valley between two isomer peaks is less than 25% of the sum of the two peak heights. Otherwise, structural isomers are identified as isomeric pairs.

7.6.1.5 Identification is hampered when sample components are not resolved chromatographically and produce mass spectra containing ions contributed by more than one analyte. When gas chromatographic peaks obviously represent more than one sample component (i.e., a broadened peak with shoulder(s) or a valley between two or more maxima), appropriate selection of analyte spectra and background spectra is important.

7.6.1.6 Examination of extracted ion current profiles of appropriate ions can aid in the selection of spectra, and in qualitative identification of compounds. When analytes coelute (i.e., only one chromatographic peak is apparent), the identification criteria may be met, but each analyte spectrum will contain extraneous ions contributed by the coeluting compound.

7.6.2 For samples containing components not associated with the calibration standards, a library search may be made for the purpose of tentative identification. The necessity to perform this type of identification will be determined by the purpose of the analyses being conducted. Data system library search routines should not use normalization routines that would misrepresent the library or unknown spectra when compared to each other.

For example, the RCRA permit or waste delisting requirements may require the reporting of non-target analytes. Only after visual comparison of sample spectra with the nearest library

searches may the analyst assign a tentative identification. Use the following guidelines for making tentative identifications:

(1) Relative intensities of major ions in the reference spectrum (ions greater than 10% of the most abundant ion) should be present in the sample spectrum.

(2) The relative intensities of the major ions should agree within ± 20%. (Example: For an ion with an abundance of 50% in the standard spectrum, the corresponding sample ion abundance must be between 30 and 70%).

(3) Molecular ions present in the reference spectrum should be present in the sample spectrum.

(4) Ions present in the sample spectrum but not in the reference spectrum should be reviewed for possible background contamination or presence of coeluting compounds.

(5) Ions present in the reference spectrum but not in the sample spectrum should be reviewed for possible subtraction from the sample spectrum because of background contamination or coeluting peaks. Data system library reduction programs can sometimes create these discrepancies.

7.7 Quantitative analysis

7.7.1 Once a compound has been identified, the quantitation of that compound will be based on the integrated abundance from the EICP of the primary characteristic ion. The internal standard used shall be the one nearest the retention time of that of a given analyte.

7.7.2 If the RSD of a compound's response factors is 15% or less, then the concentration in the extract may be determined using the average response factor ($\overline{RF}$) from initial calibration data (7.3.6). See Method 8000, Sec. 7.0, for the equations describing internal standard calibration and either linear or non-linear calibrations.

7.7.3 Where applicable, the concentration of any non-target analytes identified in the sample (Sec. 7.6.2) should be estimated. The same formulae should be used with the following modifications: The areas A_x and A_{is} should be from the total ion chromatograms, and the RF for the compound should be assumed to be 1.

7.7.4 The resulting concentration should be reported indicating: (1) that the value is an estimate, and (2) which internal standard was used to determine concentration. Use the nearest internal standard free of interferences.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for specific quality control (QC) procedures. Quality control procedures to ensure the proper operation of the various sample preparation and/or sample introduction techniques can be found in Methods 3500 and 5000. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated.

8.2 Quality control procedures necessary to evaluate the GC system operation are found in Method 8000, Sec. 7.0 and include evaluation of retention time windows, calibration verification and chromatographic analysis of samples. In addition, instrument QC requirements may be found in the following sections of Method 8260:

8.2.1 The GC/MS system must be tuned to meet the BFB specifications in Secs. 7.3.1 and 7.4.1.

8.2.2 There must be an initial calibration of the GC/MS system as described in Sec. 7.3.

8.2.3 The GC/MS system must meet the SPCC criteria described in Sec. 7.4.4 and the CCC criteria in Sec. 7.4.5, each 12 hours.

8.3 Initial Demonstration of Proficiency - Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made. See Method 8000, Sec. 8.0 for information on how to accomplish this demonstration.

8.4 Sample Quality Control for Preparation and Analysis - The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and detection limit). At a minimum, this includes the analysis of QC samples including a method blank, matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch and the addition of surrogates to each field sample and QC sample.

8.4.1 Before processing any samples, the analyst should demonstrate, through the analysis of a method blank, that interferences from the analytical system, glassware, and reagents are under control. Each time a set of samples is analyzed or there is a change in reagents, a method blank should be analyzed as a safeguard against chronic laboratory contamination. The blanks should be carried through all stages of sample preparation and measurement.

8.4.2 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, laboratories should use a matrix spike and matrix spike duplicate pair.

8.4.3 A Laboratory Control Sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

8.4.4 See Method 8000, Sec. 8.0 for the details on carrying out sample quality control procedures for preparation and analysis.

8.5 Surrogate recoveries - The laboratory must evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory. See Method 8000, Sec. 8.0 for information on evaluating surrogate data and developing and updating surrogate limits.

8.6 The experience of the analyst performing GC/MS analyses is invaluable to the success of the methods. Each day that analysis is performed, the calibration verification standard should be evaluated to determine if the chromatographic system is operating properly. Questions that should be asked are: Do the peaks look normal? Is the response obtained comparable to the response from previous calibrations? Careful examination of the standard chromatogram can indicate whether the column is still performing acceptably, the injector is leaking, the injector septum needs replacing, etc. If any changes are made to the system (e.g., the column changed), recalibration of the system must take place.

8.7 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.0 METHOD PERFORMANCE

9.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The MDL actually achieved in a given analysis will vary depending on instrument sensitivity and matrix effects.

9.2 This method has been tested using purge-and-trap (Method 5030) in a single laboratory using spiked water. Using a wide-bore capillary column, water was spiked at concentrations between 0.5 and 10 µg/L. Single laboratory accuracy and precision data are presented for the method analytes in Table 6. Calculated MDLs are presented in Table 1.

9.3 The method was tested using purge-and-trap (Method 5030) with water spiked at 0.1 to 0.5 µg/L and analyzed on a cryofocussed narrow-bore column. The accuracy and precision data for these compounds are presented in Table 7. MDL values were also calculated from these data and are presented in Table 2.

9.4 Direct injection (Method 3585) has been used for the analysis of waste motor oil samples using a wide-bore column. Single laboratory precision and accuracy data are presented in Tables 10 and 11 for TCLP volatiles in oil. The performance data were developed by spiking and analyzing seven replicates each of new and used oil. The oils were spiked at the TCLP regulatory concentrations for most analytes, except for the alcohols, ketones, ethyl acetate and chlorobenzene which are spiked at 5 ppm, well below the regulatory concentrations. Prior to spiking, the new oil (an SAE 30-weight motor oil) was heated at 80°C overnight to remove volatiles. The used oil (a mixture of used oil drained from passenger automobiles) was not heated and was contaminated with 20 - 300 ppm of BTEX compounds and isobutanol. These contaminants contributed to the extremely high recoveries of the BTEX compounds in the used oil. Therefore, the data from the deuterated analogs of these analytes represent more typical recovery values.

9.5 Single laboratory accuracy and precision data were obtained for the Method 5035 analytes in three soil matrices: sand; a soil collected 10 feet below the surface of a hazardous landfill, called C-Horizon; and a surface garden soil. Sample preparation was by Method 5035. Each

sample was fortified with the analytes at a concentration of 4 µg/kg. These data are listed in Tables 17, 18, and 19. All data were calculated using fluorobenzene as the internal standard added to the soil sample prior to extraction. This causes some of the results to be greater than 100% recovery because the precision of results is sometimes as great as 28%.

9.5.1 In general, the recoveries of the analytes from the sand matrix are the highest, the C-Horizon soil results are somewhat less, and the surface garden soil recoveries are the lowest. This is due to the greater adsorptive capacity of the garden soil. This illustrates the necessity of analyzing matrix spike samples to assess the degree of matrix effects.

9.5.2 The recoveries of some of the gases, or very volatile compounds, such as vinyl chloride, trichlorofluoromethane, and 1,1-dichloroethene, are somewhat greater than 100%. This is due to the difficulty encountered in fortifying the soil with these compounds, allowing an equilibration period, then extracting them with a high degree of precision. Also, the garden soil results in Table 19 include some extraordinarily high recoveries for some aromatic compounds, such as toluene, xylenes, and trimethylbenzenes. This is due to contamination of the soil prior to sample collection, and to the fact that no background was subtracted.

9.6 Performance data for nonpurgeable volatiles using azeotropic distillation (Method 5031) are included in Tables 12 to 16.

9.7 Performance data for volatiles prepared using vacuum distillation (Method 5032) in soil, water, oil and fish tissue matrices are included in Tables 20 to 27.

9.8 Single laboratory accuracy and precision data were obtained for the Method 5021 analytes in two soil matrices: sand and a surface garden soil. Replicate samples were fortified with the analytes at concentrations of 10 µg/kg. These data are listed in Table 30. All data were calculated using the internal standards listed for each analyte in Table 28. The recommended internal standards were selected because they generated the best accuracy and precision data for the analyte in both types of soil.

9.8.1 If a detector other than an MS is used for analysis, consideration must be given to the choice of internal standards and surrogates. They must not coelute with any other analyte and must have similar properties to the analytes. The recoveries of the analytes are 50% or higher for each matrix studied. The recoveries of the gases or very volatile compounds are greater than 100% in some cases. Also, results include high recoveries of some aromatic compounds, such as toluene, xylenes, and trimethylbenzenes. This is due to contamination of the soil prior to sample collection.

9.8.2 The method detection limits using Method 5021 listed in Table 29 were calculated from results of seven replicate analyses of the sand matrix. Sand was chosen because it demonstrated the least degree of matrix effect of the soils studied. These MDLs were calculated utilizing the procedure described in Chapter One and are intended to be a general indication of the capabilities of the method.

9.9 The MDL concentrations listed in Table 31 were determined using Method 5041 in conjunction with Method 8260. They were obtained using cleaned blank VOST tubes and reagent water. Similar results have been achieved with field samples. The MDL actually achieved in a given analysis will vary depending upon instrument sensitivity and the effects of the matrix. Preliminary spiking studies indicate that under the test conditions, the MDLs for spiked compounds in extremely complex matrices may be larger by a factor of 500 - 1000.

9.10 The EQL of sample taken by Method 0040 and analyzed by Method 8260 is estimated to be in the range of 0.03 to 0.9 ppm (See Table 33). Matrix effects may cause the individual compound detection limits to be higher.

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TABLE 1

CHROMATOGRAPHIC RETENTION TIMES AND METHOD DETECTION LIMITS (MDL)
FOR VOLATILE ORGANIC COMPOUNDS ON WIDE-BORE CAPILLARY COLUMNS

Compound	Retention Time (minutes)			MDL[d]
	Column 1[a]	Column 2[b]	Column 2'[c]	(µg/L)
Dichlorodifluoromethane	1.35	0.70	3.13	0.10
Chloromethane	1.49	0.73	3.40	0.13
Vinyl Chloride	1.56	0.79	3.93	0.17
Bromomethane	2.19	0.96	4.80	0.11
Chloroethane	2.21	1.02	--	0.10
Trichlorofluoromethane	2.42	1.19	6.20	0.08
Acrolein	3.19			
Iodomethane	3.56			
Acetonitrile	4.11			
Carbon disulfide	4.11			
Allyl chloride	4.11			
Methylene chloride	4.40	2.06	9.27	0.03
1,1-Dichloroethene	4.57	1.57	7.83	0.12
Acetone	4.57			
trans-1,2-Dichloroethene	4.57	2.36	9.90	0.06
Acrylonitrile	5.00			
1,1-Dichloroethane	6.14	2.93	10.80	0.04
Vinyl acetate	6.43			
2,2-Dichloropropane	8.10	3.80	11.87	0.35
2-Butanone	--			
cis-1,2-Dichloroethene	8.25	3.90	11.93	0.12
Propionitrile	8.51			
Chloroform	9.01	4.80	12.60	0.03
Bromochloromethane	--	4.38	12.37	0.04
Methacrylonitrile	9.19			
1,1,1-Trichloroethane	10.18	4.84	12.83	0.08
Carbon tetrachloride	11.02	5.26	13.17	0.21
1,1-Dichloropropene	--	5.29	13.10	0.10
Benzene	11.50	5.67	13.50	0.04
1,2-Dichloroethane	12.09	5.83	13.63	0.06
Trichloroethene	14.03	7.27	14.80	0.19
1,2-Dichloropropane	14.51	7.66	15.20	0.04
Bromodichloromethane	15.39	8.49	15.80	0.08
Dibromomethane	15.43	7.93	5.43	0.24
Methyl methacrylate	15.50			
1,4-Dioxane	16.17			
2-Chloroethyl vinyl ether	--			
4-Methyl-2-pentanone	17.32			
trans-1,3-Dichloropropene	17.47	--	16.70	--
Toluene	18.29	10.00	17.40	0.11
cis-1,3-Dichloropropene	19.38	--	17.90	--

TABLE 1 (cont.)

Compound	Retention Time (minutes)			MDL[d]
	Column 1[a]	Column 2[b]	Column 2"[c]	(µg/L)
1,1,2-Trichloroethane	19.59	11.05	18.30	0.10
Ethyl methacrylate	20.01			
2-Hexanone	20.30			
Tetrachloroethene	20.26	11.15	18.60	0.14
1,3-Dichloropropane	20.51	11.31	18.70	0.04
Dibromochloromethane	21.19	11.85	19.20	0.05
1,2-Dibromoethane	21.52	11.83	19.40	0.06
1-Chlorohexane	--	13.29	--	0.05
Chlorobenzene	23.17	13.01	20.67	0.04
1,1,1,2-Tetrachloroethane	23.36	13.33	20.87	0.05
Ethylbenzene	23.38	13.39	21.00	0.06
p-Xylene	23.54	13.69	21.30	0.13
m-Xylene	23.54	13.68	21.37	0.05
o-Xylene	25.16	14.52	22.27	0.11
Styrene	25.30	14.60	22.40	0.04
Bromoform	26.23	14.88	22.77	0.12
Isopropylbenzene (Cumene)	26.37	15.46	23.30	0.15
cis-1,4-Dichloro-2-butene	27.12			
1,1,2,2-Tetrachloroethane	27.29	16.35	24.07	0.04
Bromobenzene	27.46	15.86	24.00	0.03
1,2,3-Trichloropropane	27.55	16.23	24.13	0.32
n-Propylbenzene	27.58	16.41	24.33	0.04
2-Chlorotoluene	28.19	16.42	24.53	0.04
trans-1,4-Dichloro-2-butene	28.26			
1,3,5-Trimethylbenzene	28.31	16.90	24.83	0.05
4-Chlorotoluene	28.33	16.72	24.77	0.06
Pentachloroethane	29.41			
1,2,4-Trimethylbenzene	29.47	17.70	31.50	0.13
sec-Butylbenzene	30.25	18.09	26.13	0.13
tert-Butylbenzene	30.59	17.57	26.60	0.14
p-Isopropyltoluene	30.59	18.52	26.50	0.12
1,3-Dichlorobenzene	30.56	18.14	26.37	0.12
1,4-Dichlorobenzene	31.22	18.39	26.60	0.03
Benzyl chloride	32.00			
n-Butylbenzene	32.23	19.49	27.32	0.11
1,2-Dichlorobenzene	32.31	19.17	27.43	0.03
1,2-Dibromo-3-chloropropane	35.30	21.08	--	0.26
1,2,4-Trichlorobenzene	38.19	23.08	31.50	0.04
Hexachlorobutadiene	38.57	23.68	32.07	0.11
Naphthalene	39.05	23.52	32.20	0.04
1,2,3-Trichlorobenzene	40.01	24.18	32.97	0.03

TABLE 1 (cont.)

Compound	Retention Time (minutes) Column 1[a]	Column 2[b]	Column 2"[c]	MDL[d] (µg/L)
INTERNAL STANDARDS/SURROGATES				
1,4-Difluorobenzene	13.26			
Chlorobenzene-d_5	23.10			
1,4-Dichlorobenzene-d_4	31.16			
4-Bromofluorobenzene	27.83	15.71	23.63	
1,2-Dichlorobenzene-d_4	32.30	19.08	27.25	
Dichloroethane-d_4	12.08			
Dibromofluoromethane	--			
Toluene-d_8	18.27			
Pentafluorobenzene	--			
Fluorobenzene	13.00	6.27	14.06	

[a] Column 1 - 60 meter x 0.75 mm ID VOCOL capillary. Hold at 10°C for 8 minutes, then program to 180°C at 4°C/min.

[b] Column 2 - 30 meter x 0.53 mm ID DB-624 wide-bore capillary using cryogenic oven. Hold at 10°C for 5 minutes, then program to 160°C at 6°C/min.

[c] Column 2" - 30 meter x 0.53 mm ID DB-624 wide-bore capillary, cooling GC oven to ambient temperatures. Hold at 10°C for 6 minutes, program to 70°C at 10 °C/min, program to 120°C at 5°C/min, then program to 180°C at 8°C/min.

[d] MDL based on a 25-mL sample volume.

TABLE 2

CHROMATOGRAPHIC RETENTION TIMES AND METHOD DETECTION LIMITS (MDL)
FOR VOLATILE ORGANIC COMPOUNDS ON NARROW-BORE CAPILLARY COLUMNS

Compound	Retention Time (minutes) Column 3[a]	MDL[b] (µg/L)
Dichlorodifluoromethane	0.88	0.11
Chloromethane	0.97	0.05
Vinyl chloride	1.04	0.04
Bromomethane	1.29	0.03
1,1-Dichloroethane	4.03	0.03
cis-1,2-Dichloroethene	5.07	0.06
2,2-Dichloropropane	5.31	0.08
Chloroform	5.55	0.04
Bromochloromethane	5.63	0.09
1,1,1-Trichloroethane	6.76	0.04
1,2-Dichloroethane	7.00	0.02
1,1-Dichloropropene	7.16	0.12
Carbon tetrachloride	7.41	0.02
Benzene	7.41	0.03
1,2-Dichloropropane	8.94	0.02
Trichloroethene	9.02	0.02
Dibromomethane	9.09	0.01
Bromodichloromethane	9.34	0.03
Toluene	11.51	0.08
1,1,2-Trichloroethane	11.99	0.08
1,3-Dichloropropane	12.48	0.08
Dibromochloromethane	12.80	0.07
Tetrachloroethene	13.20	0.05
1,2-Dibromoethane	13.60	0.10
Chlorobenzene	14.33	0.03
1,1,1,2-Tetrachloroethane	14.73	0.07
Ethylbenzene	14.73	0.03
p-Xylene	15.30	0.06
m-Xylene	15.30	0.03
Bromoform	15.70	0.20
o-Xylene	15.78	0.06
Styrene	15.78	0.27
1,1,2,2-Tetrachloroethane	15.78	0.20
1,2,3-Trichloropropane	16.26	0.09
Isopropylbenzene	16.42	0.10
Bromobenzene	16.42	0.11
2-Chlorotoluene	16.74	0.08
n-Propylbenzene	16.82	0.10
4-Chlorotoluene	16.82	0.06

TABLE 2 (cont.)

Compound	Retention Time (minutes) Column 3[a]	MDL[b] (µg/L)
1,3,5-Trimethylbenzene	16.99	0.06
tert-Butylbenzene	17.31	0.33
1,2,4-Trimethylbenzene	17.31	0.09
sec-Butylbenzene	17.47	0.12
1,3-Dichlorobenzene	17.47	0.05
p-Isopropyltoluene	17.63	0.26
1,4-Dichlorobenzene	17.63	0.04
1,2-Dichlorobenzene	17.79	0.05
n-Butylbenzene	17.95	0.10
1,2-Dibromo-3-chloropropane	18.03	0.50
1,2,4-Trichlorobenzene	18.84	0.20
Naphthalene	19.07	0.10
Hexachlorobutadiene	19.24	0.10
1,2,3-Trichlorobenzene	19.24	0.14

[a] Column 3 - 30 meter x 0.32 mm ID DB-5 capillary with 1 µm film thickness.

[b] MDL based on a 25-mL sample volume.

TABLE 3

ESTIMATED QUANTITATION LIMITS FOR VOLATILE ANALYTES[a]

Estimated Quantitation Limits		
5-mL Ground Water Purge (µg/L)	25-mL Ground water Purge (µg/L)	Low Soil/Sediment[b] µg/kg
5	1	5

[a] Estimated Quantitation Limit (EQL) - The lowest concentration that can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions. The EQL is generally 5 to 10 times the MDL. However, it may be nominally chosen within these guidelines to simplify data reporting. For many analytes the EQL analyte concentration is selected for the lowest non-zero standard in the calibration curve. Sample EQLs are highly matrix-dependent. The EQLs listed herein are provided for guidance and may not always be achievable. See the following footnote for further guidance on matrix-dependent EQLs.

[b] EQLs listed for soil/sediment are based on wet weight. Normally data are reported on a dry weight basis; therefore, EQLs will be higher, based on the percent dry weight in each sample.

Other Matrices	Factor[c]
Water miscible liquid waste	50
High concentration soil and sludge	125
Non-water miscible waste	500

[c] EQL = [EQL for low soil sediment (Table 3)] x [Factor].

For non-aqueous samples, the factor is on a wet-weight basis.

TABLE 4

BFB (4-BROMOFLUOROBENZENE) MASS INTENSITY CRITERIA[a]

m/z	Required Intensity (relative abundance)
50	15 to 40% of m/z 95
75	30 to 60% of m/z 95
95	Base peak, 100% relative abundance
96	5 to 9% of m/z 95
173	Less than 2% of m/z 174
174	Greater than 50% of m/z 95
175	5 to 9% of m/z 174
176	Greater than 95% but less than 101% of m/z 174
177	5 to 9% of m/z 176

[a] Alternate tuning criteria may be used, (e.g. CLP, Method 524.2, or manufacturers" instructions), provided that method performance is not adversely affected.

TABLE 5

CHARACTERISTIC MASSES (m/z) FOR PURGEABLE ORGANIC COMPOUNDS

Compound	Primary Characteristic Ion	Secondary Characteristic Ion(s)
Acetone	58	43
Acetonitrile	41	40, 39
Acrolein	56	55, 58
Acrylonitrile	53	52, 51
Allyl alcohol	57	58, 39
Allyl chloride	76	41, 39, 78
Benzene	78	-
Benzyl chloride	91	126, 65, 128
Bromoacetone	136	43, 138, 93, 95
Bromobenzene	156	77, 158
Bromochloromethane	128	49, 130
Bromodichloromethane	83	85, 127
Bromoform	173	175, 254
Bromomethane	94	96
iso-Butanol	74	43
n-Butanol	56	41
2-Butanone	72	43
n-Butylbenzene	91	92, 134
sec-Butylbenzene	105	134
tert-Butylbenzene	119	91, 134
Carbon disulfide	76	78
Carbon tetrachloride	117	119
Chloral hydrate	82	44, 84, 86, 111
Chloroacetonitrile	48	75
Chlorobenzene	112	77, 114
1-Chlorobutane	56	49
Chlorodibromomethane	129	208, 206
Chloroethane	64 (49*)	66 (51*)
2-Chloroethanol	49	44, 43, 51, 80
Bis(2-chloroethyl) sulfide	109	111, 158, 160
2-Chloroethyl vinyl ether	63	65, 106
Chloroform	83	85
Chloromethane	50 (49*)	52 (51*)
Chloroprene	53	88, 90, 51
3-Chloropropionitrile	54	49, 89, 91
2-Chlorotoluene	91	126
4-Chlorotoluene	91	126
1,2-Dibromo-3-chloropropane	75	155, 157
Dibromochloromethane	129	127
1,2-Dibromoethane	107	109, 188
Dibromomethane	93	95, 174

TABLE 5 (cont.)

Compound	Primary Characteristic Ion	Secondary Characteristic Ion(s)
1,2-Dichlorobenzene	146	111, 148
1,2-Dichlorobenzene-d_4	152	115, 150
1,3-Dichlorobenzene	146	111, 148
1,4-Dichlorobenzene	146	111, 148
cis-1,4-Dichloro-2-butene	75	53, 77, 124, 89
trans-1,4-Dichloro-2-butene	53	88, 75
Dichlorodifluoromethane	85	87
1,1-Dichloroethane	63	65, 83
1,2-Dichloroethane	62	98
1,1-Dichloroethene	96	61, 63
cis-1,2-Dichloroethene	96	61, 98
trans-1,2-Dichloroethene	96	61, 98
1,2-Dichloropropane	63	112
1,3-Dichloropropane	76	78
2,2-Dichloropropane	77	97
1,3-Dichloro-2-propanol	79	43, 81, 49
1,1-Dichloropropene	75	110, 77
cis-1,3-Dichloropropene	75	77, 39
trans-1,3-Dichloropropene	75	77, 39
1,2,3,4-Diepoxybutane	55	57, 56
Diethyl ether	74	45, 59
1,4-Dioxane	88	58, 43, 57
Epichlorohydrin	57	49, 62, 51
Ethanol	31	45, 27, 46
Ethyl acetate	88	43, 45, 61
Ethylbenzene	91	106
Ethylene oxide	44	43, 42
Ethyl methacrylate	69	41, 99, 86, 114
Hexachlorobutadiene	225	223, 227
Hexachloroethane	201	166, 199, 203
2-Hexanone	43	58, 57, 100
2-Hydroxypropionitrile	44	43, 42, 53
Iodomethane	142	127, 141
Isobutyl alcohol	43	41, 42, 74
Isopropylbenzene	105	120
p-Isopropyltoluene	119	134, 91
Malononitrile	66	39, 65, 38
Methacrylonitrile	41	67, 39, 52, 66
Methyl acrylate	55	85
Methyl-t-butyl ether	73	57
Methylene chloride	84	86, 49
Methyl ethyl ketone	72	43
Methyl iodide	142	127, 141

TABLE 5 (cont.)

Compound	Primary Characteristic Ion	Secondary Characteristic Ion(s)
Methyl methacrylate	69	41, 100, 39
4-Methyl-2-pentanone	100	43, 58, 85
Naphthalene	128	-
Nitrobenzene	123	51, 77
2-Nitropropane	46	-
2-Picoline	93	66, 92, 78
Pentachloroethane	167	130, 132, 165, 169
Propargyl alcohol	55	39, 38, 53
β-Propiolactone	42	43, 44
Propionitrile (ethyl cyanide)	54	52, 55, 40
n-Propylamine	59	41, 39
n-Propylbenzene	91	120
Pyridine	79	52
Styrene	104	78
1,2,3-Trichlorobenzene	180	182, 145
1,2,4-Trichlorobenzene	180	182, 145
1,1,1,2-Tetrachloroethane	131	133, 119
1,1,2,2-Tetrachloroethane	83	131, 85
Tetrachloroethene	164	129, 131, 166
Toluene	92	91
1,1,1-Trichloroethane	97	99, 61
1,1,2-Trichloroethane	83	97, 85
Trichloroethene	95	97, 130, 132
Trichlorofluoromethane	151	101, 153
1,2,3-Trichloropropane	75	77
1,2,4-Trimethylbenzene	105	120
1,3,5-Trimethylbenzene	105	120
Vinyl acetate	43	86
Vinyl chloride	62	64
o-Xylene	106	91
m-Xylene	106	91
p-Xylene	106	91
Internal Standards/Surrogates:		
Benzene-d_6	84	83
Bromobenzene-d_5	82	162
Bromochloromethane-d_2	51	131
1,4-Difluorobenzene	114	
Chlorobenzene-d_5	117	
1,4-Dichlorobenzene-d_4	152	115, 150
1,1,2-Trichloroethane-d_3	100	
4-Bromofluorobenzene	95	174, 176
Chloroform-d_1	84	
Dibromofluoromethane	113	

TABLE 5 (cont.)

Compound	Primary Characteristic Ion	Secondary Characteristic Ion(s)
Internal Standards/Surrogates		
Dichloroethane-d_4	102	
Toluene-d_8	98	
Pentafluorobenzene	168	
Fluorobenzene	96	77

* Characteristic ion for an ion trap mass spectrometer (to be used when ion-molecule reactions are observed).

TABLE 6

SINGLE LABORATORY ACCURACY AND PRECISION DATA FOR PURGEABLE VOLATILE ORGANIC COMPOUNDS IN WATER DETERMINED WITH A WIDE-BORE CAPILLARY COLUMN (METHOD 5030)

Compound	Conc. Range (µg/L)	Number of Samples	% Recovery[a]	Standard Deviation of Recovery[b]	RSD
Benzene	0.1 - 10	31	97	6.5	5.7
Bromobenzene	0.1 - 10	30	100	5.5	5.5
Bromochloromethane	0.5 - 10	24	90	5.7	6.4
Bromodichloromethane	0.1 - 10	30	95	5.7	6.1
Bromoform	0.5 - 10	18	101	6.4	6.3
Bromomethane	0.5 - 10	18	95	7.8	8.2
n-Butylbenzene	0.5 - 10	18	100	7.6	7.6
sec-Butylbenzene	0.5 - 10	16	100	7.6	7.6
tert-Butylbenzene	0.5 - 10	18	102	7.4	7.3
Carbon tetrachloride	0.5 - 10	24	84	7.4	8.8
Chlorobenzene	0.1 - 10	31	98	5.8	5.9
Chloroethane	0.5 - 10	24	89	8.0	9.0
Chloroform	0.5 - 10	24	90	5.5	6.1
Chloromethane	0.5 - 10	23	93	8.3	8.9
2-Chlorotoluene	0.1 - 10	31	90	5.6	6.2
4-Chlorotoluene	0.1 - 10	31	99	8.2	8.3
1,2-Dibromo-3-Chloropropane	0.5 - 10	24	83	16.6	19.9
Dibromochloromethane	0.1 - 10	31	92	6.5	7.0
1,2-Dibromoethane	0.5 - 10	24	102	4.0	3.9
Dibromomethane	0.5 - 10	24	100	5.6	5.6
1,2-Dichlorobenzene	0.1 - 10	31	93	5.8	6.2
1,3-Dichlorobenzene	0.5 - 10	24	99	6.8	6.9
1,4-Dichlorobenzene	0.2 - 20	31	103	6.6	6.4
Dichlorodifluoromethane	0.5 - 10	18	90	6.9	7.7
1,1-Dichlorobenzene	0.5 - 10	24	96	5.1	5.3
1,2-Dichlorobenzene	0.1 - 10	31	95	5.1	5.4
1,1-Dichloroethene	0.1 - 10	34	94	6.3	6.7
cis-1,2-Dichloroethene	0.5 - 10	18	101	6.7	6.7
trans-1,2-Dichloroethene	0.1 - 10	30	93	5.2	5.6
1,2-Dichloropropane	0.1 - 10	30	97	5.9	6.1
1,3-Dichloropropane	0.1 - 10	31	96	5.7	6.0
2,2-Dichloropropane	0.5 - 10	12	86	14.6	16.9
1,1-Dichloropropene	0.5 - 10	18	98	8.7	8.9
Ethylbenzene	0.1 - 10	31	99	8.4	8.6
Hexachlorobutadiene	0.5 - 10	18	100	6.8	6.8
Isopropylbenzene	0.5 - 10	16	101	7.7	7.6
p-Isopropyltoluene	0.1 - 10	23	99	6.7	6.7
Methylene chloride	0.1 - 10	30	95	5.0	5.3

TABLE 6 (cont.)

Compound	Conc. Range (µg/L)	Number of Samples	% Recovery[a]	Standard Deviation of Recovery[b]	RSD
Naphthalene	0.1 -100	31	104	8.6	8.2
n-Propylbenzene	0.1 - 10	31	100	5.8	5.8
Styrene	0.1 -100	39	102	7.3	7.2
1,1,1,2-Tetrachloroethane	0.5 - 10	24	90	6.1	6.8
1,1,2,2-Tetrachloroethane	0.1 - 10	30	91	5.7	6.3
Tetrachloroethene	0.5 - 10	24	89	6.0	6.8
Toluene	0.5 - 10	18	102	8.1	8.0
1,2,3-Trichlorobenzene	0.5 - 10	18	109	9.4	8.6
1,2,4-Trichlorobenzene	0.5 - 10	18	108	9.0	8.3
1,1,1-Trichloroethane	0.5 - 10	18	98	7.9	8.1
1,1,2-Trichloroethane	0.5 - 10	18	104	7.6	7.3
Trichloroethene	0.5 - 10	24	90	6.5	7.3
Trichlorofluoromethane	0.5 - 10	24	89	7.2	8.1
1,2,3-Trichloropropane	0.5 - 10	16	108	15.6	14.4
1,2,4-Trimethylbenzene	0.5 - 10	18	99	8.0	8.1
1,3,5-Trimethylbenzene	0.5 - 10	23	92	6.8	7.4
Vinyl chloride	0.5 - 10	18	98	6.5	6.7
o-Xylene	0.1 - 31	18	103	7.4	7.2
m-Xylene	0.1 - 10	31	97	6.3	6.5
p-Xylene	0.5 - 10	18	104	8.0	7.7

[a] Recoveries were calculated using internal standard method. The internal standard was fluorobenzene.

[b] Standard deviation was calculated by pooling data from three concentrations.

TABLE 7

SINGLE LABORATORY ACCURACY AND PRECISION DATA FOR PURGEABLE VOLATILE ORGANIC COMPOUNDS IN WATER DETERMINED WITH A NARROW-BORE CAPILLARY COLUMN (METHOD 5030)

Compound	Conc. (µg/L)	Number of Samples	% Recovery[a]	Standard Deviation of Recovery[b]	RSD
Benzene	0.1	7	99	6.2	6.3
Bromobenzene	0.5	7	97	7.4	7.6
Bromochloromethane	0.5	7	97	5.8	6.0
Bromodichloromethane	0.1	7	100	4.6	4.6
Bromoform	0.5	7	101	5.4	5.3
Bromomethane	0.5	7	99	7.1	7.2
n-Butylbenzene	0.5	7	94	6.0	6.4
sec-Butylbenzene	0.5	7	110	7.1	6.5
tert-Butylbenzene	0.5	7	110	2.5	2.3
Carbon tetrachloride	0.1	7	108	6.8	6.3
Chlorobenzene	0.1	7	91	5.8	6.4
Chloroethane	0.1	7	100	5.8	5.8
Chloroform	0.1	7	105	3.2	3.0
Chloromethane	0.5	7	101	4.7	4.7
2-Chlorotoluene	0.5	7	99	4.6	4.6
4-Chlorotoluene	0.5	7	96	7.0	7.3
1,2-Dibromo-3-chloropropane	0.5	7	92	10.0	10.9
Dibromochloromethane	0.1	7	99	5.6	5.7
1,2-Dibromoethane	0.5	7	97	5.6	5.8
Dibromomethane	0.5	7	93	5.6	6.0
1,2-Dichlorobenzene	0.1	7	97	3.5	3.6
1,3-Dichlorobenzene	0.1	7	101	6.0	5.9
1,4-Dichlorobenzene	0.1	7	106	6.5	6.1
Dichlorodifluoromethane	0.1	7	99	8.8	8.9
1,1-Dichloroethane	0.5	7	98	6.2	6.3
1,2-Dichloroethane	0.1	7	100	6.3	6.3
1,1-Dichloroethene	0.1	7	95	9.0	9.5
cis-1,2-Dichloroethene	0.1	7	100	3.5	3.7
trans-1,2-Dichloroethene	0.1	7	98	7.2	7.3
1,2-Dichloropropane	0.5	7	96	6.0	6.3
1,3-Dichloropropane	0.5	7	99	5.8	5.9
2,2-Dichloropropane	0.5	7	99	4.9	4.9
1,1-Dichloropropene	0.5	7	102	7.4	7.3
Ethylbenzene	0.5	7	99	5.2	5.3
Hexachlorobutadiene	0.5	7	100	6.7	6.7
Isopropylbenzene	0.5	7	102	6.4	6.3
p-Isopropyltoluene	0.5	7	113	13.0	11.5
Methylene chloride	0.5	7	97	13.0	13.4
Naphthalene	0.5	7	98	7.2	7.3

TABLE 7 (cont.)

Compound	Conc. (µg/L)	Number of Samples	% Recovery[a]	Standard Deviation of Recovery[b]	RSD
n-Propylbenzene	0.5	7	99	6.6	6.7
Styrene	0.5	7	96	19.0	19.8
1,1,1,2-Tetrachloroethane	0.5	7	100	4.7	4.7
1,1,2,2-Tetrachloroethane	0.5	7	100	12.0	12.0
Tetrachloroethene	0.1	7	96	5.0	5.2
Toluene	0.5	7	100	5.9	5.9
1,2,3-Trichlorobenzene	0.5	7	102	8.9	8.7
1,2,4-Trichlorobenzene	0.5	7	91	16.0	17.6
1,1,1-Trichloroethane	0.5	7	100	4.0	4.0
1,1,2-Trichloroethane	0.5	7	102	4.9	4.8
Trichloroethene	0.1	7	104	2.0	1.9
Trichlorofluoromethane	0.1	7	97	4.6	4.7
1,2,3-Trichloropropane	0.5	7	96	6.5	6.8
1,2,4-Trimethylbenzene	0.5	7	96	6.5	6.8
1,3,5-Trimethylbenzene	0.5	7	101	4.2	4.2
Vinyl chloride	0.1	7	104	0.2	0.2
o-Xylene	0.5	7	106	7.5	7.1
m-Xylene	0.5	7	106	4.6	4.3
p-Xylene	0.5	7	97	6.1	6.3

[a] Recoveries were calculated using internal standard method. Internal standard was fluorobenzene.

TABLE 8

SURROGATE SPIKE RECOVERY LIMITS FOR WATER AND SOIL/SEDIMENT SAMPLES

Surrogate Compound	Water	Soil/Sediment
4-Bromofluorobenzene[a]	86-115	74-121
Dibromofluoromethane[a]	86-118	80-120
Toluene-d_8[a]	88-110	81-117
Dichloroethane-d_4[a]	80-120	80-120

[a] Single laboratory data, for guidance only.

TABLE 9

QUANTITY OF EXTRACT REQUIRED FOR ANALYSIS OF HIGH CONCENTRATION SAMPLES

Approximate Concentration Range (µg/kg)	Volume of Extract[a]
500 - 10,000	100 µL
1,000 - 20,000	50 µL
5,000 - 100,000	10 µL
25,000 - 500,000	100 µL of 1/50 dilution[b]

Calculate appropriate dilution factor for concentrations exceeding this table.

[a] The volume of solvent added to 5 mL of water being purged should be kept constant. Therefore, add to the 5-mL syringe whatever volume of solvent is necessary to maintain a volume of 100 µL added to the syringe.

[b] Dilute an aliquot of the solvent extract and then take 100 µL for analysis.

TABLE 10

DIRECT INJECTION ANALYSIS OF NEW OIL AT 5 PPM (METHOD 3585)

Compound	Recovery (%)	%RSD	Blank (ppm)	Spike (ppm)
Acetone	91	14.8	1.9	5.0
Benzene	86	21.3	0.1	0.5
n-Butanol*,**	107	27.8	0.5	5.0
iso-Butanol*,**	95	19.5	0.9	5.0
Carbon tetrachloride	86	44.7	0.0	0.5
Carbon disulfide**	53	22.3	0.0	5.0
Chlorobenzene	81	29.3	0.0	5.0
Chloroform	84	29.3	0.0	6.0
1,4-Dichlorobenzene	98	24.9	0.0	7.5
1,2-Dichloroethane	101	23.1	0.0	0.5
1,1-Dichloroethene	97	45.3	0.0	0.7
Diethyl ether	76	24.3	0.0	5.0
Ethyl acetate	113	27.4	0.0	5.0
Ethylbenzene	83	30.1	0.2	5.0
Hexachloroethane	71	30.3	0.0	3.0
Methylene chloride	98	45.3	0.0	5.0
Methyl ethyl ketone	79	24.6	0.4	5.0
MIBK	93	31.4	0.0	5.0
Nitrobenzene	89	30.3	0.0	2.0
Pyridine	31	35.9	0.0	5.0
Tetrachloroethene	82	27.1	0.0	0.7
Trichlorofluoromethane	76	27.6	0.0	5.0
1,1,2-Trichlorotrifluoroethane	69	29.2	0.0	5.0
Toluene	73	21.9	0.6	5.0
Trichloroethene	66	28.0	0.0	0.5
Vinyl chloride	63	35.2	0.0	0.2
o-Xylene	83	29.5	0.4	5.0
m/p-Xylene	84	29.5	0.6	10.0

* Alternate mass employed
** IS quantitation

Data are taken from Reference 9.

TABLE 11

SINGLE LABORATORY PERFORMANCE
DATA FOR THE DIRECT INJECTION METHOD - USED OIL (METHOD 3585)

Compound	Recovery (%)	%RSD	Blank (ppm)	Spike (ppm)
Acetone**	105	54	2.0	5.0
Benzene	3135	44	14	0.5
Benzene-d_6	56	44	2.9	0.5
n-Butanol**	100	71	12	5.0
iso-Butanol*,**	132	27	0	5.0
Carbon tetrachloride	143	68	0	0.5
Carbon tetrachloride-^{13}C	99	44	5.1	0.5
Carbon disulfide**	95	63	0	5.0
Chlorobenzene	148	71	0	5.0
Chlorobenzene-d_5	60	44	3.6	5.0
Chloroform	149	74	0	6.0
Chloroform-d_1	51	44	2.6	6.0
1,4-Dichlorobenzene	142	72	0	7.5
1,4-Dichlorobenzene-d_4	53	44	3.4	7.5
1,2-Dichloroethane**	191	54	0	0.5
1,1-Dichloroethene*	155	51	0	0.7
1,1-Dichloroethene-d_2	68	44	3.4	0.7
Diethyl ether**	95	66	0	5.0
Ethyl acetate*,**	126	39	0	5.0
Ethylbenzene	1298	44	54	5.0
Ethylbenzene-d_{10}	63	44	3.6	5.0
Hexachloroethane	132	72	0	3.0
Hexachloroethane-^{13}C	54	45	3.5	3.0
Methylene chloride**	86	65	0.3	5.0
Methyl ethyl ketone**	107	64	0	5.0
4-Methyl-2-pentanone (MIBK)**	100	74	0.1	5.0
Nitrobenzene	111	80	0	2.0
Nitrobenzene-d_5	65	53	4.0	2.0
Pyridine**	68	85	0	5.0
Pyridine-d_5	ND	--	0	5.0
Tetrachloroethene**	101	73	0	0.7
Trichlorofluoromethane**	91	70	0	5.0
1,1,2-Cl_3F_3ethane**	81	70	0	5.0
Toluene	2881	44	128	5.0
Toluene-d_8	63	44	3.6	5.0
Trichloroethene	152	57	0	0.5
Trichloroethene-d_1	55	44	2.8	0.5

TABLE 11 (cont.)

Compound	Recovery (%)	%RSD	Blank (ppm)	Spike (ppm)
Vinyl chloride**	100	69	0	0.2
o-Xylene	2292	44	105	5.0
o-Xylene-d_{10}	76	44	4.2	5.0
m-/p-Xylene	2583	44	253	10.0
p-Xylene-d_{10}	67	44	3.7	10.0

* Alternate mass employed
** IS quantitation
ND = Not Detected

Data are based on seven measurements and are taken from Reference 9.

TABLE 12

METHOD DETECTION LIMITS (METHOD 5031)

Compound	MDL (µg/L) Macro[a]	Concentration Factor Macro	Micro
Acetone	31	25-500	-
Acetonitrile	57	25-500	200
Acrolein	-	-	100
Acrylonitrile	16	25-500	100
Allyl Alcohol	7	25-500	-
1-Butanol	-	-	250
Crotonaldehyde	12	25-500	-
1,4-Dioxane	12	25-500	150
Ethyl Acetate	-	-	100
Isobutyl alcohol	7	25-500	-
Methanol	38	25-500	140
Methyl Ethyl Ketone	16	25-500	-
2-Methyl-1-propanol	-	-	250
n-Nitroso-di-n-butylamine	14	25-500	-
Paraldehyde	10	25-500	-
2-Picoline	7	25-500	-
1-Propanol	-	-	240
Propionitrile	11	25-500	200
Pyridine	4	25-500	-
o-Toluidine	13	25-500	-

[a] Produced by analysis of seven aliquots of reagent water spiked at 25 ppb at the listed compounds; calculations based on internal standard technique and use of the following equation:

MDL = 3.134 x Std. Dev. of low concentration spike (ppb).

[b] When a 40-mL sample is used, and the first 100 µL of distillate are collected.

TABLE 13

TARGET COMPOUNDS, SURROGATES, AND INTERNAL STANDARDS (METHOD 5031)

Target Compound	Surrogate	Internal Standard
Acetone	d_6-Acetone	d_8-Isopropyl alcohol
Acetonitrile	d_3-Acetonitrile	d_8-Isopropyl alcohol
Acrylonitrile	d_8-Isopropyl alcohol	
Allyl alcohol	d_7-Dimethyl formamide	
Crotonaldehyde	d_8-Isopropyl alcohol	
1,4-Dioxane	d_8-1,4-Dioxane	d_7-Dimethyl formamide
Isobutyl alcohol	d_7-Dimethyl formamide	
Methanol	d_3-Methanol	d_8-Isopropyl alcohol
Methyl ethyl ketone	d_8-Isopropyl alcohol	
N-Nitroso-di-n-butylamine	d_7-Dimethyl formamide	
Paraldehyde	d_7-Dimethyl formamide	
2-Picoline	d_7-Dimethyl formamide	
Propionitrile	d_8-Isopropyl alcohol	
Pyridine	d_5-Pyridine	d_7-Dimethyl formamide
o-Toluidine	d_7-Dimethyl formamide	

TABLE 14

RECOMMENDED CONCENTRATIONS FOR CALIBRATION SOLUTIONS (METHOD 5031)

Compound	Concentration(s) (ng/µL)
Internal Standards	
d_5-benzyl alcohol	10.0
d_{14}-Diglyme	10.0
d_7-Dimethyl formamide	10.0
d_8-Isopropyl alcohol	10.0
Surrogates	
d_6-Acetone	10.0
d_3-Acetonitrile	10.0
d_8-1,4-Dioxane	10.0
d_3-Methanol	10.0
d_5-Pyridine	10.0
Target Compounds	
Acetone	1.0, 5.0, 10.0, 25.0, 100.0
Acetonitrile	1.0, 5.0, 10.0, 25.0, 100.0
Acrylonitrile	1.0, 5.0, 10.0, 25.0, 100.0
Allyl alcohol	1.0, 5.0, 10.0, 25.0, 100.0
Crotonaldehyde	1.0, 5.0, 10.0, 25.0, 100.0
1,4-Dioxane	1.0, 5.0, 10.0, 25.0, 100.0
Isobutyl alcohol	1.0, 5.0, 10.0, 25.0, 100.0
Methanol	1.0, 5.0, 10.0, 25.0, 100.0
Methyl ethyl ketone	1.0, 5.0, 10.0, 25.0, 100.0
N-Nitroso-di-n-butylamine	1.0, 5.0, 10.0, 25.0, 100.0
Paraldehyde	1.0, 5.0, 10.0, 25.0, 100.0
2-Picoline	1.0, 5.0, 10.0, 25.0, 100.0
Propionitrile	1.0, 5.0, 10.0, 25.0, 100.0
Pyridine	1.0, 5.0, 10.0, 25.0, 100.0
o-Toluidine	1.0, 5.0, 10.0, 25.0, 100.0

TABLE 15

CHARACTERISTIC IONS AND RETENTION TIMES FOR VOCs (METHOD 5031)

Compound	Quantitation Ion[a]	Secondary Ions	Retention Time (min)[b]
Internal Standards			
d_8-Isopropyl alcohol	49		1.75
d_{14}-Diglyme	66	98,64	9.07
d_7-Dimethyl formamide	50	80	9.20
Surrogates			
d_6-Acetone	46	64,42	1.03
d_3-Methanol	33	35,30	1.75
d_3-Acetonitrile	44	42	2.63
d_8-1,4-Dioxane	96	64,34	3.97
d_5-Pyridine	84	56,79	6.73
d_5-Phenol[c]	99	71	15.43
Target Compounds			
Acetone	43	58	1.05
Methanol	31	29	1.52
Methyl ethyl ketone	43	72,57	1.53
Methacrylonitrile[c]	67	41	2.38
Acrylonitrile	53	52,51	2.53
Acetonitrile	41	40,39	2.73
Methyl isobutyl ketone[c]	85	100,58	2.78
Propionitrile	54	52,55	3.13
Crotonaldehyde	41	70	3.43
1,4-Dioxane	58	88,57	4.00
Paraldehyde	45	89	4.75
Isobutyl alcohol	43	33,42	5.05
Allyl alcohol	57	39	5.63
Pyridine	79	50,52	6.70
2-Picoline	93	66	7.27
N-Nitroso-di-n-butylamine	84	116	12.82
Aniline[c]	93	66,92	13.23
o-Toluidine	106	107	13.68
Phenol[c]	94	66,65	15.43

[a] These ions were used for quantitation in selected ion monitoring.
[b] GC column: DB-Wax, 30 meter x 0.53 mm, 1 µm film thickness.
Oven program: 45°C for 4 min, increased to 220°C at 12°C/min.
[c] Compound removed from target analyte list due to poor accuracy and precision.

TABLE 16

METHOD ACCURACY AND PRECISION BY MEAN PERCENT RECOVERY AND PERCENT RELATIVE STANDARD DEVIATION[a] (METHOD 5031 - MACRODISTILLATION TECHNIQUE)
(Single Laboratory and Single Operator)

Compound	25 ppb Spike		100 ppb Spike		500 ppb Spike	
	Mean %R	%RSD	Mean %R	%RSD	Mean %R	%RSD
d_6-Acetone	66	24	69	14	65	16
d_3-Acetonitrile	89	18	80	18	70	10
d_8-1,4-Dioxane	56	34	58	11	61	18
d_3-Methanol	43	29	48	19	56	14
d_5-Pyridine	83	6.3	84	7.8	85	9.0
Acetone	67	45	63	14	60	14
Acetonitrile	44	35	52	15	56	15
Acrylonitrile	49	42	47	27	45	27
Allyl alcohol	69	13	70	9.7	73	10
Crotonaldehyde	68	22	68	13	69	13
1,4-Dioxane	63	25	55	16	54	13
Isobutyl alcohol	66	14	66	5.7	65	7.9
Methanol	50	36	46	22	49	18
Methyl ethyl ketone	55	37	56	20	52	19
N-Nitroso-di-n-butylamine	57	21	61	15	72	18
Paraldehyde	65	20	66	11	60	8.9
Picoline	81	12	81	6.8	84	8.0
Propionitrile	67	22	69	13	68	13
Pyridine	74	7.4	72	6.7	74	7.3
o-Toluidine	52	31	54	15	58	12

[a] Data from analysis of seven aliquots of reagent water spiked at each concentration, using a quadrapole mass spectrometer in the selected ion monitoring mode.

TABLE 17

RECOVERIES IN SAND SAMPLES FORTIFIED AT 4 µg/kg (ANALYSIS BY METHOD 5035)

Compound	Recovery per Replicate (ng) 1	2	3	4	5	Mean	RSD	Mean Rec
Vinyl chloride	8.0	7.5	6.7	5.4	6.6	6.8	13.0	34.2
Trichlorofluoromethane	13.3	16.5	14.9	13.0	10.3	13.6	15.2	68.0
1,1-Dichloroethene	17.1	16.7	15.1	14.8	15.6	15.9	5.7	79.2
Methylene chloride	24.5	22.7	19.7	19.4	20.6	21.4	9.1	107
trans-1,2-Dichloroethene	22.7	23.6	19.4	18.3	20.1	20.8	0.7	104
1,2-Dichloroethane	18.3	18.0	16.7	15.6	15.9	16.9	6.4	84.4
cis-1,2-Dichloroethene	26.1	23.1	22.6	20.3	20.8	22.6	9.0	113
Bromochloromethane	24.5	25.4	20.9	20.1	20.1	22.2	10.2	111
Chloroform	26.5	26.0	22.1	18.9	22.1	23.1	12.2	116
1,1,1-Trichloroethane	21.5	23.0	23.9	16.7	31.2	23.4	21.2	117
Carbon tetrachloride	23.6	24.2	22.6	18.3	23.3	22.4	9.4	112
Benzene	22.4	23.9	20.4	17.4	19.2	20.7	11.2	103
Trichloroethene	21.5	20.5	19.2	14.4	19.1	18.9	12.7	94.6
1,2-Dichloropropane	24.9	26.3	23.1	19.0	23.3	23.3	10.5	117
Dibromomethane	25.4	26.4	21.6	20.4	23.6	23.5	9.6	117
Bromodichloromethane	25.7	26.7	24.1	17.9	23.0	23.5	13.1	117
Toluene	28.3	25.0	24.8	16.3	23.6	23.6	16.9	118
1,1,2-Trichloroethane	25.4	24.5	21.6	17.7	22.1	22.2	12.1	111
1,3-Dichloropropane	25.4	24.2	22.7	17.0	22.2	22.3	12.8	112
Dibromochloromethane	26.3	26.2	23.7	18.2	23.2	23.5	12.5	118
Chlorobenzene	22.9	22.5	19.8	14.6	19.4	19.9	15.0	99.3
1,1,1,2-Tetrachloroethane	22.4	27.7	25.1	19.4	22.6	23.4	12.0	117
Ethylbenzene	25.6	25.0	22.1	14.9	24.0	22.3	17.5	112
p-Xylene	22.5	22.0	19.8	13.9	20.3	19.7	15.7	98.5
o-Xylene	24.2	23.1	21.6	14.0	20.4	20.7	17.3	103
Styrene	23.9	21.5	20.9	14.3	20.5	20.2	15.7	101
Bromoform	26.8	25.6	26.0	20.1	23.5	24.4	9.9	122
iso-Propylbenzene	25.3	25.1	24.2	15.4	24.6	22.9	16.6	114
Bromobenzene	19.9	21.8	20.0	15.5	19.1	19.3	10.7	96.3
1,2,3-Trichloropropane	25.9	23.0	25.6	15.9	21.4	22.2	15.8	111
n-Propylbenzene	26.0	23.8	22.6	13.9	21.9	21.6	19.0	108
2-Chlorotoluene	23.6	23.8	21.3	13.0	21.5	20.6	19.2	103
4-Chlorotoluene	21.0	19.7	18.4	12.1	18.3	17.9	17.1	89.5
1,3,5-Trimethylbenzene	24.0	22.1	22.5	13.8	22.9	21.1	17.6	105
sec-Butylbenzene	25.9	25.3	27.8	16.1	28.6	24.7	18.1	124
1,2,4-Trimethylbenzene	30.6	39.2	22.4	18.0	22.7	26.6	28.2	133
1,3-Dichlorobenzene	20.3	20.6	18.2	13.0	17.6	17.9	15.2	89.7
p-iso-Propyltoluene	21.6	22.1	21.6	16.0	22.8	20.8	11.8	104
1,4-Dichlorobenzene	18.1	21.2	20.0	13.2	17.4	18.0	15.3	90.0
1,2-Dichlorobenzene	18.4	22.5	22.5	15.2	19.9	19.7	13.9	96.6
n-Butylbenzene	13.1	20.3	19.5	10.8	18.7	16.5	23.1	82.4
1,2,4-Trichlorobenzene	14.5	14.9	15.7	8.8	12.3	13.3	18.8	66.2
Hexachlorobutadiene	17.6	22.5	21.6	13.2	21.6	19.3	18.2	96.3
1,2,3-Trichlorobenzene	14.9	15.9	16.5	11.9	13.9	14.6	11.3	73.1

Data in Tables 17, 18, and 19 are from Reference 15.

TABLE 18
RECOVERIES IN C-HORIZON SOILS FORTIFIED AT 4 µg/kg (ANALYSIS BY METHOD 5035)

Compound	Recovery per Replicate (ng) 1	2	3	4	5	Mean	RSD	Mean Rec
Vinyl chloride	33.4	31.0	30.9	29.7	28.6	30.8	5.2	154
Trichlorofluoromethane	37.7	20.8	20.0	21.8	20.5	24.1	28.2	121
1,1-Dichloroethene	21.7	33.5	39.8	30.2	32.5	31.6	18.5	158
Methylene chloride	20.9	19.4	18.7	18.3	18.4	19.1	5.1	95.7
trans-1,2-Dichloroethene	21.8	18.9	20.4	17.9	17.8	19.4	7.9	96.8
1,1-Dichloroethane	23.8	21.9	21.3	21.3	20.5	21.8	5.2	109
cis-1,2-Dichloroethene	21.6	18.8	18.5	18.2	18.2	19.0	6.7	95.2
Bromochloromethane	22.3	19.5	19.3	19.0	19.2	20.0	6.0	100
Chloroform	20.5	17.1	17.3	16.5	15.9	17.5	9.2	87.3
1,1,1-Trichloroethane	16.4	11.9	10.7	9.5	9.4	11.6	22.4	57.8
Carbon tetrachloride	13.1	11.3	13.0	11.8	11.2	12.1	6.7	60.5
Benzene	21.1	19.3	18.7	18.2	16.9	18.8	7.4	94.1
Trichloroethene	19.6	16.4	16.5	16.5	15.5	16.9	8.3	84.5
1,2-Dichloropropane	21.8	19.0	18.3	18.8	16.5	18.9	9.0	94.4
Dibromomethane	20.9	17.9	17.9	17.2	18.3	18.4	6.9	92.1
Bromodichloromethane	20.9	18.0	18.9	18.2	17.3	18.6	6.6	93.2
Toluene	22.2	17.3	18.8	17.0	15.9	18.2	12.0	91.2
1,1,2-Trichloroethane	21.0	16.5	17.2	17.2	16.5	17.7	9.6	88.4
1,3-Dichloropropane	21.4	17.3	18.7	18.6	16.7	18.5	8.8	92.6
Dibromochloromethane	20.9	18.1	19.0	18.8	16.6	18.7	7.5	93.3
Chlorobenzene	20.8	18.4	17.6	16.8	14.8	17.7	11.2	88.4
1,1,1,2-Tetrachloroethane	19.5	19.0	17.8	17.2	16.5	18.0	6.2	90.0
Ethylbenzene	21.1	18.3	18.5	16.9	15.3	18.0	10.6	90.0
p-Xylene	20.0	17.4	18.2	16.3	14.4	17.3	10.9	86.3
o-Xylene	20.7	17.2	16.8	16.2	14.8	17.1	11.4	85.7
Styrene	18.3	15.9	16.2	15.3	13.7	15.9	9.3	79.3
Bromoform	20.1	15.9	17.1	17.5	16.1	17.3	8.6	86.7
iso-Propylbenzene	21.0	18.1	19.2	18.4	15.6	18.4	9.6	92.2
Bromobenzene	20.4	16.2	17.2	16.7	15.4	17.2	10.1	85.9
1,1,2,2-Tetrachloroethane	23.3	17.9	21.2	18.8	16.8	19.6	12.1	96.0
1,2,3-Trichloropropane	18.4	14.6	15.6	16.1	15.6	16.1	8.0	80.3
n-Propylbenzene	20.4	18.9	17.9	17.0	14.3	17.7	11.6	88.4
2-Chlorotoluene	19.1	17.3	16.1	16.0	14.4	16.7	9.2	83.6
4-Chlorotoluene	19.0	15.5	16.8	15.9	13.6	16.4	10.6	81.8
1,3,5-Trimethylbenzene	20.8	18.0	17.4	16.1	14.7	17.4	11.7	86.9
sec-Butylbenzene	21.4	18.3	18.9	17.0	14.9	18.1	11.8	90.5
1,2,4-Trimethylbenzene	20.5	18.6	16.8	15.3	13.7	17.0	14.1	85.0
1,3-Dichlorobenzene	17.6	15.9	15.6	14.2	14.4	15.6	7.9	77.8
p-iso-Propyltoluene	20.5	17.0	17.1	15.6	13.4	16.7	13.9	83.6
1,4-Dichlorobenzene	18.5	13.8	14.8	16.7	14.9	15.7	10.5	78.7
1,2-Dichlorobenzene	18.4	15.0	15.4	15.3	13.5	15.5	10.5	77.6
n-Butylbenzene	19.6	15.9	15.9	14.4	18.9	16.9	11.7	84.6
1,2,4-Trichlorobenzene	15.2	17.2	17.4	13.6	12.1	15.1	13.5	75.4
Hexachlorobutadiene	18.7	16.2	15.5	13.8	16.6	16.1	10.0	80.7
Naphthalene	13.9	11.1	10.2	10.8	11.4	11.5	11.0	57.4
1,2,3-Trichlorobenzene	14.9	15.2	16.8	13.7	12.7	14.7	9.5	73.2

TABLE 19
RECOVERIES IN GARDEN SOIL FORTIFIED AT 4 µg/kg (ANALYSIS BY METHOD 5035)

Compound	Recovery per Replicate (ng) 1	2	3	4	5	Mean	RSD	Mean Rec
Vinyl chloride	12.7	10.9	9.8	8.1	7.2	9.7	20.2	48.7
Trichlorofluoromethane	33.7	6.4	30.3	27.8	22.9	24.2	39.6	121
1,1-Dichloroethene	27.7	20.5	24.1	15.1	13.2	20.1	26.9	101
Methylene chloride	25.4	23.9	24.7	22.2	24.2	24.1	4.4	120
trans-1,2-Dichloroethene	2.8	3.0	3.3	2.2	2.4	2.7	15.0	13.6
1,1-Dichloroethane	24.1	26.3	27.0	20.5	21.2	23.8	11.0	119
cis-1,2-Dichloroethene	8.3	10.2	8.7	5.8	6.4	7.9	20.1	39.4
Bromochloromethane	11.1	11.8	10.2	8.8	9.0	10.2	11.2	50.9
Chloroform	16.7	16.9	17.0	13.8	15.0	15.9	7.9	79.3
1,1,1-Trichloroethane	24.6	22.8	22.1	16.2	20.9	21.3	13.4	107
Carbon tetrachloride	19.4	20.3	22.2	20.0	20.2	20.4	4.6	102
Benzene	21.4	22.0	22.4	19.6	20.4	21.2	4.9	106
Trichloroethene	12.4	16.5	14.9	9.0	9.9	12.5	22.9	62.7
1,2-Dichloropropane	19.0	18.8	19.7	16.0	17.6	18.2	7.1	91.0
Dibromomethane	7.3	8.0	6.9	5.6	6.8	6.9	11.3	34.6
Bromodichloromethane	14.9	15.9	15.9	12.8	13.9	14.7	8.3	73.3
Toluene	42.6	39.3	45.1	39.9	45.3	42.4	5.9	212
1,1,2-Trichloroethane	13.9	15.2	1.4	21.3	14.9	15.9	17.0	79.6
1,3-Dichloropropane	13.3	16.7	11.3	10.9	9.5	12.3	20.3	61.7
Dibromochloromethane	14.5	13.1	14.5	11.9	14.4	13.7	7.6	68.3
Chlorobenzene	8.4	10.0	8.3	6.9	7.8	8.3	12.1	41.3
1,1,1,2-Tetrachloroethane	16.7	16.7	15.6	15.8	15.7	16.1	3.2	80.4
Ethylbenzene	22.1	21.4	23.1	20.1	22.6	21.9	4.8	109
p-Xylene	41.4	38.4	43.8	38.3	44.0	41.2	6.1	206
o-Xylene	31.7	30.8	34.3	30.4	33.2	32.1	4.6	160
Styrene	0	0	0	0	0	0	0	0
Bromoform	8.6	8.9	9.1	7.0	7.7	8.3	9.4	41.4
iso-Propylbenzene	18.1	18.8	9.7	18.3	19.6	18.9	3.5	94.4
Bromobenzene	5.1	5.4	5.3	4.4	4.0	4.8	11.6	24.1
1,1,2,2-Tetrachloroethane	14.0	13.5	14.7	15.3	17.1	14.9	8.5	74.5
1,2,3-Trichloropropane	11.0	12.7	11.7	11.7	11.9	11.8	4.5	59.0
n-Propylbenzene	13.4	13.3	14.7	12.8	13.9	13.6	4.7	68.1
2-Chlorotoluene	8.3	9.0	11.7	8.7	7.9	9.1	14.8	45.6
4-Chlorotoluene	5.1	5.4	5.5	4.8	4.5	5.0	7.9	25.2
1,3,5-Trimethylbenzene	31.3	27.5	33.0	31.1	33.6	31.3	6.8	157
sec-Butylbenzene	13.5	13.4	16.4	13.8	15.4	14.5	8.3	72.5
1,2,4-Trimethylbenzene	38.7	32.4	40.8	34.1	40.3	37.3	9.1	186
1,3-Dichlorobenzene	3.6	3.6	3.7	3.0	3.2	3.4	8.0	17.2
p-iso-Propyltoluene	14.7	14.1	16.1	13.9	15.1	14.8	5.2	73.8
1,4-Dichlorobenzene	3.0	3.5	3.3	2.6	2.8	3.0	10.2	15.0
1,2-Dichlorobenzene	3.6	4.3	4.0	3.5	3.6	3.8	8.3	19.0
n-Butylbenzene	17.4	13.8	14.0	18.9	24.0	17.6	21.2	88.0
1,2,4-Trichlorobenzene	2.8	2.9	3.3	2.6	3.2	3.0	8.5	15.0
Hexachlorobutadiene	4.8	4.0	6.1	5.6	6.0	5.3	15.1	26.4
Naphthalene	5.5	5.1	5.5	4.7	5.6	5.3	6.2	26.5
1,2,3-Trichlorobenzene	2.2	2.3	2.4	2.2	2.3	2.3	3.5	11.4

Data in Table 19 are from Reference 15.

TABLE 20

VOLATILE ORGANIC ANALYTE RECOVERY FROM SOIL
USING VACUUM DISTILLATION (METHOD 5032)[a]

Compound	Soil/H_2O[b] Recovery Mean	RSD	Soil/Oil[c] Recovery Mean	RSD	Soil/Oil/H_2O Recovery Mean	RSD
Chloromethane	61	20	40	18	108	68
Bromomethane	58	20	47	13	74	13
Vinyl chloride	54	12	46	11	72	20
Chloroethane	46	10	41	8	52	14
Methylene chloride	60	2	65	8	76	11
Acetone	INT[e]	INT	44	8		
Carbon disulfide	47	13	53	10	47	4
1,1-Dichloroethene	48	9	47	5	58	3
1,1-Dichloroethane	61	6	58	9	61	6
trans-1,2-Trichloroethane	54	7	60	7	56	5
cis-1,2-Dichloroethene	60	4	72	6	63	8
Chloroform	104	11	93	6	114	15
1,2-Dichloroethane	177	50	117	8	151	22
2-Butanone	INT	36	38	INT		
1,1,1-Trichloroethane	124	13	72	16	134	26
Carbon tetrachloride	172	122	INT	INT		
Vinyl acetate	88	11	INT			
Bromodichloromethane	93	4	91	23	104	23
1,1,2,2-Tetrachloroethane	96	13	50	12	104	7
1,2-Dichloropropane	105	8	102	6	111	6
trans-1,3-Dichloropropene	134	10	84	16	107	8
Trichloroethene	98	9	99	10	100	5
Dibromochloromethane	119	8	125	31	142	16
1,1,2-Trichloroethane	126	10	72	16	97	4
Benzene	99	7	CONT[f]	CONT		
cis-1,3-Dichloropropene	123	12	94	13	112	9
Bromoform	131	13	58	18	102	9
2-Hexanone	155	18	164	19	173	29
4-Methyl-2-pentanone	152	20	185	20	169	18
Tetrachloroethene	90	9	123	14	128	7
Toluene	94	3	CONT	CONT		
Chlorobenzene	98	7	93	18	112	5
Ethylbenzene	114	13	CONT	CONT		
Styrene	106	8	93	18	112	5
p-Xylene	97	9	CONT	CONT		
o-Xylene	105	8	112	12	144	13

TABLE 20 (cont.)

Compound	Soil/H_2O[b] Recovery Mean	RSD	Soil/Oil[c] Recovery Mean	RSD	Soil/Oil/H_2O Recovery Mean	RSD
Surrogates						
1,2-Dichloroethane	177	50	117	8	151	22
Toluene-d_8	96	6	79	12	82	6
Bromofluorobenzene	139	13	37	13	62	5

[a] Results are for 10 min. distillations times, and condenser temperature held at -10°C. A 30 m x 0.53 mm ID stable wax column with a 1 µm film thickness was used for chromatography. Standards and samples were replicated and precision value reflects the propagated errors. Each analyte was spiked at 50 ppb. Vacuum distillation efficiencies (Method 5032) are modified by internal standard corrections. Method 8260 internal standards may introduce bias for some analytes. See Method 5032 to identify alternate internal standards with similar efficiencies to minimize bias.

[b] Soil samples spiked with 0.2 mL water containing analytes and then 5 mL water added to make slurry.

[c] Soil sample + 1 g cod liver oil, spiked with 0.2 mL water containing analytes.

[d] Soil samples + 1 g cod liver oil, spiked as above with 5 mL of water added to make slurry.

[e] Interference by co-eluting compounds prevented accurate measurement of analyte.

[f] Contamination of sample matrix by analyte prevented assessment of efficiency.

TABLE 21

VACUUM DISTILLATION EFFICIENCIES FOR VOLATILE ORGANIC ANALYTES IN FISH TISSUE (METHOD 5032)[a]

Compound	Efficiency Mean (%)	RSD (%)
Chloromethane	N/A[b]	
Bromomethane	N/A[b]	
Vinyl chloride	N/A[b]	
Chloroethane	N/A[b]	
Methylene chloride	CONT[c]	
Acetone	CONT[c]	
Carbon disulfide	79	36
1,1-Dichloroethene	122	39
1,1-Dichloroethane	126	35
trans-1,2-Trichloroethene	109	46
cis-1,2-Dichloroethene	106	22
Chloroform	111	32
1,2-Dichloroethane	117	27
2-Butanone	INT[d]	
1,1,1-Trichloroethane	106	30
Carbon tetrachloride	83	34
Vinyl acetate	INT[d]	
Bromodichloromethane	97	22
1,1,2,2-Tetrachloroethane	67	20
1,2-Dichloropropane	117	23
trans-1,3-Dichloropropene	92	22
Trichloroethene	98	31
Dibromochloromethane	71	19
1,1,2-Trichloroethane	92	20
Benzene	129	35
cis-1,3-Dichloropropene	102	24
Bromoform	58	19
2-Hexanone	INT[d]	
4-Methyl-2-pentanone	113	37
Tetrachloroethene	66	20
Toluene	CONT[c]	
Chlorobenzene	65	19
Ethylbenzene	74	19
Styrene	57	14
p-Xylene	46	13
o-Xylene	83	20

TABLE 21 (cont.)

Compound	Efficiency Mean (%)	RSD (%)
Surrogates		
1,2-Dichloroethane	115	27
Toluene-d_8	88	24
Bromofluorobenzene	52	15

[a] Results are for 10 min. distillation times and condenser temperature held at -10°C. Five replicate 10-g aliquots of fish spiked at 25 ppb were analyzed using GC/MS external standard quantitation. A 30 m x 0.53 mm ID stable wax column with a 1 µm film thickness was used for chromatography. Standards were replicated and results reflect 1 sigma propagated standard deviation.

[b] No analyses.

[c] Contamination of sample matrix by analyte prevented accurate assessment of analyte efficiency.

[d] Interfering by co-eluting compounds prevented accurate measurement of analyte.

TABLE 22

METHOD DETECTION LIMITS (MDL) FOR VOLATILE ORGANIC ANALYTES IN FISH TISSUE (METHOD 5032)[a]

Compound	Method Detection Limit (ppb) External Standard Method	Internal Standard Method
Chloromethane	7.8	7.3
Bromomethane	9.7	9.8
Vinyl chloride	9.5	9.4
Chloroethane	9.2	10.0
Methylene chloride	CONT[b]	CONT[b]
Acetone	CONT[b]	CONT[b]
Carbon disulfide	5.4	4.9
1,1-Dichloroethene	4.0	5.7
1,1-Dichloroethane	4.0	3.5
trans-1,2-Dichloroethene	4.4	4.0
cis-1,2-Dichloroethene	4.7	4.1
Chloroform	5.6	5.0
1,2-Dichloroethane	3.3	3.2
2-Butanone	INT[c]	INT[c]
1,1,1-Trichloroethane	1.1	4.2
Carbon tetrachloride	3.2	3.5
Vinyl acetate	INT[c]	INT[c]
Bromodichloromethane	3.2	2.8
1,1,2,2-Tetrachloroethane	4.4	3.8
1,2-Dichloropropane	3.8	3.7
trans-1,3-Dichloropropene	3.4	3.0
Trichloroethene	3.1	4.0
Dibromochloromethane	3.5	3.2
1,1,2-Trichloroethane	4.4	3.3
Benzene	3.6	3.2
cis-1,3-Dichloropropene	3.5	3.0
Bromoform	4.9	4.0
2-Hexanone	7.7	8.0
4-Methyl-2-pentanone	7.5	8.0
Tetrachloroethene	4.3	4.0
Toluene	3.0	2.5
Chlorobenzene	3.3	2.8
Ethylbenzene	3.6	3.5
Styrene	3.5	3.3
p-Xylene	3.7	3.5
o-Xylene	3.3	4.7

Footnotes are on the following page.

TABLE 22 (cont.)

[a] Values shown are the average MDLs for studies on three non-consecutive days, involving seven replicate analyses of 10 g of fish tissue spiked a 5 ppb. Daily MDLs were calculated as three times the standard deviation. Quantitation was performed by GC/MS Method 8260 and separation with a 30 m x 0.53 mm ID stable wax column with a 1 µm film thickness.

[b] Contamination of sample by analyte prevented determination.

[c] Interference by co-eluting compounds prevented accurate quantitation.

TABLE 23

VOLATILE ORGANIC ANALYTES RECOVERY FOR WATER
USING VACUUM DISTILLATION (METHOD 5032)[a]

Compound	5 mL H_2O[b] Recovery Mean	RSD	20 mL H_2O[c] Recovery Mean	RSD	20 mL H_2O/Oil Recovery Mean	RSD
Chloromethane	114	27	116	29	176	67
Bromomethane	131	14	121	14	113	21
Vinyl chloride	131	13	120	16	116	23
Chloroethane	110	15	99	8	96	16
Methylene chloride	87	16	105	15	77	6
Acetone	83	22	65	34	119	68
Carbon disulfide	138	17	133	23	99	47
1,1-Dichloroethene	105	11	89	4	96	18
1,1-Dichloroethane	118	10	119	11	103	25
trans-1,2-Dichloroethene	105	11	107	14	96	18
cis-1,2-Dichloroethene	106	7	99	5	104	23
Chloroform	114	6	104	8	107	21
1,2-Dichloroethane	104	6	109	8	144	19
2-Butanone	83	50	106	31	INT[c]	
1,1,1-Trichloroethane	118	9	109	9	113	23
Carbon tetrachloride	102	6	108	12	109	27
Vinyl acetate	90	16	99	7	72	36
Bromodichloromethane	104	3	110	5	99	5
1,1,2,2-Tetrachloroethane	85	17	81	7	111	43
1,2-Dichloropropane	100	6	103	2	104	7
trans-1,3-Dichloropropene	105	8	105	4	92	4
Trichloroethene	98	4	99	2	95	5
Dibromochloroethane	99	8	99	6	90	25
1,1,2-Trichloroethane	98	7	100	4	76	12
Benzene	97	4	100	5	112	10
cis-1,3-Dichloropropene	106	5	105	4	98	3
Bromoform	93	16	94	8	57	21
2-Hexanone	60	17	63	16	78	23
4-Methyl-2-pentanone	79	24	63	14	68	15
Tetrachloroethene	101	3	97	7	77	14
Toluene	100	6	97	8	85	5
Chlorobenzene	98	6	98	4	88	16
Ethylbenzene	100	3	92	8	73	13
Styrene	98	4	97	9	88	16
p-Xylene	96	4	94	8	60	12
o-Xylene	96	7	95	6	72	14

TABLE 23 (cont.)

Compound	5 mL H_2O[b] Recovery Mean	RSD	20 mL H_2O[c] Recovery Mean	RSD	20 mL H_2O/Oil Recovery Mean	RSD
Surrogates						
1,2-Dichloroethane	104	6	109	6	144	19
Toluene-d_8	104	5	102	2	76	7
Bromofluorobenzene	106	6	106	9	40	8

[a] Results are for 10 min. distillation times, and condenser temperature held at -10°C. A 30 m x 0.53 mm ID stable wax column with a 1 µm film thickness was used for chromatography. Standards and samples were replicated and precision values reflect the propagated errors. Concentrations of analytes were 50 ppb for 5-mL samples and 25 ppb for 20-mL samples. Recovery data generated with comparison to analyses of standards without the water matrix.

[b] Sample contained 1 gram cod liver oil and 20 mL water. An emulsion was created by adding 0.2 mL of water saturated with lecithin.

[c] Interference by co-eluting compounds prevented accurate assessment of recovery.

TABLE 24

METHOD DETECTION LIMITS (MDL) FOR VOLATILE ORGANIC ANALYTES USING VACUUM DISTILLATION (METHOD 5032) (INTERNAL STANDARD METHOD)[a]

Compound	Water[b] (µg/L)	Soil[c] (µg/kg)	Tissue[d] (µg/kg)	Oil[e] (mg/kg)
Chloromethane	3.2	8.0	7.3	N/A[f]
Bromomethane	2.8	4.9	9.8	N/A[f]
Vinyl chloride	3.5	6.0	9.4	N/A[f]
Chloroethane	5.9	6.0	10.0	N/A[f]
Methylene chloride	3.1	4.0	CONT[g]	0.05
Acetone	5.6	CONT[g]	CONT[g]	0.06
Carbon disulfide	2.5	2.0	4.9	0.18
1,1-Dichloroethene	2.9	3.2	5.7	0.18
1,1-Dichloroethane	2.2	2.0	3.5	0.14
trans-1,2-Dichloroethene	2.2	1.4	4.0	0.10
cis-1,2-Dichloroethene	2.0	2.3	4.1	0.07
Chloroform	2.4	1.8	5.0	0.07
1,2-Dichloroethane	1.7	1.5	3.2	0.06
2-Butanone	7.4	INT[h]	INT[h]	INT[h]
1,1,1-Trichloroethane	1.8	1.7	4.2	0.10
Carbon tetrachloride	1.4	1.5	3.5	0.13
Vinyl acetate	11.8	INT[h]	INT[h]	INT[h]
Bromodichloromethane	1.6	1.4	2.8	0.06
1,1,2,2-Tetrachloroethane	2.5	2.1	3.8	0.02
1,2-Dichloropropane	2.2	2.1	3.7	0.15
trans-1,3-Dichloropropene	1.5	1.7	3.0	0.05
Trichloroethene	1.6	1.7	4.0	0.04
Dibromochloromethane	1.7	1.5	3.2	0.07
1,1,2-Trichloroethane	2.1	1.7	3.3	0.05
Benzene	0.5	1.5	3.2	0.05
cis-1,3-Dichloropropene	1.4	1.7	3.0	0.04
Bromoform	1.8	1.5	4.0	0.05
2-Hexanone	4.6	3.6	8.0	INT[h]
4-Methyl-2-pentanone	3.5	4.6	8.0	INT[h]
Tetrachloroethene	1.4	1.6	4.0	0.10
Toluene	1.0	3.3	2.5	0.05
Chlorobenzene	1.4	1.4	2.8	0.06
Ethylbenzene	1.5	2.8	3.5	0.04
Styrene	1.4	1.4	3.3	0.18
p-Xylene	1.5	2.9	3.5	0.20
o-Xylene	1.7	3.4	4.7	0.07

Footnotes are found on the following page.

TABLE 24 (cont.)

[a] Quantitation was performed using GC/MS Method 8260 and chromatographic separation with a 30 m x 0.53 mm ID stable wax column with a 1 µm film thickness. Method detection limits are the average MDLs for studies on three non-consecutive days.

[b] Method detection limits are the average MDLs for studies of three non-consecutive days. Daily studies were seven replicated analyses of 5 mL aliquots of 4 ppb soil. Daily MDLs were three times the standard deviation.

[c] Daily studies were seven replicated analyses of 10 g fish tissue spiked at 5 ppb. Daily MDLs were three times the standard deviation. Quantitation was performed using GC/MS Method 8260 and chromatographic separation with a 30 m x 0.53 mm ID stable wax column with a 1 µm film thickness.

[d] Method detection limits are estimated analyzing 1 g of cod liver oil samples spiked at 250 ppm. Five replicates were analyzed using Method 8260.

[e] No analyses.

[f] Contamination of sample by analyte prevented determination.

[g] Interference by co-eluting compounds prevented accurate quantitation.

TABLE 25

METHOD DETECTION LIMITS (MDL) FOR VOLATILE ORGANIC ANALYTES
(METHOD 5032) (EXTERNAL STANDARD METHOD)[a]

Compound	Water[b] (µg/L)	Soil[c] (µg/kg)	Tissue[d] (µg/kg)	Oil[e] (mg/kg)
Chloromethane	3.1	8.6[f]	7.8	N/A[g]
Bromomethane	2.5	4.9[f]	9.7	N/A[g]
Vinyl chloride	4.0	7.1[f]	9.5	N/A[g]
Chloroethane	6.1	7.5[f]	9.2	N/A[g]
Methylene chloride	3.1	3.3	CONT[h]	0.08
Acetone	33.0[f]	CONT[h]	CONT[h]	0.12
Carbon disulfide	2.5	3.2	5.4	0.19
1,1-Dichloroethene	3.4	3.8	4.0	0.19
1,1-Dichloroethane	2.3	1.7	4.0	0.13
trans-1,2-Dichloroethene	3.0	3.2	4.4	0.09
cis-1,2-Dichloroethene	2.4	2.7	4.7	0.08
Chloroform	2.7	2.6	5.6	0.06
1,2-Dichloroethane	1.6	1.7	3.3	0.06
2-Butanone	57.0[f]	INT[i]	INT[i]	INT[i]
1,1,1-Trichloroethane	1.6	2.4	1.1	0.08
Carbon tetrachloride	1.5	1.7	3.2	0.15
Vinyl acetate	23.0[f]	INT[i]	INT[i]	INT[i]
Bromodichloromethane	2.0	2.3	3.2	0.05
1,1,2,2-Tetrachloroethane	3.6	3.2	4.4	0.09
1,2-Dichloropropane	2.9	3.7	3.8	0.12
trans-1,3-Dichloropropene	2.3	2.4	3.8	0.08
Trichloroethene	2.5	3.0	3.1	0.06
Dibromochloromethane	2.1	2.9	3.5	0.04
1,1,2-Trichloroethane	2.7	2.8	4.4	0.07
Benzene	1.7	2.9	3.6	0.03
cis-1,3-Dichloropropene	2.1	2.5	3.5	0.06
Bromoform	2.3	2.5	4.9	0.10
2-Hexanone	4.6	4.6	7.7	INT[i]
4-Methyl-2-pentanone	3.8	3.9	7.5	INT[i]
Tetrachloroethene	1.8	2.6	4.3	0.12
Toluene	1.8	4.4	3.0	0.09
Chlorobenzene	2.4	2.6	3.3	0.07
Ethylbenzene	2.4	4.1	3.6	0.09
Styrene	2.0	2.5	3.5	0.16
p-Xylene	2.3	3.9	3.7	0.18
o-Xylene	2.4	4.1	3.3	0.08

TABLE 25 (cont.)

[a] Method detection limits are the average MDLs for studies on three non-consecutive days. Daily studies were seven replicate analyses of 5-mL aliquots of water spiked at 4 ppb. Daily MDLs were three times the standard deviation.

[b] Daily studies were seven replicate analyses of 5-mL aliquots of water spiked at 4 ppb.

[c] These studies were seven replicate analyses of 5-g aliquots of soil spiked at 4 ppb.

[d] These studies were seven replicate analyses of 10-g aliquots of fish tissue spiked at 5 ppb.

[e] Method detection limits were estimated by analyzing cod liver oil samples spiked at 250 ppb. Five replicates were analyzed using Method 8260.

[f] Method detection limits were estimated by analyzing replicate 50 ppb standards five times over a single day.

[g] No analyses.

[h] Contamination of sample by analyte prevented determination.

[i] Interference by co-eluting compound prevented accurate quantitation.

TABLE 26

VOLATILE ORGANIC ANALYTE RECOVERY FROM OIL
USING VACUUM DISTILLATION (METHOD 5032)[a]

Compound	Recovery Mean (%)	RSD (%)
Chloromethane	N/A[b]	
Bromomethane	N/A[b]	
Vinyl chloride	N/A[b]	
Chloroethane	N/A[b]	
Methylene chloride	62	32
Acetone	108	55
Carbon disulfide	98	46
1,1-Dichloroethene	97	24
1,1-Dichloroethane	96	22
trans-1,2-Trichloroethene	86	23
cis-1,2-Dichloroethene	99	11
Chloroform	93	14
1,2-Dichloroethane	138	31
2-Butanone	INT[c]	
1,1,1-Trichloroethane	89	14
Carbon tetrachloride	129	23
Vinyl acetate	INT[c]	
Bromodichloromethane	106	14
1,1,2,2-Tetrachloroethane	205	46
1,2-Dichloropropane	107	24
trans-1,3-Dichloropropene	98	13
Trichloroethene	102	8
Dibromochloromethane	168	21
1,1,2-Trichloroethane	95	7
Benzene	146	10
cis-1,3-Dichloropropene	98	11
Bromoform	94	18
2-Hexanone	INT[c]	
4-Methyl-2-pentanone	INT[c]	
Tetrachloroethene	117	22
Toluene	108	8
Chlorobenzene	101	12
Ethylbenzene	96	10
Styrene	120	46
p-Xylene	87	23
o-Xylene	90	10

TABLE 26 (cont.)

Compound	Recovery Mean (%)	RSD (%)
Surrogates		
1,2-Dichloroethane	137	30
Toluene-d_8	84	6
Bromofluorobenzene	48	2

[a] Results are for 10 min. distillation times and condenser temperature held at -10°C. Five replicates of 10-g fish aliquots spiked at 25 ppb were analyzed. Quantitation was performed with a 30 m x 0.53 mm ID stable wax column with a 1 µm film thickness. Standards and samples were replicated and precision value reflects the propagated errors. Vacuum distillation efficiencies (Method 5032) are modified by internal standard corrections. Method 8260 internal standards may bias for some analytes. See Method 5032 to identify alternate internal standards with similar efficiencies to minimize bias.

[b] Not analyzed.

[c] Interference by co-evaluating compounds prevented accurate measurement of analyte.

TABLE 27

METHOD DETECTION LIMITS (MDL) FOR VOLATILE ORGANIC ANALYTES IN OIL (METHOD 5032)[a]

Compound	Method Detection Limit (ppb) External Standard Method	Internal Standard Method
Chloromethane	N/A[b]	N/A[b]
Bromomethane	N/A[b]	N/A[b]
Vinyl chloride	N/A[b]	N/A[b]
Chloroethane	N/A[b]	N/A[b]
Methylene chloride	80	50
Acetone	120	60
Carbon disulfide	190	180
1,1-Dichloroethene	190	180
1,1-Dichloroethane	130	140
trans-1,2-Dichloroethene	90	100
cis-1,2-Dichloroethene	80	70
Chloroform	60	70
1,2-Dichloroethane	60	60
2-Butanone	INT[c]	INT[c]
1,1,1-Trichloroethane	80	100
Carbon tetrachloride	150	130
Vinyl acetate	INT[c]	INT[c]
Bromodichloromethane	50	60
1,1,2,2-Tetrachloroethane	90	20
1,2-Dichloropropane	120	150
trans-1,3-Dichloropropene	80	50
Trichloroethene	60	40
Dibromochloromethane	40	70
1,1,2-Trichloroethane	70	50
Benzene	30	50
cis-1,3-Dichloropropene	60	40
Bromoform	100	50
2-Hexanone	INT[c]	INT[c]
4-Methyl-2-pentanone	INT[c]	INT[c]
Tetrachloroethene	120	100
Toluene	90	50
Chlorobenzene	70	60
Ethylbenzene	90	40
Styrene	160	180
p-Xylene	180	200
o-Xylene	80	70

TABLE 27 (cont.)

[a] Method detection limits are estimated as the result of five replicated analyses of 1 g cod liver oil spiked at 25 ppb. MDLs were calculated as three times the standard deviation. Quantitation was performed using a 30 m x 0.53 mm ID stable wax column with a 1 µm film thickness.

[b] No analyses.

[c] Interference by co-eluting compounds prevented accurate quantitation.

TABLE 28

INTERNAL STANDARDS FOR ANALYTES AND SURROGATES PREPARED USING EQUILIBRIUM HEADSPACE ANALYSIS (METHOD 5021)

Chloroform-d_1	1,1,2-TCA-d_3	Bromobenzene-d_5
Dichlorodifluoromethane	1,1,1-Trichloroethane	Chlorobenzene
Chloromethane	1,1-Dichloropropene	Bromoform
Vinyl chloride	Carbon tetrachloride	Styrene
Bromomethane	Benzene	iso-Propylbenzene
Chloroethane	Dibromomethane	Bromobenzene
Trichlorofluoromethane	1,2-Dichloropropane	n-Propylbenzene
1,1-Dichloroethene	Trichloroethene	2-Chlorotoluene
Methylene chloride	Bromodichloromethane	4-Chlorotoluene
trans-1,2-Dichloroethene	cis-1,3-Dichloropropene	1,3,5-Trimethylbenzene
1,1-Dichloroethane	trans-1,3-Dichloropropene	tert-Butylbenzene
cis-1,2-Dichloroethene	1,1,2-Trichloroethane	1,2,4-Trimethylbenzene
Bromochloromethane	Toluene	sec-Butylbenzene
Chloroform	1,3-Dichloropropane	1,3-Dichlorobenzene
2,2-Dichloropropane	Dibromochloromethane	1,4-Dichlorobenzene
1,2-Dichloroethane	1,2-Dibromoethane	p-iso-Propyltoluene
	Tetrachloroethene	1,2-Dichlorobenzene
	1,1,2-Trichloroethane	n-Butylbenzene
	Ethylbenzene	1,2-Dibromo-3-chloropropane
	m-Xylene	1,2,4-Trichlorobenzene
	p-Xylene	Naphthalene
	o-Xylene	Hexachlorobutadiene
	1,1,2,2-Tetrachloroethane	1,2,3-Trichlorobenzene
	1,2,3-Trichloropropane	

TABLE 29

PRECISION AND MDL DETERMINED FOR ANALYSIS OF FORTIFIED SAND[a] (METHOD 5021)

Compound	% RSD	MDL (µg/kg)
Benzene	3.0	0.34
Bromochloromethane	3.4	0.27
Bromodichloromethane	2.4	0.21
Bromoform	3.9	0.30
Bromomethane	11.6	1.3
Carbon tetrachloride	3.6	0.32
Chlorobenzene	3.2	0.24
Chloroethane	5.6	0.51
Chloroform	3.1	0.30
Chloromethane	4.1	3.5[b]
1,2-Dibromo-3-chloropropane	5.7	0.40
1,2-Dibromoethane	3.2	0.29
Dibromomethane	2.8	0.20
1,2-Dichlorobenzene	3.3	0.27
1,3-Dichlorobenzene	3.4	0.24
1,4-Dichlorobenzene	3.7	0.30
Dichlorodifluoromethane	3.0	0.28
1,1-Dichloroethane	4.5	0.41
1,2-Dichloroethane	3.0	0.24
1,1-Dichloroethene	3.3	0.28
cis-1,2-Dichloroethene	3.2	0.27
trans-1,2-Dichloroethene	2.6	0.22
1,2-Dichloropropane	2.6	0.21
1,1-Dichloropropene	3.2	0.30
cis-1,3-Dichloropropene	3.4	0.27
Ethylbenzene	4.8	0.47
Hexachlorobutadiene	4.1	0.38
Methylene chloride	8.2	0.62[c]
Naphthalene	16.8	3.4[c]
Styrene	7.9	0.62
1,1,1,2-Tetrachloroethane	3.6	0.27
1,1,2,2-Tetrachloroethane	2.6	0.20
Tetrachloroethene	9.8	1.2[c]
Toluene	3.5	0.38
1,2,4-Trichlorobenzene	4.2	0.44
1,1,1-Trichloroethane	2.7	0.27
1,1,2-Trichloroethane	2.6	0.20
Trichloroethene	2.3	0.19

TABLE 29 (cont.)

Compound	% RSD	MDL (µg/kg)
Trichlorofluoromethane	2.7	0.31
1,2,3-Trichloropropane	1.5	0.11
Vinyl chloride	4.8	0.45
m-Xylene/p-Xylene	3.6	0.37
o-Xylene	3.6	0.33

[a] Most compounds spiked at 2 ng/g (2 µg/kg)
[b] Incorrect ionization due to methanol
[c] Compound detected in unfortified sand at >1 ng

TABLE 30

RECOVERIES IN GARDEN SOIL FORTIFIED AT 20 µg/kg (ANALYSIS BY METHOD 5021)

Compound	Recovery per Replicate (ng) Sample 1	Sample 2	Sample 3	Mean (ng)	RSD	Recovery (%)
Benzene	37.6	35.2	38.4	37.1	3.7	185[a]
Bromochloromethane	20.5	19.4	20.0	20.0	2.3	100
Bromodichloromethane	21.1	20.3	22.8	21.4	4.9	107
Bromoform	23.8	23.9	25.1	24.3	2.4	121
Bromomethane	21.4	19.5	19.7	20.2	4.2	101
Carbon tetrachloride	27.5	26.6	28.6	27.6	3.0	138
Chlorobenzene	25.6	25.4	26.4	25.8	1.7	129
Chloroethane	25.0	24.4	25.3	24.9	1.5	125
Chloroform	21.9	20.9	21.7	21.5	2.0	108
Chloromethane	21.0	19.9	21.3	20.7	2.9	104[a]
1,2-Dibromo-3-chloro-propane	20.8	20.8	21.0	20.9	0.5	104
1,2-Dibromoethane	20.1	19.5	20.6	20.1	2.2	100
Dibromomethane	22.2	21.0	22.8	22.0	3.4	110
1,2-Dichlorobenzene	18.0	17.7	17.1	17.6	2.1	88.0
1,3-Dichlorobenzene	21.2	21.0	20.1	20.8	2.3	104
1,4-Dichlorobenzene	20.1	20.9	19.9	20.3	2.1	102
Dichlorodifluoromethane	25.3	24.1	25.4	24.9	2.4	125
1,1-Dichloroethane	23.0	22.0	22.7	22.6	1.9	113
1,2-Dichloroethane	20.6	19.5	19.8	20.0	2.3	100
1,1-Dichloroethene	24.8	23.8	24.4	24.3	1.7	122
cis-1,2-Dichloroethene	21.6	20.0	21.6	21.1	3.6	105
trans-1,2-Dichloroethene	22.4	21.4	22.2	22.0	2.0	110
1,2-Dichloropropane	22.8	22.2	23.4	22.8	2.1	114
1,1-Dichloropropene	26.3	25.7	28.0	26.7	3.7	133
cis-1,3-Dichloropropene	20.3	19.5	21.1	20.3	3.2	102
Ethylbenzene	24.7	24.5	25.5	24.9	1.7	125
Hexachlorobutadiene	23.0	25.3	25.2	24.5	4.3	123
Methylene chloride	26.0	25.7	26.1	25.9	0.7	130[a]
Naphthalene	13.8	12.7	11.8	12.8	6.4	63.8[a]
Styrene	24.2	23.3	23.3	23.6	1.8	118
1,1,1,2-Tetrachloroethane	21.4	20.2	21.3	21.0	2.6	105
1,1,2,2-Tetrachloroethane	18.6	17.8	19.0	18.5	2.7	92.3
Tetrachloroethene	25.2	24.8	26.4	25.5	2.7	127
Toluene	28.6	27.9	30.9	29.1	4.4	146[a]
1,2,4-Trichlorobenzene	15.0	14.4	12.9	14.1	6.3	70.5
1,1,1-Trichloroethane	28.1	27.2	29.9	28.4	4.0	142
1,1,2-Trichloroethane	20.8	19.6	21.7	20.7	4.2	104

TABLE 30 (cont.)

Compound	Recovery per Replicate (ng) Sample 1	Sample 2	Sample 3	Mean (ng)	RSD	Recovery (%)
Trichloroethene	26.3	24.9	26.8	26.0	3.1	130
Trichlorofluoromethane	25.9	24.8	26.5	25.7	2.7	129
1,2,3-Trichloropropane	18.8	18.3	19.3	18.8	2.2	94.0
Vinyl chloride	24.8	23.2	23.9	24.0	2.7	120
m-Xylene/p-Xylene	24.3	23.9	25.3	24.5	2.4	123
o-Xylene	23.1	22.3	23.4	22.9	2.0	115

[a] Compound found in unfortified garden soil matrix at >5 ng.

TABLE 31

METHOD DETECTION LIMITS AND BOILING POINTS
FOR VOLATILE ORGANICS (ANALYSIS BY METHOD 5041)[a]

Compound	Detection Limit (ng)	Boiling Point (°C)
Chloromethane	58	-24
Bromomethane	26	4
Vinyl chloride	14	-13
Chloroethane	21	13
Methylene chloride	9	40
Acetone	35	56
Carbon disulfide	11	46
1,1-Dichloroethene	14	32
1,1-Dichloroethane	12	57
trans-1,2-Dichloroethene	11	48
Chloroform	11	62
1,2-Dichloroethane	13	83
1,1,1-Trichloroethane	8	74
Carbon tetrachloride	8	77
Bromodichloromethane	11	88
1,1,2,2-Tetrachloroethane**	23	146
1,2-Dichloropropane	12	95
trans-1,3-Dichloropropene	17	112
Trichloroethene	11	87
Dibromochloromethane	21	122
1,1,2-Trichloroethane	26	114
Benzene	26	80
cis-1,3-Dichloropropene	27	112
Bromoform**	26	150
Tetrachloroethene	11	121
Toluene	15	111
Chlorobenzene	15	132
Ethylbenzene**	21	136
Styrene**	46	145
Trichlorofluoromethane	17	24
Iodomethane	9	43
Acrylonitrile	13	78
Dibromomethane	14	97
1,2,3-Trichloropropane**	37	157
total Xylenes**	22	138-144

Footnotes are found on the following page.

* The method detection limit (MDL) is defined in Chapter One. The detection limits cited above were determined according to 40 CFR, Part 136, Appendix B, using standards spiked onto clean VOST tubes. Since clean VOST tubes were used, the values cited above represent the best that the methodology can achieve. The presence of an emissions matrix will affect the ability of the methodology to perform at its optimum level.

** Boiling Point greater than 130°C. Not appropriate for quantitative sampling by Method 0030.

TABLE 32

VOLATILE INTERNAL STANDARDS WITH CORRESPONDING ANALYTES
ASSIGNED FOR QUANTITATION (METHOD 5041)

Bromochloromethane	1,4-Difluorobenzene
Acetone	Benzene
Acrylonitrile	Bromodichloromethane
Bromomethane	Bromoform
Carbon disulfide	Carbon tetrachloride
Chloroethane	Chlorodibromomethane
Chloroform	Dibromomethane
Chloromethane	1,2-Dichloropropane
1,1-Dichloroethane	cis-1,3-Dichloropropene
1,2-Dichloroethane	trans-1,3-Dichloropropene
1,2-Dichloroethane-d_4 (surrogate)	1,1,1-Trichloroethane
1,1-Dichloroethene	1,1,2-Trichloroethane
Trichloroethene	
trans-1,2-Dichloroethene	
Iodomethane	
Methylene chloride	
Trichlorofluoromethane	
Vinyl chloride	

Chlorobenzene-d_5

4-Bromofluorobenzene (surrogate)
Chlorobenzene
Ethylbenzene
Styrene
1,1,2,2-Tetrachloroethane
Tetrachloroethene
Toluene
Toluene-d_8 (surrogate)
1,2,3-Trichloropropane
Xylenes

TABLE 33

METHOD 0040 - COMPOUNDS DEMONSTRATED TO BE APPLICABLE TO THE METHOD

Compound	Boiling Point (°C)	Condensation Point at 20°C (%)	Estimated Detection Limit[a] (ppm)
Dichlorodifluoromethane	-30	Gas	0.20
Vinyl chloride	-19	Gas	0.11
1,3-Butadiene	-4	Gas	0.90
1,2-Dichloro-1,1,2,2-tetrafluoroethane	4	Gas	0.14
Methyl bromide	4	Gas	0.14
Trichlorofluoromethane	24	88	0.18
1,1-Dichloroethene	31	22	0.07
Methylene chloride	40	44	0.05
1,1,2-Trichloro-trifluoroethane	48	37	0.13
Chloroform	61	21	0.04
1,1,1-Trichloroethane	75	13	0.03
Carbon tetrachloride	77	11	0.03
Benzene	80	10	0.16
Trichloroethene	87	8	0.04
1,2-Dichloropropane	96	5	0.05
Toluene	111	3	0.08
Tetrachloroethene	121	2	0.03

[a] Since this value represents a direct injection (no concentration) from the Tedlar® bag, these values are directly applicable as stack detection limits.

FIGURE 1
GAS CHROMATOGRAM OF VOLATILE ORGANICS

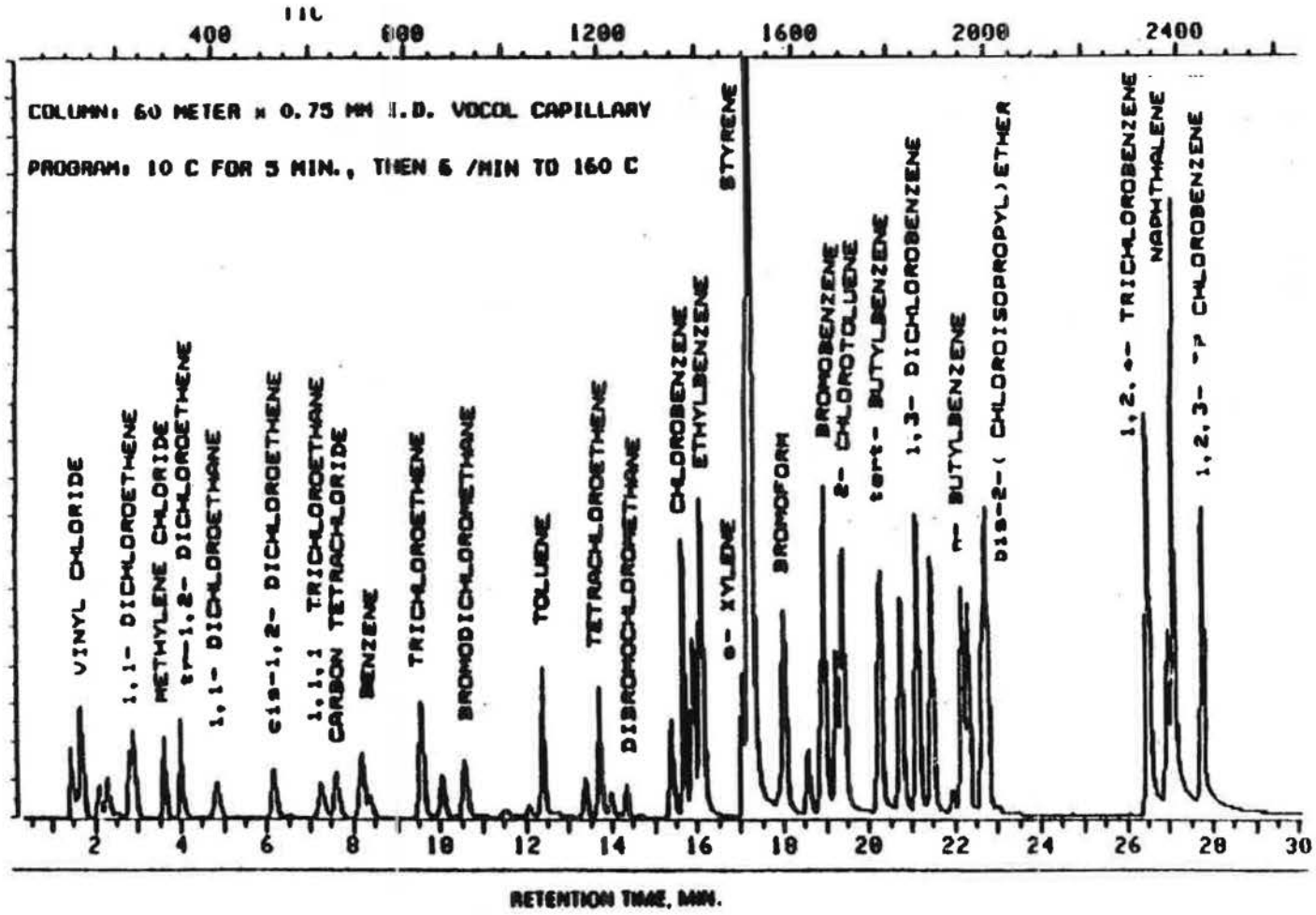


FIGURE 2
GAS CHROMATOGRAM OF VOLATILE ORGANICS

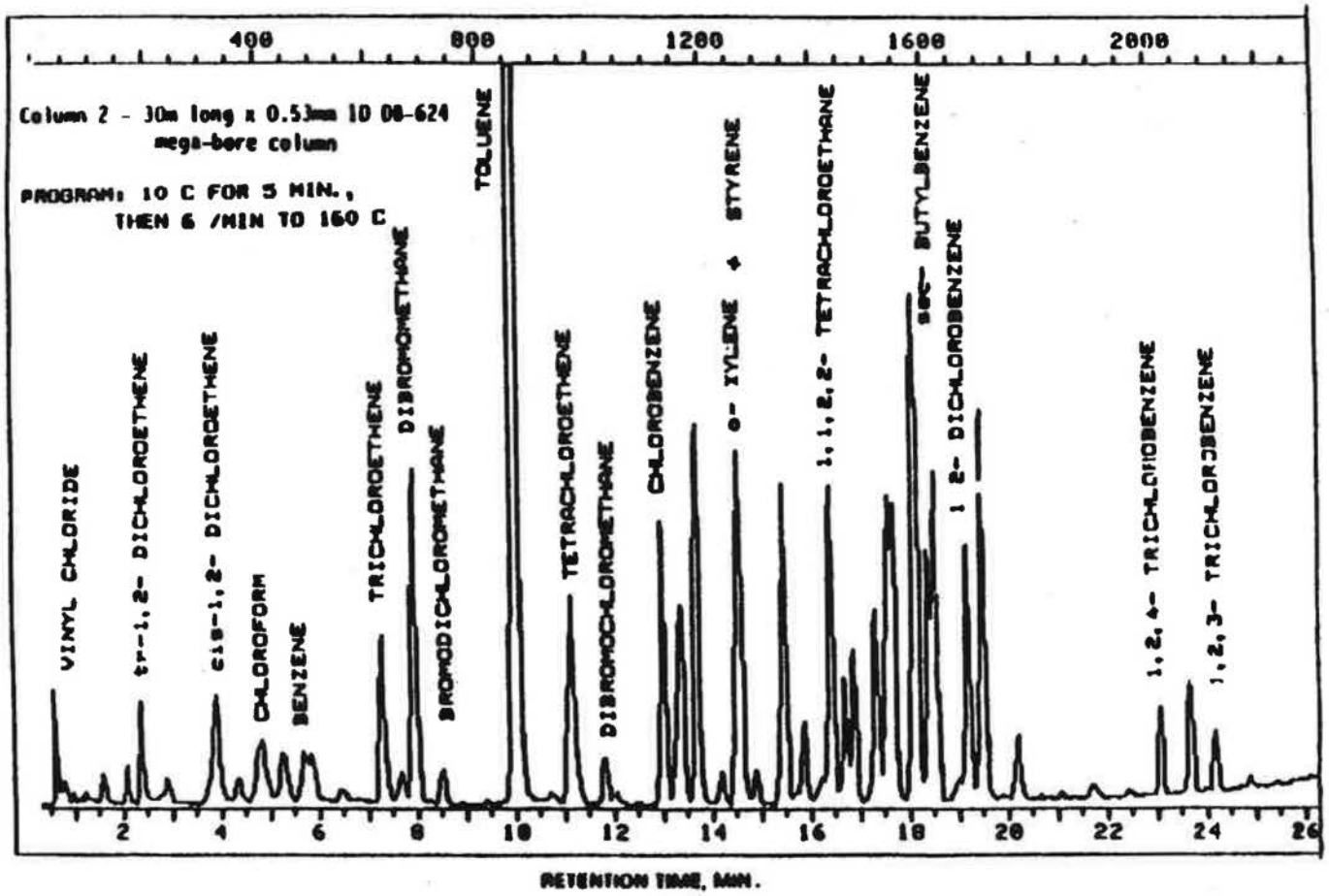


FIGURE 3
GAS CHROMATOGRAM OF VOLATILE ORGANICS

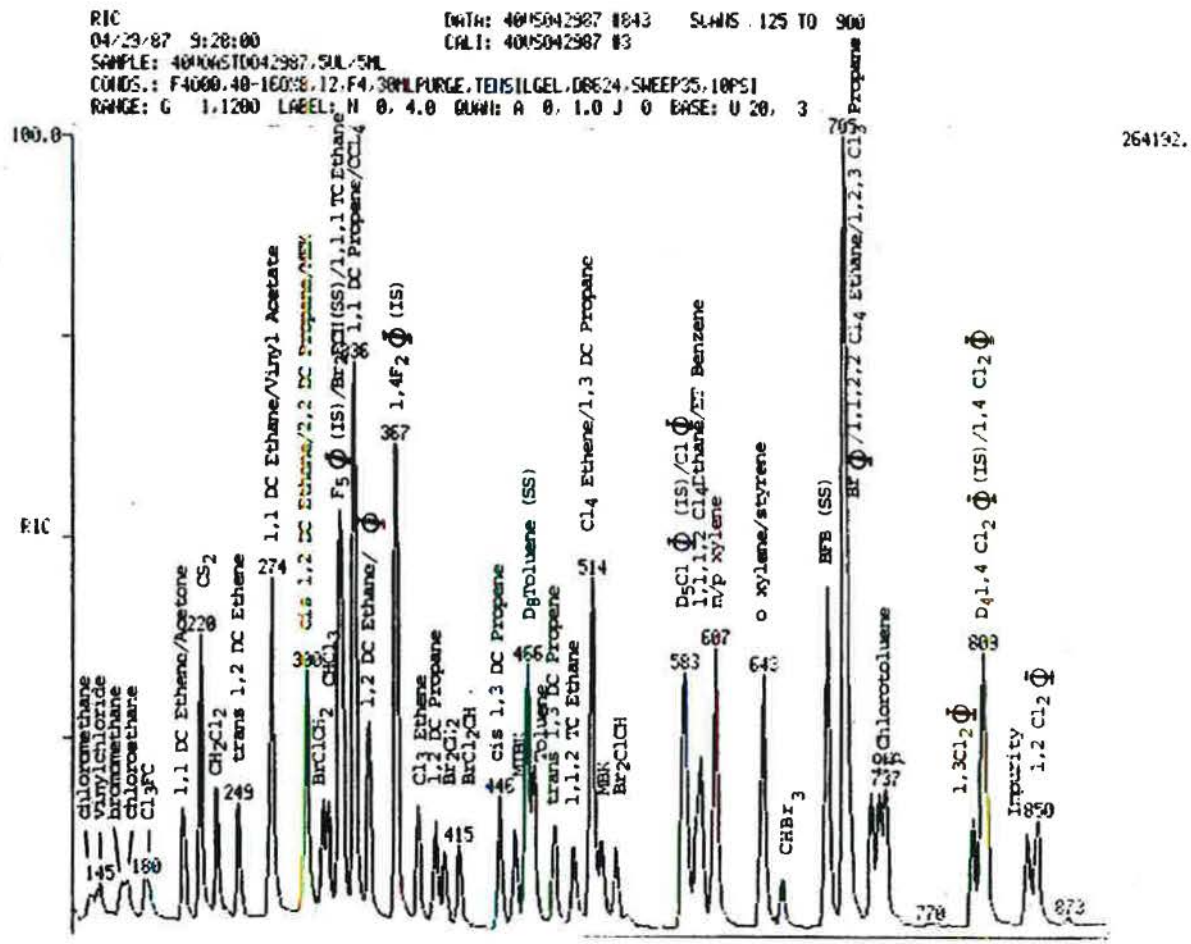
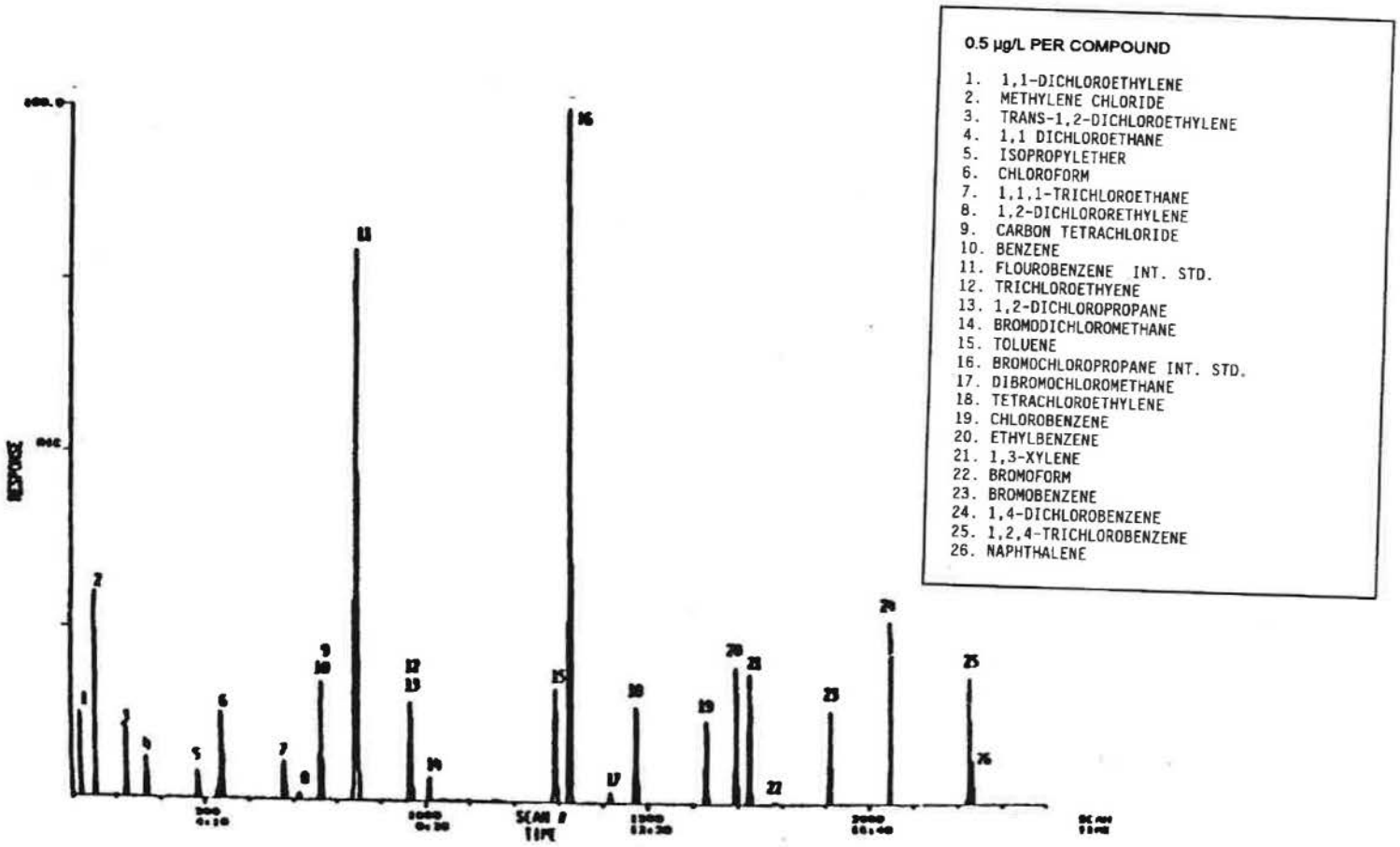
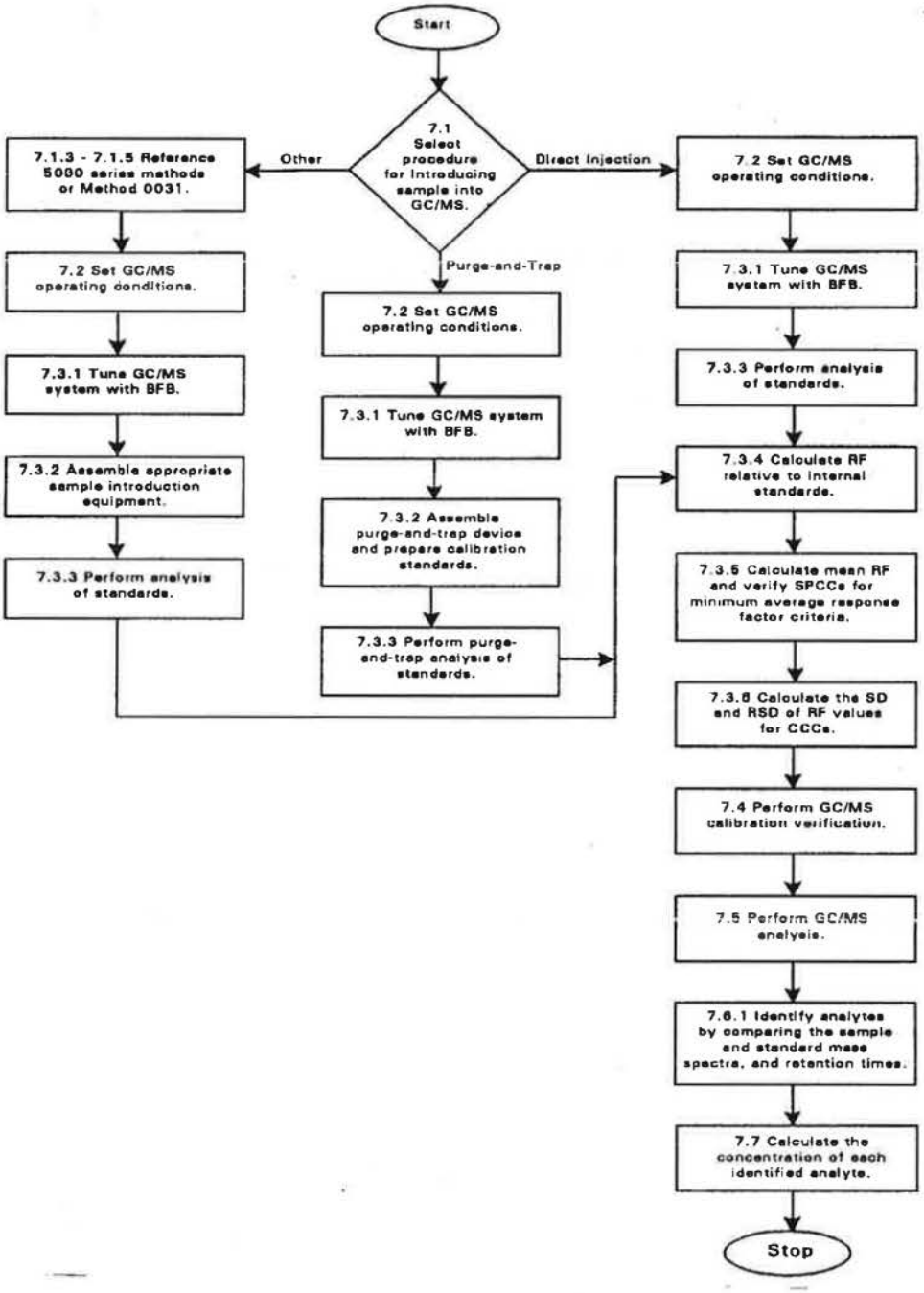


FIGURE 4
GAS CHROMATOGRAM OF TEST MIXTURE




Start
7.1 Select procedure for introducing sample into GC/MS.
Other
Purge-and-Trap
Direct Injection
7.1.3 - 7.1.5 Reference 5000 series methods or Method 0031.
7.2 Set GC/MS operating conditions.
7.3.1 Tune GC/MS system with BFB.
7.3.2 Assemble appropriate sample introduction equipment.
7.3.3 Perform analysis of standards.
7.2 Set GC/MS operating conditions.
7.3.1 Tune GC/MS system with BFB.
7.3.2 Assemble purge-and-trap device and prepare calibration standards.
7.3.3 Perform purge-and-trap analysis of standards.
7.2 Set GC/MS operating conditions.
7.3.1 Tune GC/MS system with BFB.
7.3.3 Perform analysis of standards.
7.3.4 Calculate RF relative to internal standards.
7.3.5 Calculate mean RF and verify SPCCs for minimum average response factor criteria.
7.3.6 Calculate the SD and RSD of RF values for CCCs.
7.4 Perform GC/MS calibration verification.
7.5 Perform GC/MS analysis.
7.6.1 Identify analytes by comparing the sample and standard mass spectra, and retention times.
7.7 Calculate the concentration of each identified analyte.
Stop

METHOD 8270D

SEMIVOLATILE ORGANIC COMPOUNDS
BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS)

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required method use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed Standard Operating Procedure (SOP), either for its own general use or for a specific project application. The performance data included in this method are for guidance purposes only, and are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

1.0 SCOPE AND APPLICATION

1.1 This method is used to determine the concentration of semivolatile organic compounds in extracts prepared from many types of solid waste matrices, soils, air sampling media and water samples. Direct injection of a sample may be used in limited applications. The following RCRA analytes have been determined by this method:

		Appropriate Preparation Techniques[b]				
Compounds	CAS No[a]	3510	3520	3540/ 3541	3550	3580
Acenaphthene	83-32-9	X	X	X	X	X
Acenaphthylene	208-96-8	X	X	X	X	X
Acetophenone	98-86-2	X	ND	ND	ND	X
2-Acetylaminofluorene	53-96-3	X	ND	ND	ND	X
1-Acetyl-2-thiourea	591-08-2	LR	ND	ND	ND	LR
Aldrin	309-00-2	X	X	X	X	X
2-Aminoanthraquinone	117-79-3	X	ND	ND	ND	X
Aminoazobenzene	60-09-3	X	ND	ND	ND	X
4-Aminobiphenyl	92-67-1	X	ND	ND	ND	X
3-Amino-9-ethylcarbazole	132-32-1	X	X	ND	ND	ND
Anilazine	101-05-3	X	ND	ND	ND	X
Aniline	62-53-3	X	X	ND	X	X
o-Anisidine	90-04-0	X	ND	ND	ND	X
Anthracene	120-12-7	X	X	X	X	X
Aramite	140-57-8	HS	ND	ND	ND	X
Aroclor 1016	12674-11-2	X	X	X	X	X
Aroclor 1221	11104-28-2	X	X	X	X	X
Aroclor 1232	11141-16-5	X	X	X	X	X
Aroclor 1242	53469-21-9	X	X	X	X	X
Aroclor 1248	12672-29-6	X	X	X	X	X

Compounds	CAS No[a]	Appropriate Preparation Techniques[b]				
		3510	3520	3540/ 3541	3550	3580
Aroclor 1254	11097-69-1	X	X	X	X	X
Aroclor 1260	11096-82-5	X	X	X	X	X
Azinphos-methyl	86-50-0	HS	ND	ND	ND	X
Barban	101-27-9	LR	ND	ND	ND	LR
Benzidine	92-87-5	CP	CP	CP	CP	CP
Benzoic acid	65-85-0	X	X	ND	X	X
Benz(a)anthracene	56-55-3	X	X	X	X	X
Benzo(b)fluoranthene	205-99-2	X	X	X	X	X
Benzo(k)fluoranthene	207-08-9	X	X	X	X	X
Benzo(g,h,i)perylene	191-24-2	X	X	X	X	X
Benzo(a)pyrene	50-32-8	X	X	X	X	X
p-Benzoquinone	106-51-4	OE	ND	ND	ND	X
Benzyl alcohol	100-51-6	X	X	ND	X	X
α-BHC	319-84-6	X	X	X	X	X
β-BHC	319-85-7	X	X	X	X	X
δ-BHC	319-86-8	X	X	X	X	X
γ-BHC (Lindane)	58-89-9	X	X	X	X	X
Bis(2-chloroethoxy)methane	111-91-1	X	X	X	X	X
Bis(2-chloroethyl) ether	111-44-4	X	X	X	X	X
Bis(2-chloroisopropyl) ether	39638-32-9	X	X	X	X	X
Bis(2-ethylhexyl) phthalate	117-81-7	X	X	X	X	X
4-Bromophenyl phenyl ether	101-55-3	X	X	X	X	X
Bromoxynil	1689-84-5	X	ND	ND	ND	X
Butyl benzyl phthalate	85-68-7	X	X	X	X	X
Captafol	2425-06-1	HS	ND	ND	ND	X
Captan	133-06-2	HS	ND	ND	ND	X
Carbaryl	63-25-2	X	ND	ND	ND	X
Carbofuran	1563-66-2	X	ND	ND	ND	X
Carbophenothion	786-19-6	X	ND	ND	ND	X
Chlordane (NOS)	57-74-9	X	X	X	X	X
Chlorfenvinphos	470-90-6	X	ND	ND	ND	X
4-Chloroaniline	106-47-8	X	ND	ND	ND	X
Chlorobenzilate	510-15-6	X	ND	ND	ND	X
5-Chloro-2-methylaniline	95-79-4	X	ND	ND	ND	X
4-Chloro-3-methylphenol	59-50-7	X	X	X	X	X
3-(Chloromethyl)pyridine hydrochloride	6959-48-4	X	ND	ND	ND	X
1-Chloronaphthalene	90-13-1	X	X	X	X	X
2-Chloronaphthalene	91-58-7	X	X	X	X	X
2-Chlorophenol	95-57-8	X	X	X	X	X
4-Chloro-1,2-phenylenediamine	95-83-0	X	X	ND	ND	ND
4-Chloro-1,3-phenylenediamine	5131-60-2	X	X	ND	ND	ND
4-Chlorophenyl phenyl ether	7005-72-3	X	X	X	X	X

Compounds	CAS No[a]	Appropriate Preparation Techniques[b]				
		3510	3520	3540/ 3541	3550	3580
Chrysene	218-01-9	X	X	X	X	X
Coumaphos	56-72-4	X	ND	ND	ND	X
p-Cresidine	120-71-8	X	ND	ND	ND	X
Crotoxyphos	7700-17-6	X	ND	ND	ND	X
2-Cyclohexyl-4,6-dinitro-phenol	131-89-5	X	ND	ND	ND	LR
4,4'-DDD	72-54-8	X	X	X	X	X
4,4'-DDE	72-55-9	X	X	X	X	X
4,4'-DDT	50-29-3	X	X	X	X	X
Demeton-O	298-03-3	HS	ND	ND	ND	X
Demeton-S	126-75-0	X	ND	ND	ND	X
Diallate (*cis* or *trans*)	2303-16-4	X	ND	ND	ND	X
2,4-Diaminotoluene	95-80-7	DC, OE	ND	ND	ND	X
Dibenz(a,j)acridine	224-42-0	X	ND	ND	ND	X
Dibenz(a,h)anthracene	53-70-3	X	X	X	X	X
Dibenzofuran	132-64-9	X	X	ND	X	X
Dibenzo(a,e)pyrene	192-65-4	ND	ND	ND	ND	X
1,2-Dibromo-3-chloropropane	96-12-8	X	X	ND	ND	ND
Di-n-butyl phthalate	84-74-2	X	X	X	X	X
Dichlone	117-80-6	OE	ND	ND	ND	X
1,2-Dichlorobenzene	95-50-1	X	X	X	X	X
1,3-Dichlorobenzene	541-73-1	X	X	X	X	X
1,4-Dichlorobenzene	106-46-7	X	X	X	X	X
3,3'-Dichlorobenzidine	91-94-1	X	X	X	X	X
2,4-Dichlorophenol	120-83-2	X	X	X	X	X
2,6-Dichlorophenol	87-65-0	X	ND	ND	ND	X
Dichlorovos	62-73-7	X	ND	ND	ND	X
Dicrotophos	141-66-2	X	ND	ND	ND	X
Dieldrin	60-57-1	X	X	X	X	X
Diethyl phthalate	84-66-2	X	X	X	X	X
Diethylstilbestrol	56-53-1	AW, OS	ND	ND	ND	X
Diethyl sulfate	64-67-5	LR	ND	ND	ND	LR
Dimethoate	60-51-5	HE, HS	ND	ND	ND	X
3,3'-Dimethoxybenzidine	119-90-4	X	ND	ND	ND	LR
Dimethylaminoazobenzene	60-11-7	X	ND	ND	ND	X
7,12-Dimethylbenz(a)-anthracene	57-97-6	CP	ND	ND	ND	CP
3,3'-Dimethylbenzidine	119-93-7	X	ND	ND	ND	X
α,α-Dimethylphenethylamine	122-09-8	ND	ND	ND	ND	X
2,4-Dimethylphenol	105-67-9	X	X	X	X	X
Dimethyl phthalate	131-11-3	X	X	X	X	X
1,2-Dinitrobenzene	528-29-0	X	ND	ND	ND	X
1,3-Dinitrobenzene	99-65-0	X	ND	ND	ND	X
1,4-Dinitrobenzene	100-25-4	HE	ND	ND	ND	X
4,6-Dinitro-2-methylphenol	534-52-1	X	X	X	X	X

Compounds	CAS No[a]	Appropriate Preparation Techniques[b]				
		3510	3520	3540/ 3541	3550	3580
2,4-Dinitrophenol	51-28-5	X	X	X	X	X
2,4-Dinitrotoluene	121-14-2	X	X	X	X	X
2,6-Dinitrotoluene	606-20-2	X	X	X	X	X
Dinocap	39300-45-3	CP, HS	ND	ND	ND	CP
Dinoseb	88-85-7	X	ND	ND	ND	X
Diphenylamine	122-39-4	X	X	X	X	X
5,5-Diphenylhydantoin	57-41-0	X	ND	ND	ND	X
1,2-Diphenylhydrazine	122-66-7	X	X	X	X	X
Di-n-octyl phthalate	117-84-0	X	X	X	X	X
Disulfoton	298-04-4	X	ND	ND	ND	X
Endosulfan I	959-98-8	X	X	X	X	X
Endosulfan II	33213-65-9	X	X	X	X	X
Endosulfan sulfate	1031-07-8	X	X	X	X	X
Endrin	72-20-8	X	X	X	X	X
Endrin aldehyde	7421-93-4	X	X	X	X	X
Endrin ketone	53494-70-5	X	X	ND	X	X
EPN	2104-64-5	X	ND	ND	ND	X
Ethion	563-12-2	X	ND	ND	ND	X
Ethyl carbamate	51-79-6	DC	ND	ND	ND	X
Ethyl methanesulfonate	62-50-0	X	ND	ND	ND	X
Famphur	52-85-7	X	ND	ND	ND	X
Fensulfothion	115-90-2	X	ND	ND	ND	X
Fenthion	55-38-9	X	ND	ND	ND	X
Fluchloralin	33245-39-5	X	ND	ND	ND	X
Fluoranthene	206-44-0	X	X	X	X	X
Fluorene	86-73-7	X	X	X	X	X
2-Fluorobiphenyl (surr)	321-60-8	X	X	X	X	X
2-Fluorophenol (surr)	367-12-4	X	X	X	X	X
Heptachlor	76-44-8	X	X	X	X	X
Heptachlor epoxide	1024-57-3	X	X	X	X	X
Hexachlorobenzene	118-74-1	X	X	X	X	X
Hexachlorobutadiene	87-68-3	X	X	X	X	X
Hexachlorocyclopentadiene	77-47-4	X	X	X	X	X
Hexachloroethane	67-72-1	X	X	X	X	X
Hexachlorophene	70-30-4	AW, CP	ND	ND	ND	CP
Hexachloropropene	1888-71-7	X	ND	ND	ND	X
Hexamethylphosphoramide	680-31-9	X	ND	ND	ND	X
Hydroquinone	123-31-9	ND	ND	ND	ND	X
Indeno(1,2,3-cd)pyrene	193-39-5	X	X	X	X	X
Isodrin	465-73-6	X	ND	ND	ND	X
Isophorone	78-59-1	X	X	X	X	X
Isosafrole	120-58-1	DC	ND	ND	ND	X
Kepone	143-50-0	X	ND	ND	ND	X

Compounds	CAS No[a]	Appropriate Preparation Techniques[b]				
		3510	3520	3540/ 3541	3550	3580
Leptophos	21609-90-5	X	ND	ND	ND	X
Malathion	121-75-5	HS	ND	ND	ND	X
Maleic anhydride	108-31-6	HE	ND	ND	ND	X
Mestranol	72-33-3	X	ND	ND	ND	X
Methapyrilene	91-80-5	X	ND	ND	ND	X
Methoxychlor	72-43-5	X	ND	ND	ND	X
3-Methylcholanthrene	56-49-5	X	ND	ND	ND	X
4,4'-Methylenebis (2-chloroaniline)	101-14-4	OE, OS	ND	ND	ND	LR
4,4'-Methylenebis(*N*,*N*-dimethyl-aniline)	101-61-1	X	X	ND	ND	ND
Methyl methanesulfonate	66-27-3	X	ND	ND	ND	X
2-Methylnaphthalene	91-57-6	X	X	ND	X	X
Methyl parathion	298-00-0	X	ND	ND	ND	X
2-Methylphenol	95-48-7	X	ND	ND	ND	X
3-Methylphenol	108-39-4	X	ND	ND	ND	X
4-Methylphenol	106-44-5	X	ND	ND	ND	X
Mevinphos	7786-34-7	X	ND	ND	ND	X
Mexacarbate	315-18-4	HE, HS	ND	ND	ND	X
Mirex	2385-85-5	X	ND	ND	ND	X
Monocrotophos	6923-22-4	HE	ND	ND	ND	X
Naled	300-76-5	X	ND	ND	ND	X
Naphthalene	91-20-3	X	X	X	X	X
1,4-Naphthoquinone	130-15-4	X	ND	ND	ND	X
1-Naphthylamine	134-32-7	OS	ND	ND	ND	X
2-Naphthylamine	91-59-8	X	ND	ND	ND	X
Nicotine	54-11-5	DC	ND	ND	ND	X
5-Nitroacenaphthene	602-87-9	X	ND	ND	ND	X
2-Nitroaniline	88-74-4	X	X	ND	X	X
3-Nitroaniline	99-09-2	X	X	ND	X	X
4-Nitroaniline	100-01-6	X	X	ND	X	X
5-Nitro-*o*-anisidine	99-59-2	X	ND	ND	ND	X
Nitrobenzene	98-95-3	X	X	X	X	X
4-Nitrobiphenyl	92-93-3	X	ND	ND	ND	X
Nitrofen	1836-75-5	X	ND	ND	ND	X
2-Nitrophenol	88-75-5	X	X	X	X	X
4-Nitrophenol	100-02-7	X	X	X	X	X
5-Nitro-o-toluidine	99-55-8	X	X	ND	ND	X
Nitroquinoline-1-oxide	56-57-5	X	ND	ND	ND	X
N-Nitrosodi-*n*-butylamine	924-16-3	X	ND	ND	ND	X
N-Nitrosodiethylamine	55-18-5	X	ND	ND	ND	X
N-Nitrosodimethylamine	62-75-9	X	X	X	X	X
N-Nitrosodiphenylamine	86-30-6	X	X	X	X	X
N-Nitrosodi-*n*-propylamine	621-64-7	X	X	X	X	X

Compounds	CAS No[a]	Appropriate Preparation Techniques[b]				
		3510	3520	3540/ 3541	3550	3580
N-Nitrosomethylethylamine	10595-95-6	X	ND	ND	ND	X
N-Nitrosomorpholine	59-89-2	ND	ND	ND	ND	X
N-Nitrosopiperidine	100-75-4	X	ND	ND	ND	X
N-Nitrosopyrrolidine	930-55-2	X	ND	ND	ND	X
Octamethyl pyrophosphoramide	152-16-9	LR	ND	ND	ND	LR
4,4'-Oxydianiline	101-80-4	X	ND	ND	ND	X
Parathion	56-38-2	X	X	ND	ND	X
Pentachlorobenzene	608-93-5	X	ND	ND	ND	X
Pentachloronitrobenzene	82-68-8	X	ND	ND	ND	X
Pentachlorophenol	87-86-5	X	X	X	X	X
Phenacetin	62-44-2	X	ND	ND	ND	X
Phenanthrene	85-01-8	X	X	X	X	X
Phenobarbital	50-06-6	X	ND	ND	ND	X
Phenol	108-95-2	DC	X	X	X	X
1,4-Phenylenediamine	106-50-3	X	ND	ND	ND	X
Phorate	298-02-2	X	ND	ND	ND	X
Phosalone	2310-17-0	HS	ND	ND	ND	X
Phosmet	732-11-6	HS	ND	ND	ND	X
Phosphamidon	13171-21-6	HE	ND	ND	ND	X
Phthalic anhydride	85-44-9	CP, HE	ND	ND	ND	CP
2-Picoline (2-Methylpyridine)	109-06-8	X	X	ND	ND	ND
Piperonyl sulfoxide	120-62-7	X	ND	ND	ND	X
Pronamide	23950-58-5	X	ND	ND	ND	X
Propylthiouracil	51-52-5	LR	ND	ND	ND	LR
Pyrene	129-00-0	X	X	X	X	X
Resorcinol	108-46-3	DC, OE	ND	ND	ND	X
Safrole	94-59-7	X	ND	ND	ND	X
Strychnine	57-24-9	AW, OS	ND	ND	ND	X
Sulfallate	95-06-7	X	ND	ND	ND	X
Terbufos	13071-79-9	X	ND	ND	ND	X
1,2,4,5-Tetrachlorobenzene	95-94-3	X	ND	ND	ND	X
2,3,4,6-Tetrachlorophenol	58-90-2	X	ND	ND	ND	X
Tetrachlorvinphos	961-11-5	X	ND	ND	ND	X
Tetraethyl dithiopyrophosphate	3689-24-5	X	X	ND	ND	ND
Tetraethyl pyrophosphate	107-49-3	X	ND	ND	ND	X
Thionazine	297-97-2	X	ND	ND	ND	X
Thiophenol (Benzenethiol)	108-98-5	X	ND	ND	ND	X
Toluene diisocyanate	584-84-9	HE	ND	ND	ND	X
o-Toluidine	95-53-4	X	ND	ND	ND	X
Toxaphene	8001-35-2	X	X	X	X	X
1,2,4-Trichlorobenzene	120-82-1	X	X	X	X	X
2,4,5-Trichlorophenol	95-95-4	X	X	ND	X	X
2,4,6-Trichlorophenol	88-06-2	X	X	X	X	X

Compounds	CAS No[a]	Appropriate Preparation Techniques[b]				
		3510	3520	3540/ 3541	3550	3580
Trifluralin	1582-09-8	X	ND	ND	ND	X
2,4,5-Trimethylaniline	137-17-7	X	ND	ND	ND	X
Trimethyl phosphate	512-56-1	HE	ND	ND	ND	X
1,3,5-Trinitrobenzene	99-35-4	X	ND	ND	ND	X
Tris(2,3-dibromopropyl) phosphate	126-72-7	X	ND	ND	ND	LR
Tri-*p*-tolyl phosphate	78-32-0	X	ND	ND	ND	X
O,O,O-Triethyl phosphorothioate	126-68-1	X	ND	ND	ND	X

[a] Chemical Abstract Service Registry Number
[b] See Sec. 1.2 for other acceptable preparation methods.

KEY TO ANALYTE LIST

AW = Adsorption to walls of glassware during extraction and storage.
CP = Nonreproducible chromatographic performance.
DC = Unfavorable distribution coefficient.
HE = Hydrolysis during extraction accelerated by acidic or basic conditions.
HS = Hydrolysis during storage potential.
LR = Low response.
ND = Not determined.
OE = Oxidation during extraction accelerated by basic conditions.
OS = Oxidation during storage potential.
X = Historically, adequate recovery can be obtained by this technique. However, actual recoveries may vary depending on the extraction efficiency, the number of constituents being analyzed concurrently, and the analytical instrumentation.

1.2 In addition to the sample preparation methods listed in the above analyte list, Method 3535 describes a solid-phase extraction procedure that may be applied to the extraction of semivolatiles from TCLP leachates (see Tables 16 and 17 of this method for performance data). Method 3542 describes sample preparation for semivolatile organic compounds in air sampled by Method 0010 (see Table 11 of this method for surrogate performance data), Method 3545 describes an automated solvent extraction device for semivolatiles in solids (see Table 12 of this method for performance data), Method 3561 describes a supercritical fluid device for the extraction of PAHs from solids (see Tables 13, 14, and 15 of this method for performance data), and Method 3546 provides an extraction procedure employing commercially available microwave equipment to extract semivolatiles while using less solvent and taking less time than procedures such as a Soxhlet extraction (see Tables 19 through 23 of this method for the applicable performance data). (The tabulated data are provided for guidance purposes only.)

1.3 This method can be used to quantitate most neutral, acidic, and basic organic compounds that are soluble in methylene chloride (or other suitable solvents provided that the desired performance data can be generated) and are capable of being eluted, without derivatization, as sharp peaks from a gas chromatographic fused-silica capillary column coated with a slightly polar silicone. Such compounds include polynuclear aromatic hydrocarbons, chlorinated hydrocarbons and pesticides, phthalate esters, organophosphate esters, nitrosamines, haloethers, aldehydes, ethers, ketones, anilines, pyridines, quinolines, aromatic

nitro compounds, and phenols, including nitrophenols. See Table 1 for a list of compounds and their characteristic ions that have been evaluated.

In most cases, this method is not appropriate for the quantitation of multicomponent analytes, e.g., Aroclors, Toxaphene, Chlordane, etc., because of limited sensitivity for those analytes. When these analytes have been identified by another technique, Method 8270 may be appropriate for confirmation of the identification of these analytes when concentration in the extract permits. Refer to Methods 8081 and 8082 for guidance on calibration and quantitation of multicomponent analytes such as the Aroclors, Toxaphene, and Chlordane.

1.4 The following compounds may require special treatment when being determined by this method:

1.4.1 Benzidine may be subject to oxidative losses during solvent concentration and its chromatographic behavior is poor.

1.4.2 Under the alkaline conditions of the extraction step from aqueous matrices, α-BHC, γ-BHC, Endosulfan I and II, and Endrin are subject to decomposition. Neutral extraction should be performed if these compounds are expected to be present.

1.4.3 Hexachlorocyclopentadiene is subject to thermal decomposition in the inlet of the gas chromatograph, chemical reaction in acetone solution, and photochemical decomposition.

1.4.4 N-nitrosodimethylamine is difficult to separate from the solvent under the chromatographic conditions described.

1.4.5 N-nitrosodiphenylamine decomposes in the gas chromatographic inlet and cannot be separated from diphenylamine. For this reason, it is acceptable to report the combined result for n-nitrosodiphenylamine and diphenylamine for either of these compounds as a combined concentration.

1.4.6 1,2-Diphenylhydrazine is unstable even at room temperature and readily converts to azobenzene. Given the stability problems, it would be acceptable to calibrate for 1,2-diphenylhydrazine using azobenzene. Under these poor compound separation circumstances the results for either of these compounds should be reported as a combined concentration.

1.4.7 Pentachlorophenol, 2,4-dinitrophenol, 4-nitrophenol, benzoic acid, 4,6-dinitro-2-methylphenol, 4-chloro-3-methylphenol, 2-nitroaniline, 3-nitroaniline, 4-nitroaniline, and benzyl alcohol are subject to erratic chromatographic behavior, especially if the GC system is contaminated with high boiling material.

1.4.8 Pyridine may perform poorly at the GC injection port temperatures listed in this method. Lowering the injection port temperature may reduce the amount of degradation. However, the analyst must use caution in modifying the injection port temperature, as the performance of other analytes may be adversely affected. Therefore, if pyridine is to be determined in addition to other target analytes, it may be necessary to perform separate analyses. In addition, pyridine may be lost during the evaporative concentration of the sample extract. As a result, many of the extraction methods listed above may yield low recoveries unless great care is exercised during the concentration steps. For this reason, analysts may wish to consider the use of extraction techniques such as pressurized fluid extraction (Method 3545), microwave extraction (Method 3546),

or supercritical fluid extraction, which involve smaller extract volumes, thereby reducing or eliminating the need for evaporative concentration techniques for many applications.

1.4.9 Toluene diisocyanate rapidly hydrolyzes in water (half-life of less than 30 min). Therefore, recoveries of this compound from aqueous matrices should not be expected. In addition, in solid matrices, toluene diisocyanate often reacts with alcohols and amines to produce urethane and ureas and consequently cannot usually coexist in a solution containing these materials.

1.4.10 In addition, analytes in the list provided above are flagged when there are limitations caused by sample preparation and/or chromatographic problems.

1.5 The lower limits of quantitation for this method when determining an individual compound are approximately 660 µg/kg (wet weight) for soil/sediment samples, 1-200 mg/kg for wastes (dependent on matrix and method of preparation), and 10 µg/L for ground water samples (see Table 2). Lower limits of quantitation will be proportionately higher for sample extracts that require dilution to avoid saturation of the detector. The lower limits of quantitation listed in Table 2 are provided for guidance and may not always be achievable.

1.6 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3500, 3600, 5000, and 8000) for additional information on quality control procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives for the intended application.

1.7 Use of this method is restricted to use by, or under supervision of, personnel appropriately experienced and trained in the use of gas chromatograph/mass spectrometers and skilled in the interpretation of mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 The samples are prepared for analysis by gas chromatography/mass spectrometry (GC/MS) using the appropriate sample preparation (refer to Method 3500) and, if necessary, sample cleanup procedures (refer to Method 3600).

2.2 The semivolatile compounds are introduced into the GC/MS by injecting the sample extract into a gas chromatograph (GC) equipped with a narrow-bore fused-silica capillary column. The GC column is temperature-programmed to separate the analytes, which are then detected with a mass spectrometer (MS) connected to the gas chromatograph.

2.3 Analytes eluted from the capillary column are introduced into the mass spectrometer via a jet separator or a direct connection. Identification of target analytes is

accomplished by comparing their mass spectra with the electron impact (or electron impact-like) spectra of authentic standards. Quantitation is accomplished by comparing the response of a major (quantitation) ion relative to an internal standard using an appropriate calibration curve for the intended application.

2.4 This method includes specific calibration and quality control steps that supersede the general recommendations provided in Method 8000.

3.0 DEFINITIONS

Refer to Chapter One and the manufacturer's instructions for definitions that may be relevant to this procedure.

4.0 INTERFERENCES

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be necessary. Refer to each method to be used for specific guidance on quality control procedures and to Chapter Four for general guidance on the cleaning of glassware. Also refer to Method 8000 for a discussion of interferences.

4.2 Raw GC/MS data from all blanks, samples, and spikes must be evaluated for interferences. Determine if the source of interference is in the preparation and/or cleanup of the samples and take corrective action to eliminate the problem.

4.3 Contamination by carryover can occur whenever high-concentration and low-concentration samples are sequentially analyzed. To reduce carryover, the sample syringe must be rinsed with solvent between sample injections. Whenever an unusually concentrated sample is encountered, it should be followed by the analysis of solvent to check for cross-contamination.

5.0 SAFETY

This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals listed in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this manual is for illustrative purposes only, and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those products and settings used during method development or subsequently evaluated by the Agency. Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented.

This section does not list common laboratory glassware (e.g., beakers and flasks).

6.1 Gas chromatograph/mass spectrometer system

6.1.1 Gas chromatograph -- An analytical system equipped with a temperature-programmable gas chromatograph suitable for splitless injection and all required accessories, including syringes, analytical columns, and gases. The capillary column should be directly coupled to the source.

6.1.2 Column -- 30-m x 0.25-mm ID (or 0.32-mm ID) 0.25, 0.5, or 1-µm film thickness silicone-coated fused-silica capillary column (J&W Scientific DB-5 or equivalent). The columns listed in this section were the columns used in developing the method. The listing of these columns in this method is not intended to exclude the use of other columns that may be developed. Laboratories may use these columns or other capillary columns provided that the laboratories document method performance data (e.g., chromatographic resolution, analyte breakdown, and sensitivity) that are appropriate for the intended application.

6.1.3 Mass spectrometer

6.1.3.1 Capable of scanning from 35 to 500 amu every 1 sec or less, using 70 volts (nominal) electron energy in the electron impact ionization mode. The mass spectrometer must be capable of producing a mass spectrum for decafluorotriphenylphosphine (DFTPP) which meets the criteria as outlined in Sec. 11.3.1.

6.1.3.2 An ion trap mass spectrometer may be used if it is capable of axial modulation to reduce ion-molecule reactions and can produce electron impact-like spectra that match those in the EPA/NIST Library. The mass spectrometer must be capable of producing a mass spectrum for DFTPP which meets the criteria as outlined in Sec. 11.3.1

6.1.4 GC/MS interface -- Any GC-to-MS interface may be used that gives acceptable calibration points for each compound of interest and achieves acceptable tuning performance criteria. For a narrow-bore capillary column, the interface is usually capillary-direct into the mass spectrometer source.

6.1.5 Data system -- A computer system should be interfaced to the mass spectrometer. The system must allow the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program. The computer should have software that can search any GC/MS data file for ions of a specific mass and that can plot such ion abundances versus time or scan number. This type of plot is defined as an Extracted Ion Current Profile (EICP). Software should also be available that allows integrating the abundances in any EICP between specified time or scan-number limits. The most recent version of the EPA/NIST Mass Spectral Library should also be available.

6.1.6 Guard column (optional) -- (J&W deactivated fused-silica, 0.25-mm ID x 6-m, or equivalent) between the injection port and the analytical column joined with column connectors (Agilent Catalog No. 5062-3556, or equivalent).

6.2 Syringe -- 10-µL.

6.3 Volumetric flasks, Class A -- Appropriate sizes equipped with ground-glass stoppers.

6.4 Balance -- Analytical, capable of weighing 0.0001 g.

6.5 Bottles -- Glass equipped with polytetrafluoroethylene (PTFE)-lined screw caps or crimp tops.

7.0 REAGENTS AND STANDARDS

7.1 Reagent-grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. Reagents should be stored in glass to prevent the leaching of contaminants from plastic containers.

7.2 Organic-free reagent water -- All references to water in this method refer to organic-free reagent water.

7.3 Standard solutions

The following sections describe the preparation of stock, intermediate, and working standards for the compounds of interest. This discussion is provided as an example, and other approaches and concentrations of the target compounds may be used, as appropriate for the intended application. See Method 8000 for additional information on the preparation of calibration standards.

7.4 Stock standard solutions (1000 mg/L) -- Standard solutions can be prepared from pure standard materials or purchased as certified solutions.

7.4.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide quality acetone or other suitable solvent and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is assayed to be 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially-prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

7.4.2 Transfer the stock standard solutions into bottles equipped with PTFE-lined screw-caps. Store, protected from light, at • 6 • C or as recommended by the standard manufacturer. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

7.4.3 Stock standard solutions must be replaced after 1 year or sooner if comparison with quality control check samples indicates a problem.

7.4.4 It is recommended that nitrosamine compounds be placed together in a separate calibration mix and not combined with other calibration mixes. When using a premixed certified standard, consult the manufacturer's instructions for additional guidance.

7.4.5 Mixes with hydrochloride salts may contain hydrochloric acid, which can cause analytical difficulties. When using a premixed certified standard, consult the manufacturer's instructions for additional guidance.

7.5 Internal standard solutions -- The internal standards recommended are 1,4-dichlorobenzene-d_4, naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12} (see Table 5). Other compounds may be used as internal standards as long as the criteria in Sec. 11.3.2 are met.

7.5.1 Dissolve 0.200 g of each compound with a small volume of carbon disulfide. Transfer to a 50-mL volumetric flask and dilute to volume with methylene chloride so that the final solvent is approximately 20% carbon disulfide. Most of the compounds are also soluble in small volumes of methanol, acetone, or toluene, except for perylene-d_{12}. The resulting solution will contain each standard at a concentration of 4,000 ng/µL. Each 1-mL sample extract undergoing analysis should be spiked with 10 µL of the internal standard solution, resulting in a concentration of 40 ng/µL of each internal standard. Store away from any light source at • 6 • C when not in use (-10 • C is recommended). When using premixed certified solutions, store according to the manufacturer's documented holding time and storage temperature recommendations.

7.5.2 If a more sensitive mass spectrometer is employed to achieve lower quantitation levels, a more dilute internal standard solution may be required. Area counts of the internal standard peaks should be between 50-200% of the area of the target analytes in the mid-point calibration analysis.

7.6 GC/MS tuning standard -- A methylene chloride solution containing 50 ng/µL of decafluorotriphenylphosphine (DFTPP) should be prepared. The standard should also contain 50 ng/µL each of 4,4'-DDT, pentachlorophenol, and benzidine to verify injection port inertness and GC column performance. Alternate concentrations may be used to compensate for different injection volumes if the total amount injected is 50 ng or less. Store away from any light source at • 6 • C when not in use (-10 • C is recommended). If a more sensitive mass spectrometer is employed to achieve lower quantitation levels, a more dilute tuning solution may be necessary. When using premixed certified solutions, store according to the manufacturer's documented holding time and storage temperature recommendations.

7.7 Calibration standards -- A minimum of five calibration standards should be prepared at different concentrations. At least one of the calibration standards should correspond to a sample concentration at or below that necessary to meet the data quality objectives of the project. The remaining standards should correspond to the range of concentrations found in actual samples but should not exceed the working range of the GC/MS system. Each standard and/or series of calibration standards prepared at a given concentration should contain all the desired project-specific target analytes for which quantitation and quantitative results are to be reported by this method.

7.7.1 It is the intent of EPA that all target analytes for a particular analysis be included in the calibration standard(s). These target analytes may not include the entire list of analytes (Sec. 1.1) for which the method has been demonstrated. However, the laboratory shall not report a quantitative result for a target analyte that was not included in the calibration standard(s).

7.7.2 Each 1-mL aliquot of calibration standard should be spiked with 10 µL of the internal standard solution prior to analysis. All standards should be stored away from any light source at • 6 • C when not in use (-10 • C is recommended), and should be freshly prepared once a year, or sooner if check standards indicate a problem. The calibration

verification standard should be prepared, as necessary, and stored at • 6 • €. When using premixed certified solutions, store according to the manufacturer's documented holding time and storage temperature recommendations.

7.8 Surrogate standards -- The recommended surrogates are phenol-d_6, 2-fluorophenol, 2,4,6-tribromophenol, nitrobenzene-d_5, 2-fluorobiphenyl, and p-terphenyl-d_{14}. See Method 3500 for instructions on preparing the surrogate solutions.

NOTE: In the presence of samples containing residual chlorine, phenol-d_6 has been known to react to form chlorinated phenolic compounds that are not detected as the original spiked surrogate. Sample preservation precautions outlined in Chapter Four should be used when residual chlorine is known to be present in order to minimize degradation of deuterated phenols or any other susceptible target analyte.

7.8.1 Surrogate standard check -- Determine what the appropriate concentration should be for the blank extracts after all extraction, cleanup, and concentration steps. Inject this concentration into the GC/MS to determine recovery of surrogate standards. It is recommended that this check be done whenever a new surrogate spiking solution is prepared.

NOTE: Method 3561 (SFE Extraction of PAHs) recommends the use of bromobenzene and p-quaterphenyl to better cover the range of PAHs listed in the method.

7.8.2 If a more sensitive mass spectrometer is employed to achieve lower quantitation levels, a more dilute surrogate solution may be necessary.

7.9 Matrix spike and laboratory control standards -- See Method 3500 for instructions on preparing the matrix spike standard. The same standard may be used as the laboratory control standard (LCS) and the spiking solution should be the same source as used for the initial calibration standards to restrict the influence of standard accuracy on the determination of recovery through preparation and analysis.

7.9.1 Matrix spike check -- Determine what concentration should be in the blank extracts after all extraction, cleanup, and concentration steps. Inject this concentration into the GC/MS to determine recovery. It is recommended that this check be done whenever a new matrix spiking solution is prepared.

7.9.2 If a more sensitive mass spectrometer is employed to achieve lower quantitation levels, a more dilute matrix and LCS spiking solution may be necessary.

7.9.3 Some projects may require the spiking of the specific compounds of interest, since the spiking compounds listed in Method 3500 would not be representative of the compounds of interest required for the project. When this occurs, the matrix and LCS spiking standards should be prepared in methanol, with each compound present at a concentration appropriate for the project.

7.10 Solvents -- Acetone, hexane, methylene chloride, isooctane, carbon disulfide, toluene, and other appropriate solvents. All solvents should be pesticide quality or equivalent. Solvents may be degassed prior to use.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 See the introductory material to Chapter Four, "Organic Analytes."

8.2 Store the sample extracts at • 6 • €, protected from light, in sealed vials (e.g., screw-cap vials or crimp-capped vials) equipped with unpierced PTFE-lined septa.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for guidance on quality assurance (QA) and quality control (QC) protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and those criteria given in Chapter One, and technique-specific QC criteria take precedence over the criteria in Chapter One. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and quality control data should be maintained for reference or inspection.

9.2 Refer to Method 8000 for specific determinative method QC procedures. Refer to Method 3500 or 5000 for QC procedures to ensure the proper operation of the various sample preparation techniques. If an extract cleanup procedure is performed, refer to Method 3600 for the appropriate QC procedures. Any more specific QC procedures provided in this method will supersede those noted in Methods 8000, 5000, 3500, or 3600.

9.3 Quality control procedures necessary to evaluate the GC system operation are found in Method 8000 and include evaluation of retention time windows, calibration verification and chromatographic analysis of samples. In addition, discussions regarding the instrument QC requirements listed below can be found in the referenced sections of this method:

- The GC/MS must be tuned to meet the recommended DFTPP criteria prior to the initial calibration and for each 12-hr period during which analyses are performed. See Secs. 11.3.1 and 11.4.1 for further details.

- There must be an initial calibration of the GC/MS system as described in Sec. 11.3. In addition, the initial calibration curve should be verified immediately after performing the standard analyses using a second source standard (prepared using standards different from the calibration standards). The suggested acceptance limits for this initial calibration verification analysis are 70 - 130%. Alternative acceptance limits may be appropriate based on the desired project-specific data quality objectives. Quantitative sample analyses should not proceed for those analytes that fail the second source standard initial calibration verification. However, analyses may continue for those analytes that fail the criteria with an understanding these results could be used for screening purposes and would be considered estimated values.

- The GC/MS system must meet the calibration verification acceptance criteria in Sec. 11.4, each 12 hrs.

- The RRT of the sample component must fall within the RRT window of the standard component provided in Sec. 11.6.1.

9.4 Initial demonstration of proficiency

Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff members are trained or significant changes in instrumentation are made. See Method 8000 for information on how to accomplish a demonstration of proficiency.

9.5 Initially, before processing any samples, the analyst should demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. As a continuing check, each time samples are extracted, cleaned up, and analyzed, a method blank must be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. If a peak is observed within the retention time window of any analyte that would prevent the determination of that analyte, determine the source and eliminate it, if possible, before processing the samples. The blanks should be carried through all stages of sample preparation and analysis. When new reagents or chemicals are received, the lab should monitor the preparation and/or analysis blanks associated with samples for any signs of contamination. It is not necessary to test every new batch of reagents or chemicals prior to sample preparation if the source shows no prior problems. However, if reagents are changed during a preparation batch, separate blanks need to be prepared for each set of reagents.

9.6 Sample quality control for preparation and analysis

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, method sensitivity). At a minimum, this should include the analysis of QC samples including a method blank, a matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch and the addition of surrogates to each field sample and QC sample when surrogates are used. Any method blanks, matrix spike samples, and replicate samples should be subjected to the same analytical procedures (Sec. 11.0) as those used on actual samples.

9.6.1 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, laboratories may use a matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, then laboratories should use a matrix spike and matrix spike duplicate pair. Consult Method 8000 for information on developing acceptance criteria for the MS/MSD.

9.6.2 A laboratory control sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike, when appropriate. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix. Consult Method 8000 for information on developing acceptance criteria for the LCS.

9.6.3 Also see Method 8000 for the details on carrying out sample quality control procedures for preparation and analysis. In-house method performance criteria for

evaluating method performance should be developed using the guidance found in Method 8000.

9.7 Surrogate recoveries

If surrogates are used, the laboratory should evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory. See Method 8000 for information on evaluating surrogate data and developing and updating surrogate limits. Procedures for evaluating the recoveries of multiple surrogates and the associated corrective actions should be defined in an approved project plan.

9.8 The experience of the analyst performing GC/MS analyses is invaluable to the success of the methods. Each day that analysis is performed, the calibration verification standard should be evaluated to determine if the chromatographic system is operating properly. Questions that should be asked are: Do the peaks look normal? Is the response obtained comparable to the response from previous calibrations? Careful examination of the standard chromatogram can indicate whether the column is still performing acceptably, the injector is leaking, the injector septum needs replacing, etc. When any changes are made to the system (e.g., the column is changed, a septum is changed), see the guidance in Method 8000 regarding whether recalibration of the system must take place.

9.9 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

10.0 CALIBRATION AND STANDARDIZATION

See Sec 11.3 for information on calibration and standardization.

11.0 PROCEDURE

11.1 Sample preparation

11.1.1 Samples are normally prepared by one of the following methods prior to GC/MS analysis.

Matrix	Methods
Air (particulates and sorbent resin)	3542
Water (including TCLP leachates)	3510, 3520, 3535
Soil/sediment	3540, 3541, 3545, 3546, 3550, 3560, 3561
Waste	3540, 3541, 3545, 3546, 3550, 3560, 3561, 3580

11.1.2 In very limited applications, direct injection of the sample into the GC/MS system with a 10-µL syringe may be appropriate. The quantitation limit is very high (approximately 10,000 µg/L). Therefore, it is only appropriate where concentrations in excess of 10,000 µg/L are expected.

11.2 Extract cleanup -- Cleanup procedures may not be necessary for a relatively clean sample matrix, but most extracts from environmental and waste samples will require additional preparation before analysis. The specific cleanup procedure used will depend on the nature of the sample to be analyzed and the data quality objectives for the measurements. General guidance for sample extract cleanup is provided in this section and in Method 3600.

Extracts may be cleaned up by any of the following methods prior to GC/MS analysis.

Analytes of Interest	Methods
Aniline and aniline derivatives	3620
Phenols	3630, 3640, 8041[a]
Phthalate esters	3610, 3620, 3640
Nitrosamines	3610, 3620, 3640
Organochlorine pesticides	3610, 3620, 3630, 3640, 3660
PCBs	3620, 3630, 3660, 3665
Nitroaromatics and cyclic ketones	3620, 3640
Polynuclear aromatic hydrocarbons	3611, 3630, 3640
Haloethers	3620, 3640
Chlorinated hydrocarbons	3620, 3640
Organophosphorus pesticides	3620
Petroleum waste	3611, 3650
All base, neutral, and acid priority pollutants	3640

[a] Method 8041 includes a derivatization technique and a GC/ECD analysis, if interferences are encountered on GC/FID.

11.3 Initial calibration

Establish the GC/MS operating conditions, using the following recommendations as guidance.

Mass range:	35-500 amu
Scan time:	• ¶ sec/scan
Initial temperature:	40 • €, hold for 4 min
Temperature program:	40-320 • € at 10 • €/min
Final temperature:	320 • €, hold until 2 min after benzo[g,h,i]perylene elutes
Injector temperature:	250-300 • €
Transfer line temperature:	250-300 • €
Source temperature:	According to manufacturer's specifications
Injector:	Grob-type, splitless
Injection volume:	1-2 µL
Carrier gas:	Hydrogen at 50 cm/sec or helium at 30 cm/sec
Ion trap only:	Set axial modulation, manifold temperature, and emission current to manufacturer's recommendations

Split injection is allowed if the sensitivity of the mass spectrometer is sufficient.

11.3.1 The GC/MS system must be hardware-tuned such that injecting 50 ng or less of DFTPP meets the manufacturer's specified acceptance criteria or as listed in Table 3. The tuning criteria as outlined in Table 3 were developed using quadrupole mass spectrometer instrumentation and it is recognized that other tuning criteria may be more effective depending on the type of instrumentation, e.g., Time-of-Flight, Ion Trap, etc. In

these cases it would be appropriate to follow the manufacturer's tuning instructions or some other consistent tuning criteria. However, no matter which tuning criteria is selected, the system calibration must not begin until the tuning acceptance criteria are met with the sample analyses performed under the same conditions as the calibration standards.

11.3.1.1 In the absence of specific recommendations on how to acquire the mass spectrum of DFTPP from the instrument manufacturer, the following approach should be used: Three scans (the peak apex scan and the scans immediately preceding and following the apex) are acquired and averaged. Background subtraction is required, and must be accomplished using a single scan acquired within 20 scans of the elution of DFTPP. The background subtraction should be designed only to eliminate column bleed or instrument background ions. Do not subtract part of the DFTPP peak or any other discrete peak that does not coelute with DFTPP.

11.3.1.2 Use the DFTPP mass intensity criteria in the manufacturer's instructions as primary tuning acceptance criteria or those in Table 3 as default tuning acceptance criteria if the primary tuning criteria are not available. Alternatively, other documented tuning criteria may be used (e.g. CLP, or Method 625), provided that method performance is not adversely affected. The analyst is always free to choose criteria that are tighter than those included in this method or to use other documented criteria provided they are used consistently throughout the initial calibration, calibration verification, and sample analyses.

NOTE: All subsequent standards, samples, MS/MSDs, and blanks associated with a DFTPP analysis must use the identical mass spectrometer instrument conditions.

11.3.1.3 The GC/MS tuning standard solution should also be used to assess GC column performance and injection port inertness. Degradation of DDT to DDE and DDD should not exceed 20%. (See Method 8081 for the percent breakdown calculation.) Benzidine and pentachlorophenol should be present at their normal responses, and should not exceed a tailing factor of 2 given by the following equation:

$$\text{TailingFactor} = \frac{BC}{AB}$$

Where the peak is defined as follows: AC is the width at 10% height; DE is the height of peak and B is the height at 10% of DE. This equation compares the width of the back half of the peak to the width of the front half of the peak at 10% of the height. (See Figure 1 for an example tailing factor calculation.)

11.3.1.4 If degradation is excessive and/or poor chromatography is noted, the injection port may require cleaning. It may also be necessary to break off the first 6 to12 in. of the capillary column. The use of a guard column (Sec. 6.1.6) between the injection port and the analytical column may help prolong analytical column performance life.

11.3.2 The internal standards selected in Sec. 7.5 should permit most of the components of interest in a chromatogram to have retention times of 0.80-1.20 relative to one of the internal standards. Use the base peak ion from the specific internal standard as the primary ion for quantitation (see Table 1). If interferences are noted, use the next most intense ion as the quantitation ion (e.g., for 1,4-dichlorobenzene-d_4, use m/z 150 for quantitation).

11.3.3 Analyze 1-2 µL of each calibration standard (containing the compounds for quantitation and the appropriate surrogates and internal standards) and tabulate the area of the primary ion against concentration for each target analyte (as indicated in Table 1). A set of at least five calibration standards is necessary (see Sec. 7.7 and Method 8000). Alternate injection volumes may be used if the applicable quality control requirements for using this method are met. The injection volume must be the same for all standards and sample extracts. Figure 2 shows a chromatogram of a calibration standard containing base/neutral and acid analytes.

11.3.4 Initial calibration calculations

Calculate response factors (RFs) for each target analyte relative to one of the internal standards (see Table 5) as follows:

$$RF = \frac{A_s \times C_{is}}{A_{is} \times C_s}$$

where:

A_s = Peak area (or height) of the analyte or surrogate.
A_{is} = Peak area (or height) of the internal standard.
C_s = Concentration of the analyte or surrogate, in µg/L.
C_{is} = Concentration of the internal standard, in µg/L.

11.3.4.1 Calculate the mean response factor and the relative standard deviation (RSD) of the response factors for each target analyte using the following equations. The RSD should be less than or equal to 20% for each target analyte. It is also recommended that a minimum response factor for the most common target analytes, as noted in Table 4, be demonstrated for each individual calibration level as a means to ensure that these compounds are behaving as expected. In addition, meeting the minimum response factor criteria for the lowest calibration standard is critical in establishing and demonstrating the desired sensitivity. Due to the large number of compounds that may be analyzed by this method, some compounds will fail to meet this criteria. For these occasions, it is acknowledged that the failing compounds may not be critical to the specific project and therefore they may be used as qualified data or estimated values for screening purposes. The analyst should also strive to place more emphasis on meeting the calibration criteria for those compounds that are critical project compounds, rather than meeting the criteria for those less important compounds.

$$\text{mean RF} = \overline{RF} = \frac{\sum_{i=1}^{n} RF_i}{n} \qquad SD = \sqrt{\frac{\sum_{i=1}^{n} (RF_i - \overline{RF})^2}{n-1}}$$

$$RSD = \frac{SD}{\overline{RF}} \times 100$$

where:

RF_i = RF for each of the calibration standards
$\overline{RF}$ = mean RF for each compound from the initial calibration
n = Number of calibration standards, e.g., 5

11.3.4.2 If more than 10% of the compounds included with the initial calibration exceed the 20% RSD limit and do not meet the minimum correlation coefficient (0.99) for alternate curve fits, then the chromatographic system is considered too reactive for analysis to begin. Clean or replace the injector liner and/or capillary column, then repeat the calibration procedure beginning with Sec. 11.3.

11.3.5 Evaluation of retention times -- The relative retention time (RRT) of each target analyte in each calibration standard should agree within 0.06 RRT units. Late-eluting target analytes usually have much better agreement.

$$RRT = \frac{\text{Retention time of the analyte}}{\text{Retention time of the internal standard}}$$

11.3.6 Linearity of target analytes -- If the RSD of any target analyte is 20% or less, then the relative response factor is assumed to be constant over the calibration range, and the average relative response factor may be used for quantitation (Sec. 11.7.2).

11.3.6.1 If the RSD of any target analyte is greater than 20%, refer to Method 8000 for additional calibration options. One of the options must be applied to GC/MS calibration in this situation, or a new initial calibration must be performed. The average RF should not be used for compounds that have an RSD greater than 20% unless the concentration is reported as estimated.

11.3.6.2 When the RSD exceeds 20%, the plotting and visual inspection of a calibration curve can be a useful diagnostic tool. The inspection may indicate analytical problems, including errors in standard preparation, the presence of active sites in the chromatographic system, analytes that exhibit poor chromatographic behavior, etc.

11.3.6.3 Due to the large number of compounds that may be analyzed by this method, some compounds may fail to meet either the 20% RSD, minimum correlation coefficient criteria (0.99), or the acceptance criteria for alternative calibration procedures in Method 8000. Any calibration method described in Method 8000 may be used, but it should be used consistently. It is considered inappropriate once the calibration analyses are completed to select an alternative calibration procedure in order to pass the recommended criteria on a case-by-case basis. If compounds fail to meet these criteria, the associated concentrations may still be determined but they must be reported as estimated. In order to report non-detects, it must be demonstrated that there is adequate sensitivity to detect the failed compounds at the applicable lower quantitation limit.

11.4 GC/MS calibration verification -- Calibration verification consists of three steps that are performed at the beginning of each 12-hr analytical shift.

11.4.1 Prior to the analysis of samples or calibration standards, inject 50 ng or less of the DFTPP standard into the GC/MS system. The resultant mass spectrum for DFTPP must meet the criteria as outlined in Sec. 11.3.1 before sample analysis begins. These criteria must be demonstrated each 12-hr shift during which samples are analyzed.

11.4.2 The initial calibration function for each target analyte should be checked immediately after the first occurrence in the region of the middle of the calibration range with a standard from a source different from that used for the initial calibration. The value determined from the second source check should be within 30% of the expected concentration. An alternative recovery limit may be appropriate based on the desired project-specific data quality objectives. Quantitative sample analyses should not proceed for those analytes that fail the second source standard initial calibration verification. However, analyses may continue for those analytes that fail the criteria with an understanding these results could be used for screening purposes and would be considered estimated values.

11.4.3 The initial calibration (Sec. 11.3) for each compound of interest should be verified once every 12 hrs prior to sample analysis, using the introduction technique and conditions used for samples. This is accomplished by analyzing a calibration standard (containing all the compounds for quantitation) at a concentration either near the midpoint concentration for the calibrating range of the GC/MS or near the action level for the project. The results must be compared against the most recent initial calibration curve and should meet the verification acceptance criteria provided in Secs. 11.4.5 through 11.4.7.

NOTE: The DFTPP and calibration verification standard may be combined into a single standard as long as both tuning and calibration verification acceptance criteria for the project can be met without interferences.

11.4.4 A method blank should be analyzed prior to sample analyses in order to ensure that the total system (introduction device, transfer lines and GC/MS system) is free of contaminants. If the method blank indicates contamination, then it may be appropriate to analyze a solvent blank to demonstrate that the contamination is not a result of carryover from standards or samples. See Method 8000 for information regarding method blank performance criteria.

11.4.5 Calibration verification standard criteria

11.4.5.1 Each of the most common target analytes in the calibration verification standard should meet the minimum response factors as noted in Table 4. This criteria is particularly important when the common target analytes are also critical project-required compounds. This is the same check that is applied during the initial calibration.

11.4.5.2 If the minimum response factors are not met, the system should be evaluated, and corrective action should be taken before sample analysis begins. Possible problems include standard mixture degradation, injection port inlet contamination, contamination at the front end of the analytical column, and active sites in the column or chromatographic system.

11.4.5.3 All target compounds of interest must be evaluated using a 20% criterion. Use percent difference when performing the average response factor model calibration. Use percent drift when calibrating using a regression fit model. Refer to Method 8000 for guidance on calculating percent difference and drift.

11.4.5.4 If the percent difference or percent drift for a compound is less than or equal to 20%, then the initial calibration for that compound is assumed to be valid. Due to the large numbers of compounds that may be analyzed by this method, it is expected that some compounds will fail to meet the criterion. If the criterion is not met (i.e., greater than 20% difference or drift) for more than 20% of the compounds included in the initial calibration, then corrective action must be taken prior to the analysis of samples. In cases where compounds fail, they may still be reported as non-detects if it can be demonstrated that there was adequate sensitivity to detect the compound at the applicable quantitation limit. For situations when the failed compound is present, the concentrations must be reported as estimated values.

11.4.5.5 Problems similar to those listed under initial calibration could affect the ability to pass the calibration verification standard analysis. If the problem cannot be corrected by other measures, a new initial calibration must be generated. The calibration verification criteria must be met before sample analysis begins.

11.4.5.6 The method of linear regression analysis has the potential for a significant bias to the lower portion of a calibration curve, while the relative percent difference and quadratic methods of calibration do not have this potential bias. When calculating the calibration curves using the linear regression model, a minimum quantitation check on the viability of the lowest calibration point should be performed by re-fitting the response from the low concentration calibration standard back into the curve (see Method 8000 for additional details). It is not necessary to re-analyze a low concentration standard, rather the data system can recalculate the concentrations as if it were an unknown sample. The recalculated concentration of the low calibration point should be within ± 30% of the standard's true concentration. Other recovery criteria may be applicable depending on the project's data quality objectives and for those situations the minimum quantitation check criteria should be outlined in a laboratory standard operating procedure, or a project-specific Quality Assurance Project Plan. Analytes which do not meet the minimum quantitation calibration re-fitting criteria should be considered "out of control" and corrective action such as redefining the lower limit of quantitation

and/or reporting those "out of control" target analytes as estimated when the concentration is at or near the lowest calibration point may be appropriate.

11.4.6 Internal standard retention time -- The retention times of the internal standards in the calibration verification standard must be evaluated immediately after or during data acquisition If the retention time for any internal standard changes by more than 30 sec from that in the mid-point standard level of the most recent initial calibration sequence, then the chromatographic system must be inspected for malfunctions and corrections must be made, as required. When corrections are made, reanalysis of samples analyzed while the system was malfunctioning is required.

11.4.7 Internal standard response -- If the EICP area for any of the internal standards in the calibration verification standard changes by a factor of two (-50% to +100%) from that in the mid-point standard level of the most recent initial calibration sequence, the mass spectrometer must be inspected for malfunctions and corrections must be made, as appropriate. When corrections are made, reanalysis of samples analyzed while the system was malfunctioning is required.

11.5 GC/MS analysis of samples

11.5.1 It is highly recommended that sample extracts be screened on a GC/FID or GC/PID using the same type of capillary column used in the GC/MS system. This will minimize contamination of the GC/MS system from unexpectedly high concentrations of organic compounds.

11.5.2 Allow the sample extract to warm to room temperature. Just prior to analysis, add 10 µL of the internal standard solution to the 1 mL of concentrated sample extract obtained from sample preparation.

11.5.3 Inject an aliquot of the sample extract into the GC/MS system, using the same operating conditions that were used for the calibration (Sec. 11.3). The volume to be injected should include an appropriate concentration that is within the calibration range of base/neutral and acid surrogates using the surrogate solution as noted in Sec. 7.8. The injection volume must be the same volume that was used for the calibration standards.

11.5.4 If the response for any quantitation ion exceeds the initial calibration range of the GC/MS system, the sample extract must be diluted and reanalyzed. Additional internal standard solution must be added to the diluted extract to maintain the same concentration as in the calibration standards (usually 40 ng/µL, or other concentrations as appropriate, if a more sensitive GC/MS system is being used). Secondary ion quantitation should be used only when there are sample interferences with the primary ion.

NOTE: It may be a useful diagnostic tool to monitor internal standard retention times in all samples, spikes, blanks, and standards to effectively check drifting, method performance, poor injection execution, and anticipate the need for system inspection and/or maintenance. Internal standard responses (area counts) must be monitored in all samples, spikes, blanks for similar reasons. If the EICP area for any of the internal standards in samples, spikes and blanks changes by a factor of two (-50% to +100%) from the areas determined in the continuing calibration analyzed that day, corrective action must be taken. The samples, spikes or blanks should be reanalyzed or the data should be qualified.

11.5.4.1 When ions from a compound in the sample saturate the detector, this analysis should be followed by the analysis of an instrument blank consisting of clean solvent. If the blank analysis is not free of interferences, then the system must be decontaminated. Sample analysis may not resume until the blank analysis is demonstrated to be free of interferences. Contamination from one sample to the next on the instrument usually takes place in the syringe. If adequate syringe washes are employed, then carryover from high concentration samples can usually be avoided.

11.5.4.2 All dilutions should keep the response of the major constituents (previously saturated peaks) in the upper half of the linear range of the curve.

11.5.5 The use of selected ion monitoring (SIM) is acceptable for applications requiring quantitation limits below the normal range of electron impact mass spectrometry. However, SIM may provide a lesser degree of confidence in the compound identification, since less mass spectral information is available. Using the primary ion for quantitation and the secondary ions for confirmation set up the collection groups based on their retention times. The selected ions are nominal ions and most compounds have small mass defect, usually less than 0.2 amu, in their spectra. These mass defects should be used in the acquisition table. The dwell time may be automatically calculated by the laboratory's GC/MS software or manually calculated using the following formula. The total scan time should be less than 1,000 msec and produce at least 5 to 10 scans per chromatographic peak. The start and stop times for the SIM groups are determined from the full scan analysis using the formula below:

$$\text{Dwell Time for the Group} = \frac{\text{Scan Time (msec)}}{\text{Total Ions in the Group}}$$

Additional guidance for performing SIM analyses, in particular for PAHs and phenol target analyte compounds, can be found in the most recent CLP semivolatile organic methods statement of work (SOW). See the SIM sections from the following CLP SOW for further details: EPA CLP Organics SOW. (Reference 14)

11.6 Analyte identification

11.6.1 The qualitative identification of compounds determined by this method is based on retention time and on comparison of the sample mass spectrum, after background correction, with characteristic ions in a reference mass spectrum. The reference mass spectrum must be generated by the laboratory using the conditions of this method. The characteristic ions from the reference mass spectrum are defined as the three ions of greatest relative intensity, or any ions over 30% relative intensity, if less than three such ions occur in the reference spectrum. Compounds are identified when the following criteria are met.

11.6.1.1 The intensities of the characteristic ions of a compound must maximize in the same scan or within one scan of each other. Selection of a peak by a data system target compound search routine where the search is based on the presence of a target chromatographic peak containing ions specific for the

target compound at a compound-specific retention time will be accepted as meeting this criterion.

11.6.1.2 The RRT of the sample component is within ± 0.06 RRT units of the RRT of the standard component.

11.6.1.3 The relative intensities of the characteristic ions agree within 30% of the relative intensities of these ions in the reference spectrum. (Example: For an ion with an abundance of 50% in the reference spectrum, the corresponding abundance in a sample spectrum can range between 20% and 80%.) Use professional judgement in interpretation where interferences are observed.

11.6.1.4 Structural isomers that produce very similar mass spectra should be identified as individual isomers if they have sufficiently different GC retention times. Sufficient GC resolution is achieved if the height of the valley between two isomer peaks is less than 50% of the average of the two peak heights. Otherwise, structural isomers are identified as isomeric pairs. The resolution should be verified on the mid-point concentration of the initial calibration as well as the laboratory designated continuing calibration verification level if closely eluting isomers are to be reported (e.g., benzo(b)fluoranthene and benzo(k)fluoranthene).

11.6.1.5 Identification is hampered when sample components are not resolved chromatographically and produce mass spectra containing ions contributed by more than one analyte. When gas chromatographic peaks obviously represent more than one sample component (i.e., a broadened peak with shoulder(s) or a valley between two or more maxima), appropriate selection of analyte spectra and background spectra is important.

11.6.1.6 Examination of extracted ion current profiles of appropriate ions can aid in the selection of spectra and in qualitative identification of compounds. When analytes coelute (i.e., only one chromatographic peak is apparent), the identification criteria may be met, but each analyte spectrum will contain extraneous ions contributed by the coeluting compound.

11.6.2 For samples containing components not associated with the calibration standards, a library search may be made for the purpose of tentative identification. The necessity to perform this type of identification will be determined by the purpose of the analyses being conducted. Data system library search routines should not use normalization routines that would misrepresent the library or unknown spectra when compared to each other.

For example, the RCRA permit or waste delisting requirements may require the reporting of non-target analytes. Only after visual comparison of sample spectra with the nearest library searches may the analyst assign a tentative identification. Guidelines for tentative identification are:

(1) Relative intensities of major ions in the reference spectrum (ions > 10% of the most abundant ion) should be present in the sample spectrum.

(2) The relative intensities of the major ions should agree within ± 30%. (Example: For an ion with an abundance of 50% in the standard spectrum, the corresponding sample ion abundance must be between 20 and 80%.)

(3) Molecular ions present in the reference spectrum should be present in the sample spectrum.

(4) Ions present in the sample spectrum but not in the reference spectrum should be reviewed for possible background contamination or presence of coeluting compounds.

(5) Ions present in the reference spectrum but not in the sample spectrum should be reviewed for possible subtraction from the sample spectrum because of background contamination or coeluting peaks. Data system library reduction programs can sometimes create these discrepancies.

11.7 Quantitation

11.7.1 Once a target compound has been identified, the quantitation of that compound will be based on the integrated abundance of the primary characteristic ion from the EICP.

11.7.1.1 It is highly recommended to use the integration produced by the software if the integration is correct because the software should produce more consistent integrations. However, manual integrations may be necessary when the software does not produce proper integrations because baseline selection is improper; the correct peak is missed; a coelution is integrated; the peak is partially integrated; etc. The analyst is responsible for ensuring that the integration is correct whether performed by the software or done manually.

11.7.1.2 Manual integrations should not be substituted for proper maintenance of the instrument or setup of the method (e.g. retention time updates, integration parameter files, etc). The analyst should seek to minimize manual integration by properly maintaining the instrument, updating retention times, and configuring peak integration parameters.

11.7.2 If the RSD of a compound's response factor is 20% or less, then the concentration in the extract may be determined using the average response factor (RF) from initial calibration data (Sec. 11.3.4). See Method 8000 for the equations describing internal standard calibration and either linear or non-linear calibrations.

11.7.3 Where applicable, the concentration of any non-target analytes identified in the sample (Sec. 11.6.2) should be estimated. The same formula as in Sec. 11.3.4 should be used with the following modifications: The areas A_x and A_{is} should be from the total ion chromatograms, and the RF for the compound should be assumed to be 1.

11.7.4 The resulting concentration should be reported indicating that the value is an estimate. Use the nearest internal standard free of interferences.

11.7.5 Quantitation of multicomponent compounds (e.g., Toxaphene, Aroclors, etc.) is beyond the scope of Method 8270. Normally, quantitation is performed using a GC/ECD, for example by using Methods 8081 or 8082. However, this method (8270) may be used to confirm the identification of these compounds, when the concentrations are at least 10 ng/µL in the concentrated sample extract.

11.7.6 Quantitation of multicomponent parameters such as diesel range organics (DROs) and total petroleum hydrocarbons (TPH) using the Method 8270 recommended internal standard quantitation technique is beyond the scope of this method. Typically,

analyses for these parameters are performed using GC/FID or GC with a MS detector capability that is available with Method 8015.

11.7.7 Structural isomers that produce very similar mass spectra should be quantitated as individual isomers if they have sufficiently different GC retention times. Sufficient GC resolution is achieved if the height of the valley between two isomer peaks is less than 50% of the average of the two peak heights. Otherwise, structural isomers are identified as isomeric pairs. The resolution should be verified on the mid-point concentration of the initial calibration as well as the laboratory designated continuing calibration verification level if closely eluting isomers are to be reported (e.g., benzo(b)fluoranthene and benzo(k)fluoranthene).

12.0 DATA ANALYSIS AND CALCULATIONS

See Sec. 11.7 and Method 8000 for information on data analysis and calculations.

13.0 METHOD PERFORMANCE

13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance criteria for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method. These performance data are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

13.2 Single laboratory initial demonstration of capability data were generated from five replicate measurements using a modified continuous liquid-liquid extractor (Method 3520) with hydrophobic membrane. In this case only a single acid pH extraction was performed using the CLP calibration criteria and the applicable CLP target analytes. These data are presented in Table 6. Laboratories should generate their own acceptance criteria depending on the extraction and instrument conditions. (See Method 8000.)

13.3 Chromatograms from calibration standards analyzed with Day 0 and Day 7 samples were compared to detect possible deterioration of GC performance. These recoveries (using Method 3510 extraction) are presented in Table 7. These data are provided for guidance purposes only.

13.4 Method performance data using Method 3541 (automated Soxhlet extraction) are presented in Tables 8 and 9. Single laboratory accuracy and precision data were obtained for semivolatile organics in a clay soil by spiking at a concentration of 6 mg/kg for each compound. The spiking solution was mixed into the soil during addition and then allowed to equilibrate for approximately 1 hour prior to extraction. The spiked samples were then extracted by Method 3541 (Automated Soxhlet). Three extractions were performed and each extract was analyzed by gas chromatography/mass spectrometry following Method 8270. The low recovery of the more volatile compounds is probably due to volatilization losses during equilibration. These data as listed were taken from Reference 7 and are provided for guidance purposes only.

13.5 Surrogate precision and accuracy data are presented in Table 10 from a field dynamic spiking study based on air sampling by Method 0010. The trapping media were prepared for analysis by Method 3542 and subsequently analyzed by this method (8270). These data are provided for guidance purposes only.

13.6 Single laboratory precision and bias data using Method 3545 (pressurized fluid extraction) for semivolatile organic compounds are presented in Table 11. The samples were conditioned spiked samples prepared and certified by a commercial supplier that contained 57 semivolatile organics at three concentrations (250, 2500, and 12,500 µg/kg) on three types of soil (clay, loam and sand). Spiked samples were extracted both by the Dionex Accelerated Solvent Extraction system and by the Perstorp Environmental Soxtec™ (automated Soxhlet). The data in Table 11 represent seven replicate extractions and analyses for each individual sample and were taken from Reference 9. The average recoveries from the three matrices for all analytes and all replicates relative to the automated Soxhlet data are as follows: clay 96.8%, loam 98.7% and sand 102.1%. The average recoveries from the three concentrations also relative to the automated Soxhlet data are as follows: low - 101.2%, mid - 97.2% and high - 99.2%. These data are provided for guidance purposes only.

13.7 Single laboratory precision and bias data using Method 3561 (SFE extraction of PAHs with a variable restrictor and solid trapping material) were obtained for the method analytes by the extraction of two certified reference materials (EC-1, a lake sediment from Environment Canada and HS-3, a marine sediment from the National Science and Engineering Research Council of Canada, both naturally-contaminated with PAHs). The SFE instrument used for these extractions was a Hewlett-Packard Model 7680. Analysis was by GC/MS. Average recoveries from six replicate extractions range from 85 to 148% (overall average of 100%) based on the certified value (or a Soxhlet value if a certified value was unavailable for a specific analyte) for the lake sediment. Average recoveries from three replicate extractions range from 73 to 133% (overall average of 92%) based on the certified value for the marine sediment. The data are found in Tables 12 and 13 and were taken from Reference 10. These data are provided for guidance purposes only.

13.8 Single laboratory precision and accuracy data using Method 3561 (SFE extraction of PAHs with a fixed restrictor and liquid trapping) were obtained for twelve of the method analytes by the extraction of a certified reference material (a soil naturally contaminated with PAHs). The SFE instrument used for these extractions was a Dionex Model 703-M. Analysis was by GC/MS. Average recoveries from four replicate extractions range from 60 to 122% (overall average of 89%) based on the certified value. The instrument conditions that were utilized to extract a 3.4 g sample were as follows: Pressure -- 300 atm; time -- 60 min.; extraction fluid -- CO_2; modifier -- 10% 1:1 (v/v) methanol/methylene chloride; Oven temperature -- 80 •€; Restrictor temperature -- 120 •€; and, trapping fluid -- chloroform (methylene chloride has also been used). The data are found in Table 14 and were taken from Reference 11. These data are provided for guidance purposes only.

13.9 Tables 15 and 16 contain single-laboratory precision and accuracy data for solid-phase extraction of TCLP buffer solutions spiked at two levels and extracted using Method 3535. These data are provided for guidance purposes only.

13.10 Table 17 contains multiple-laboratory data for solid-phase extraction of spiked TCLP soil leachates extracted using Method 3535. These data are provided for guidance purposes only.

13.11 Tables 18 through 22 contain single-laboratory PAH recovery data for microwave extraction of contaminated soils and standard reference materials using Method 3546. These data are provided for guidance purposes only.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction* available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th St., N.W. Washington, D.C. 20036, http://www.acs.org.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available from the American Chemical Society at the address listed in Sec. 14.2.

16.0 REFERENCES

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14. USEPA, Superfund Analytical Services/Contract Laboratory Program (CLP), Multi-Media, Multi-Concentration Organics Analysis, SOM01.X, Exhibit D - Analytical Methods, "Analytical Method for the Analysis of Semivolatile Organic Compounds," November, 2003

17.0 TABLES, DIAGRAMS, FLOW CHARTS, AND VALIDATION DATA

The following pages contain the tables and figures referenced by this method.

TABLE 1

CHARACTERISTIC IONS FOR SEMIVOLATILE COMPOUNDS IN APPROXIMATE RETENTION TIME ORDER [a]

Compound	Primary Ion	Secondary Ion(s)
2-Picoline	93	66,92
Aniline	93	66,65
Phenol	94	65,66
Bis(2-chloroethyl) ether	93	63,95
2-Chlorophenol	128	64,130
1,3-Dichlorobenzene	146	148,111
1,4-Dichlorobenzene-d_4 (IS)	152	150,115
1,4-Dichlorobenzene	146	148,111
Benzyl alcohol	108	79,77
1,2-Dichlorobenzene	146	148,111
N-Nitrosomethylethylamine	88	42,43,56
Bis(2-chloroisopropyl) ether	45	77,121
Ethyl carbamate	62	44,45,74
Thiophenol (Benzenethiol)	110	66,109,84
Methyl methanesulfonate	80	79,65,95
N-Nitrosodi-n-propylamine	70	42,101,130
Hexachloroethane	117	201,199
Maleic anhydride	54	98,53,44
Nitrobenzene	77	123,65
Isophorone	82	95,138
N-Nitrosodiethylamine	102	42,57,44,56
2-Nitrophenol	139	109,65
2,4-Dimethylphenol	122	107,121
p-Benzoquinone	108	54,82,80
Bis(2-chloroethoxy)methane	93	95,123
Benzoic acid	122	105,77
2,4-Dichlorophenol	162	164,98
Trimethyl phosphate	110	79,95,109,140
Ethyl methanesulfonate	79	109,97,45,65
1,2,4-Trichlorobenzene	180	182,145
Naphthalene-d_8 (IS)	136	68
Naphthalene	128	129,127
Hexachlorobutadiene	225	223,227
Tetraethyl pyrophosphate	99	155,127,81,109
Diethyl sulfate	139	45,59,99,111,125
4-Chloro-3-methylphenol	107	144,142
2-Methylnaphthalene	142	141
2-Methylphenol	107	108,77,79,90
Hexachloropropene	213	211,215,117,106,141
Hexachlorocyclopentadiene	237	235,272
N-Nitrosopyrrolidine	100	41,42,68,69
Acetophenone	105	71,51,120
3/4-Methylphenol[b]	107	108,77,79,90

TABLE 1
(continued)

Compound	Primary Ion	Secondary Ion(s)
2,4,6-Trichlorophenol	196	198,200
o-Toluidine	106	107,77,51,79
2-Chloronaphthalene	162	127,164
N-Nitrosopiperidine	114	42,55,56,41
1,4-Phenylenediamine	108	80,53,54,52
1-Chloronaphthalene	162	127,164
2-Nitroaniline	65	92,138
5-Chloro-2-methylaniline	106	141,140,77,89
Dimethyl phthalate	163	194,164
Acenaphthylene	152	151,153
2,6-Dinitrotoluene	165	63,89
Phthalic anhydride	104	76,50,148
o-Anisidine	108	80,123,52
3-Nitroaniline	138	108,92
Acenaphthene-d_{10} (IS)	164	162,160
Acenaphthene	154	153,152
2,4-Dinitrophenol	184	63,154
2,6-Dinitrophenol	162	164,126,98,63
4-Chloroaniline	127	129,65,92
Isosafrole	162	131,104,77,51
Dibenzofuran	168	139
2,4-Diaminotoluene	121	122,94,77,104
2,4-Dinitrotoluene	165	63,89
4-Nitrophenol	139	109,65
2-Naphthylamine	143	115,116
1,4-Naphthoquinone	158	104,102,76,50,130
p-Cresidine	122	94,137,77,93
Dichlorovos	109	185,79,145
Diethyl phthalate	149	177,150
Fluorene	166	165,167
2,4,5-Trimethylaniline	120	135,134,91,77
N-Nitrosodi-n-butylamine	84	57,41,116,158
4-Chlorophenyl phenyl ether	204	206,141
Hydroquinone	110	81,53,55
4,6-Dinitro-2-methylphenol	198	51,105
Resorcinol	110	81,82,53,69
N-Nitrosodiphenylamine	169	168,167
Safrole	162	104,77,103,135
Hexamethyl phosphoramide	135	44,179,92,42
3-(Chloromethyl)pyridine hydrochloride	92	127,129,65,39
Diphenylamine	169	168,167
1,2,4,5-Tetrachlorobenzene	216	214,179,108,143,218
1-Naphthylamine	143	115,89,63
1-Acetyl-2-thiourea	118	43,42,76
4-Bromophenyl phenyl ether	248	250,141

TABLE 1
(continued)

Compound	Primary Ion	Secondary Ion(s)
Toluene diisocyanate	174	145,173,146,132,91
2,4,5-Trichlorophenol	196	198,97,132,99
Hexachlorobenzene	284	142,249
Nicotine	84	133,161,162
Pentachlorophenol	266	264,268
5-Nitro-o-toluidine	152	77,79,106,94
Thionazine	107	96,97,143,79,68
4-Nitroaniline	138	65,108,92,80,39
Phenanthrene-d_{10} (IS)	188	94,80
Phenanthrene	178	179,176
Anthracene	178	176,179
1,4-Dinitrobenzene	168	75,50,76,92,122
Mevinphos	127	192,109,67,164
Naled	109	145,147,301,79,189
1,3-Dinitrobenzene	168	76,50,75,92,122
Diallate (cis or trans)	86	234,43,70
1,2-Dinitrobenzene	168	50,63,74
Diallate (trans or cis)	86	234,43,70
Pentachlorobenzene	250	252,108,248,215,254
5-Nitro-o-anisidine	168	79,52,138,153,77
Pentachloronitrobenzene	237	142,214,249,295,265
4-Nitroquinoline-1-oxide	174	101,128,75,116
Di-n-butyl phthalate	149	150,104
2,3,4,6-Tetrachlorophenol	232	131,230,166,234,168
Dihydrosaffrole	135	64,77
Demeton-O	88	89,60,61,115,171
Fluoranthene	202	101,203
1,3,5-Trinitrobenzene	75	74,213,120,91,63
Dicrotophos	127	67,72,109,193,237
Benzidine	184	92,185
Trifluralin	306	43,264,41,290
Bromoxynil	277	279,88,275,168
Pyrene	202	200,203
Monocrotophos	127	192,67,97,109
Phorate	75	121,97,93,260
Sulfallate	188	88,72,60,44
Demeton-S	88	60,81,89,114,115
Phenacetin	108	180,179,109,137,80
Dimethoate	87	93,125,143,229
Phenobarbital	204	117,232,146,161
Carbofuran	164	149,131,122
Octamethyl pyrophosphoramide	135	44,199,286,153,243
4-Aminobiphenyl	169	168,170,115
Dioxathion	97	125,270,153
Terbufos	231	57,97,153,103

TABLE 1
(continued)

Compound	Primary Ion	Secondary Ion(s)
α,α-Dimethylphenylamine	58	91,65,134,42
Pronamide	173	175,145,109,147
Aminoazobenzene	197	92,120,65,77
Dichlone	191	163,226,228,135,193
Dinoseb	211	163,147,117,240
Disulfoton	88	97,89,142,186
Fluchloralin	306	63,326,328,264,65
Mexacarbate	165	150,134,164,222
4,4'-Oxydianiline	200	108,171,80,65
Butyl benzyl phthalate	149	91,206
4-Nitrobiphenyl	199	152,141,169,151
Phosphamidon	127	264,72,109,138
2-Cyclohexyl-4,6-Dinitrophenol	231	185,41,193,266
Methyl parathion	109	125,263,79,93
Carbaryl	144	115,116,201
Dimethylaminoazobenzene	225	120,77,105,148,42
Propylthiouracil	170	142,114,83
Benz(a)anthracene	228	229,226
Chrysene-d_{12} (IS)	240	120,236
3,3'-Dichlorobenzidine	252	254,126
Chrysene	228	226,229
Malathion	173	125,127,93,158
Kepone	272	274,237,178,143,270
Fenthion	278	125,109,169,153
Parathion	109	97,291,139,155
Anilazine	239	241,143,178,89
Bis(2-ethylhexyl) phthalate	149	167,279
3,3'-Dimethylbenzidine	212	106,196,180
Carbophenothion	157	97,121,342,159,199
5-Nitroacenaphthene	199	152,169,141,115
Methapyrilene	97	50,191,71
Isodrin	193	66,195,263,265,147
Captan	79	149,77,119,117
Chlorfenvinphos	267	269,323,325,295
Crotoxyphos	127	105,193,166
Phosmet	160	77,93,317,76
EPN	157	169,185,141,323
Tetrachlorvinphos	329	109,331,79,333
Di-n-octyl phthalate	149	167,43
2-Aminoanthraquinone	223	167,195
Barban	222	51,87,224,257,153
Aramite	185	191,319,334,197,321
Benzo(b)fluoranthene	252	253,125
Nitrofen	283	285,202,139,253
Benzo(k)fluoranthene	252	253,125

TABLE 1
(continued)

Compound	Primary Ion	Secondary Ion(s)
Chlorobenzilate	251	139,253,111,141
Fensulfothion	293	97,308,125,292
Ethion	231	97,153,125,121
Diethylstilbestrol	268	145,107,239,121,159
Famphur	218	125,93,109,217
Tri-p-tolyl phosphate[c]	368	367,107,165,198
Benzo(a)pyrene	252	253,125
Perylene-d_{12} (IS)	264	260,265
7,12-Dimethylbenz(a)anthracene	256	241,239,120
5,5-Diphenylhydantoin	180	104,252,223,209
Captafol	79	77,80,107
Dinocap	69	41,39
Methoxychlor	227	228,152,114,274,212
2-Acetylaminofluorene	181	180,223,152
4,4'-Methylenebis(2-chloroaniline)	231	266,268,140,195
3,3'-Dimethoxybenzidine	244	201,229
3-Methylcholanthrene	268	252,253,126,134,113
Phosalone	182	184,367,121,379
Azinphos-methyl	160	132,93,104,105
Leptophos	171	377,375,77,155,379
Mirex	272	237,274,270,239,235
Tris(2,3-dibromopropyl) phosphate	201	137,119,217,219,199
Dibenz(a,j)acridine	279	280,277,250
Mestranol	277	310,174,147,242
Coumaphos	362	226,210,364,97,109
Indeno(1,2,3-cd)pyrene	276	138,277
Dibenz(a,h)anthracene	278	139,279
Benzo(g,h,i)perylene	276	138,277
1,2:4,5-Dibenzopyrene	302	151,150,300
Strychnine	334	334,335,333
Piperonyl sulfoxide	162	135,105,77
Hexachlorophene	196	198,209,211,406,408
Aldrin	66	263,220
Aroclor 1016	222	260,292
Aroclor 1221	190	224,260
Aroclor 1232	190	224,260
Aroclor 1242	222	256,292
Aroclor 1248	292	362,326
Aroclor 1254	292	362,326
Aroclor 1260	360	362,394
α-BHC	183	181,109
β-BHC	181	183,109
δ-BHC	183	181,109
γ-BHC (Lindane)	183	181,109
4,4'-DDD	235	237,165

TABLE 1
(continued)

Compound	Primary Ion	Secondary Ion(s)
4,4'-DDE	246	248,176
4,4'-DDT	235	237,165
Dieldrin	79	263,279
1,2-Diphenylhydrazine	77	105,182
Endosulfan I	195	339,341
Endosulfan II	337	339,341
Endosulfan sulfate	272	387,422
Endrin	263	82,81
Endrin aldehyde	67	345,250
Endrin ketone	317	67,319
2-Fluorobiphenyl (surr)	172	171
2-Fluorophenol (surr)	112	64
Heptachlor	100	272,274
Heptachlor epoxide	353	355,351
Nitrobenzene-d_5 (surr)	82	128,54
N-Nitrosodimethylamine	42	74,44
Phenol-d_6 (surr)	99	42,71
Terphenyl-d_{14} (surr)	244	122,212
2,4,6-Tribromophenol (surr)	330	332,141
Toxaphene	159	231,233

IS = internal standard
surr = surrogate
[a] The data presented are representative of DB-5 type analytical columns
[b] Compounds cannot be separated for quantitation
[c] Substitute for the non-specific mixture, tricresyl phosphate

TABLE 2

EXAMPLE LOWER LIMITS OF QUANTITATION FOR SEMIVOLATILE ORGANICS

Compound	Lower Limits of Quantitation[a] Ground water (µg/L)	Low Soil/Sediment[b] (µg/kg)
Acenaphthene	10	660
Acenaphthylene	10	660
Acetophenone	10	ND
2-Acetylaminofluorene	20	ND
1-Acetyl-2-thiourea	1000	ND
2-Aminoanthraquinone	20	ND
Aminoazobenzene	10	ND
4-Aminobiphenyl	20	ND
Anilazine	100	ND
o-Anisidine	10	ND
Anthracene	10	660
Aramite	20	ND
Azinphos-methyl	100	ND
Barban	200	ND
Benz(a)anthracene	10	660
Benzo(b)fluoranthene	10	660
Benzo(k)fluoranthene	10	660
Benzoic acid	50	3300
Benzo(g,h,i)perylene	10	660
Benzo(a)pyrene	10	660
p-Benzoquinone	10	ND
Benzyl alcohol	20	1300
Bis(2-chloroethoxy)methane	10	660
Bis(2-chloroethyl) ether	10	660
Bis(2-chloroisopropyl) ether	10	660
4-Bromophenyl phenyl ether	10	660
Bromoxynil	10	ND
Butyl benzyl phthalate	10	660
Captafol	20	ND
Captan	50	ND
Carbaryl	10	ND
Carbofuran	10	ND
Carbophenothion	10	ND
Chlorfenvinphos	20	ND
4-Chloroaniline	20	1300
Chlorobenzilate	10	ND
5-Chloro-2-methylaniline	10	ND
4-Chloro-3-methylphenol	20	1300

TABLE 2
(continued)

Compound	Lower Limits of Quantitation[a] Ground water (µg/L)	Low Soil/Sediment[b] (µg/kg)
3-(Chloromethyl)pyridine hydrochloride	100	ND
2-Chloronaphthalene	10	660
2-Chlorophenol	10	660
4-Chlorophenyl phenyl ether	10	660
Chrysene	10	660
Coumaphos	40	ND
p-Cresidine	10	ND
Crotoxyphos	20	ND
2-Cyclohexyl-4,6-dinitrophenol	100	ND
Demeton-O	10	ND
Demeton-S	10	ND
Diallate (cis or trans)	10	ND
Diallate (trans or cis)	10	ND
2,4-Diaminotoluene	20	ND
Dibenz(a,j)acridine	10	ND
Dibenz(a,h)anthracene	10	660
Dibenzofuran	10	660
Dibenzo(a,e)pyrene	10	ND
Di-n-butyl phthalate	10	ND
Dichlone	NA	ND
1,2-Dichlorobenzene	10	660
1,3-Dichlorobenzene	10	660
1,4-Dichlorobenzene	10	660
3,3'-Dichlorobenzidine	20	1300
2,4-Dichlorophenol	10	660
2,6-Dichlorophenol	10	ND
Dichlorovos	10	ND
Dicrotophos	10	ND
Diethyl phthalate	10	660
Diethylstilbestrol	20	ND
Diethyl sulfate	100	ND
Dimethoate	20	ND
3,3'-Dimethoxybenzidine	100	ND
Dimethylaminoazobenzene	10	ND
7,12-Dimethylbenz(a)anthracene	10	ND
3,3'-Dimethylbenzidine	10	ND
2,4-Dimethylphenol	10	660
Dimethyl phthalate	10	660
1,2-Dinitrobenzene	40	ND

TABLE 2
(continued)

Compound	Lower Limits of Quantitation[a] Ground water (µg/L)	Low Soil/Sediment[b] (µg/kg)
1,3-Dinitrobenzene	20	ND
1,4-Dinitrobenzene	40	ND
4,6-Dinitro-2-methylphenol	50	3300
2,4-Dinitrophenol	50	3300
2,4-Dinitrotoluene	10	660
2,6-Dinitrotoluene	10	660
Dinocap	100	ND
Dinoseb	20	ND
5,5-Diphenylhydantoin	20	ND
Di-n-octyl phthalate	10	660
Disulfoton	10	ND
EPN	10	ND
Ethion	10	ND
Ethyl carbamate	50	ND
Bis(2-ethylhexyl) phthalate	10	660
Ethyl methanesulfonate	20	ND
Famphur	20	ND
Fensulfothion	40	ND
Fenthion	10	ND
Fluchloralin	20	ND
Fluoranthene	10	660
Fluorene	10	660
Hexachlorobenzene	10	660
Hexachlorobutadiene	10	660
Hexachlorocyclopentadiene	10	660
Hexachloroethane	10	660
Hexachlorophene	50	ND
Hexachloropropene	10	ND
Hexamethylphosphoramide	20	ND
Indeno(1,2,3-cd)pyrene	10	660
Isodrin	20	ND
Isophorone	10	660
Isosafrole	10	ND
Kepone	20	ND
Leptophos	10	ND
Malathion	50	ND
Mestranol	20	ND
Methapyrilene	100	ND
Methoxychlor	10	ND

TABLE 2
(continued)

Compound	Lower Limits of Quantitation[a] Ground water (µg/L)	Low Soil/Sediment[b] (µg/kg)
3-Methylcholanthrene	10	ND
Methyl methanesulfonate	10	ND
2-Methylnaphthalene	10	660
Methyl parathion	10	ND
2-Methylphenol	10	660
3-Methylphenol	10	ND
4-Methylphenol	10	660
Mevinphos	10	ND
Mexacarbate	20	ND
Mirex	10	ND
Monocrotophos	40	ND
Naled	20	ND
Naphthalene	10	660
1,4-Naphthoquinone	10	ND
1-Naphthylamine	10	ND
2-Naphthylamine	10	ND
Nicotine	20	ND
5-Nitroacenaphthene	10	ND
2-Nitroaniline	50	3300
3-Nitroaniline	50	3300
4-Nitroaniline	20	ND
5-Nitro-o-anisidine	10	ND
Nitrobenzene	10	660
4-Nitrobiphenyl	10	ND
Nitrofen	20	ND
2-Nitrophenol	10	660
4-Nitrophenol	50	3300
5-Nitro-o-toluidine	10	ND
4-Nitroquinoline-1-oxide	40	ND
N-Nitrosodi-n-butylamine	10	ND
N-Nitrosodiethylamine	20	ND
N-Nitrosodiphenylamine	10	660
N-Nitroso-di-n-propylamine	10	660
N-Nitrosopiperidine	20	ND
N-Nitrosopyrrolidine	40	ND
Octamethyl pyrophosphoramide	200	ND
4,4'-Oxydianiline	20	ND
Parathion	10	ND
Pentachlorobenzene	10	ND

TABLE 2
(continued)

Compound	Lower Limits of Quantitation[a] Ground water (µg/L)	Low Soil/Sediment[b] (µg/kg)
Pentachloronitrobenzene	20	ND
Pentachlorophenol	50	3300
Phenacetin	20	ND
Phenanthrene	10	660
Phenobarbital	10	ND
Phenol	10	660
1,4-Phenylenediamine	10	ND
Phorate	10	ND
Phosalone	100	ND
Phosmet	40	ND
Phosphamidon	100	ND
Phthalic anhydride	100	ND
2-Picoline	ND	ND
Piperonyl sulfoxide	100	ND
Pronamide	10	ND
Propylthiouracil	100	ND
Pyrene	10	660
Resorcinol	100	ND
Safrole	10	ND
Strychnine	40	ND
Sulfallate	10	ND
Terbufos	20	ND
1,2,4,5-Tetrachlorobenzene	10	ND
2,3,4,6-Tetrachlorophenol	10	ND
Tetrachlorvinphos	20	ND
Tetraethyl pyrophosphate	40	ND
Thionazine	20	ND
Thiophenol (Benzenethiol)	20	ND
o-Toluidine	10	ND
1,2,4-Trichlorobenzene	10	660
2,4,5-Trichlorophenol	10	660
2,4,6-Trichlorophenol	10	660
Trifluralin	10	ND
2,4,5-Trimethylaniline	10	ND
Trimethyl phosphate	10	ND
1,3,5-Trinitrobenzene	10	ND
Tris(2,3-dibromopropyl) phosphate	200	ND
Tri-p-tolyl phosphate(h)	10	ND

TABLE 2
(continued)

[a] Sample lower limits of quantitation are highly matrix-dependent and those listed here are provided for guidance and may not always be achievable.

[b] Lower limits of quantitation listed for soil/sediment are based on wet weight. When data are reported on a dry weight basis, the lower limits will be higher based on the % dry weight of each sample. These lower limits are based on a 30-g sample and gel permeation chromatography cleanup.

ND = Not Determined

NA = Not Applicable

Other Matrices	Factor[c]
High-concentration soil and sludges by ultrasonic extractor	7.5
Non-water miscible waste	75

[c]Lower limit of quantitation = (Lower limit of quantitation for low soil/sediment given above in Table 2) x (Factor)

TABLE 3

DFTPP KEY IONS AND ION ABUNDANCE CRITERIA[a,b]

Mass	Ion Abundance Criteria
51	10-80% of Base Peak
68	< 2% of mass 69
70	< 2% of mass 69
127	10-80% of Base Peak
197	< 2% of mass 198
198	Base peak, or > 50% of Mass 442
199	5-9% of mass 198
275	10-60% of Base Peak
365	> 1% of mass 198
441	present but < 24% of mass 442
442	Base Peak, or > 50% of mass 198
443	15-24% of mass 442

[a] The majority of the data are taken from Reference 13 (Method 525.2).

[b] The criteria in this table are intended to be used as default criteria for quadrupole instrumentation if optimized manufacturer's operating conditions are not available. Alternate tuning criteria may be employed (e.g., CLP or Method 625), provided that method performance is not adversely affected. See Sec. 11.3.1

TABLE 4

RECOMMENDED MINIMUM RESPONSE FACTOR CRITERIA FOR INITIAL AND CONTINUING CALIBRATION VERIFICATION USING THE SUGGESTED IONS FROM TABLE 1

Semivolatile Compounds	Minimum Response Factor (RF)
Benzaldehyde	0.010
Phenol	0.800
Bis(2-chloroethyl)ether	0.700
2-Chlorophenol	0.800
2-Methylphenol	0.700
2,2'-Oxybis-(1-chloropropane)	0.010
Acetophenone	0.010
4-Methylphenol	0.600
N-Nitroso-di-n-propylamine	0.500
Hexachloroethane	0.300
Nitrobenzene	0.200
Isophorone	0.400
2-Nitrophenol	0.100
2,4-Dimethylphenol	0.200
Bis(2-chloroethoxy)methane	0.300
2,4-Dichlorophenol	0.200
Naphthalene	0.700
4-Chloroaniline	0.010
Hexachlorobutadiene	0.010
Caprolactam	0.010
4-Chloro-3-methylphenol	0.200
2-Methylnaphthalene	0.400
Hexachlorocyclopentadiene	0.050
2,4,6-Trichlorophenol	0.200
2,4,5-Trichlorophenol	0.200
1,1'-Biphenyl	0.010
2-Chloronaphthalene	0.800

TABLE 4
(continued)

Semivolatile Compounds	Minimum Response Factor (RF)
2-Nitroaniline	0.010
Dimethyl phthalate	0.010
2,6-Dinitrotoluene	0.200
Acenaphthylene	0.900
3-Nitroaniline	0.010
Acenaphthene	0.900
2,4-Dinitrophenol	0.010
4-Nitrophenol	0.010
Dibenzofuran	0.800
2,4-Dinitrotoluene	0.200
Diethyl phthalate	0.010
1,2,4,5-Tetrachlorobenzene	0.010
4-Chlorophenyl-phenyl ether	0.400
Fluorene	0.900
4-Nitroaniline	0.010
4,6-Dinitro-2-methylphenol	0.010
4-Bromophenyl-phenyl ether	0.100
N-Nitrosodiphenylamine	0.010
Hexachlorobenzene	0.100
Atrazine	0.010
Pentachlorophenol	0.050
Phenanthrene	0.700
Anthracene	0.700
Carbazole	0.010
Di-n-butyl phthalate	0.010
Fluoranthene	0.600
Pyrene	0.600
Butyl benzyl phthalate	0.010
3,3'-Dichlorobenzidine	0.010
Benzo(a)anthracene	0.800

TABLE 4
(continued)

Semivolatile Compounds	Minimum Response Factor (RF)
Chrysene	0.700
Bis-(2-ethylhexyl)phthalate	0.010
Di-n-octyl phthalate	0.010
Benzo(b)fluoranthene	0.700
Benzo(k)fluoranthene	0.700
Benzo(a)pyrene	0.700
Indeno(1,2,3-cd)pyrene	0.500
Dibenz(a,h)anthracene	0.400
Benzo(g,h,i)perylene	0.500
2,3,4,6-Tetrachlorophenol	0.010

TABLE 5

SEMIVOLATILE INTERNAL STANDARDS WITH CORRESPONDING ANALYTES ASSIGNED FOR QUANTITATION

1,4-Dichlorobenzene-d_4	Naphthalene-d_8	Acenaphthene-d_{10}
Aniline	Acetophenone	Acenaphthene
Benzyl alcohol	Benzoic acid	Acenaphthylene
Bis(2-chloroethyl) ether	Bis(2-chloroethoxy)methane	1-Chloronaphthalene
Bis(2-chloroisopropyl) ether	4-Chloroaniline	2-Chloronaphthalene
2-Chlorophenol	4-Chloro-3-methylphenol	4-Chlorophenyl phenyl ether
1,3-Dichlorobenzene	2,4-Dichlorophenol	Dibenzofuran
1,4-Dichlorobenzene	2,6-Dichlorophenol	Diethyl phthalate
1,2-Dichlorobenzene	α,α-Dimethyl-	Dimethyl phthalate
Ethyl methanesulfonate	phenethylamine	2,4-Dinitrophenol
2-Fluorophenol (surr)	2,4-Dimethylphenol	2,4-Dinitrotoluene
Hexachloroethane	Hexachlorobutadiene	2,6-Dinitrotoluene
Methyl methanesulfonate	Isophorone	Fluorene
2-Methylphenol	2-Methylnaphthalene	2-Fluorobiphenyl (surr)
4-Methylphenol	Naphthalene	Hexachlorocyclopentadiene
N-Nitrosodimethylamine	Nitrobenzene	1-Naphthylamine
N-Nitroso-di-n-propylamine	Nitrobenzene-d_8 (surr)	2-Naphthylamine
Phenol	2-Nitrophenol	2-Nitroaniline
Phenol-d_6 (surr)	N-Nitrosodi-n-butylamine	3-Nitroaniline
2-Picoline	N-Nitrosopiperidine	4-Nitroaniline
	1,2,4-Trichlorobenzene	4-Nitrophenol
		Pentachlorobenzene
		1,2,4,5-Tetrachlorobenzene
		2,3,4,6-Tetrachlorophenol
		2,4,6-Tribromophenol (surr)
		2,4,6-Trichlorophenol
		2,4,5-Trichlorophenol

(surr) = surrogate

TABLE 5
(continued)

Phenanthrene-d_{10}	Chrysene-d_{12}	Perylene-d_{12}
4-Aminobiphenyl	Benzidine	Benzo(b)fluoranthene
Anthracene	Benzo(a)anthracene	Benzo(k)fluoranthene
4-Bromophenyl phenyl ether	Bis(2-ethylhexyl) phthalate	Benzo(g,h,i)perylene
Di-n-butyl phthalate	Butyl benzyl phthalate	Benzo(a)pyrene
4,6-Dinitro-2-methylphenol	Chrysene	Dibenz(a,j)acridine
Diphenylamine	3,3'-Dichlorobenzidine	Dibenz(a,h)anthracene
Fluoranthene	p-Dimethyl aminoazobenzene	7,12-Dimethylbenz(a) anthracene
Hexachlorobenzene	Pyrene	Di-n-octyl phthalate
N-Nitrosodiphenylamine	Terphenyl-d_{14} (surr)	Indeno(1,2,3-cd) pyrene
Pentachlorophenol		3-Methylcholanthrene
Pentachloronitrobenzene		
Phenacetin		
Phenanthrene		
Pronamide		

(surr) = surrogate

TABLE 6

EXAMPLE SINGLE LABORATORY PERFORMANCE DATA[a]

Compound	Test conc. (µg/L)	x of 5 replicates (µg/L)	% Recovery of Avg.
Acenaphthene	50	46.7	93.4
Acenaphthylene	50	46.1	92.2
Aniline	50	8.3	16.7
Anthracene	50	48.4	96.8
Benzoic acid	50	43.7	87.4
Benz(a)anthracene	50	49.6	99.2
Benzo(b)fluoranthene	50	49.8	99.6
Benzo(k)fluoranthene	50	50.6	101
Benzo(a)pyrene	50	47.7	95.5
Benzo(g,h,i)perylene	50	52.6	105
Benzyl alcohol	50	44.4	88.8
Bis(2-chloroethyl) ether	50	44.2	88.4
Bis(2-chloroethoxy)methane	50	46.6	93.1
Bis(2-chloroisopropyl) ether	50	43.4	86.8
Bis(2-ethylhexyl) phthalate	50	50.2	100
4-Bromophenyl phenyl ether	50	48.6	97.2
Butyl benzyl phthalate	50	49.6	99.3
Carbazole	50	52.1	104
2-Chloroaniline	50	38.9	77.7
4-Chloro-3-methylphenol	50	47.3	94.6
2-Chloronaphthalene	50	45.3	90.8
2-Chlorophenol	50	43.1	86.2
4-Chlorophenyl phenyl ether	50	47.3	94.6
Chrysene	50	50.3	101
Dibenzofuran	50	47.4	94.7
Dibenz(a,h)anthracene	50	51.6	103
Di-n-butyl phthalate	50	50.5	101
1,2-Dichlorobenzene	50	35.8	71.6
1,3-Dichlorobenzene	50	33.3	66.7
1,4-Dichlorobenzene	50	34.4	68.7
3,3'-Dichlorobenzidine	50	32.0	64.0
2,4-Dichlorophenol	50	47.4	94.8
Diethyl phthalate	50	50.0	99.9
Dimethyl phthalate	50	48.5	97.0
2,4-Dimethylphenol	50	31.2	62.3
4,6-Dinitro-2-methylphenol	50	57.6	115
2,4-Dinitrophenol	50	58.7	117
2,4-Dinitrotoluene	50	51.3	103

TABLE 6
(continued)

Compound	Test conc. (µg/L)	x• of 5 replicates (µg/L)	% Recovery of Avg.
2,6-Dinitrotoluene	50	50.2	100
Di-n-octyl phthalate	50	51.1	102
Fluoranthene	50	51.0	102
Fluorene	50	48.5	97.0
Hexachlorobenzene	50	49.0	97.9
Hexachlorobutadiene	50	34.7	69.5
Hexachlorocyclopentadiene	50	1.9	3.8
Hexachloroethane	50	29.9	58.8
Indeno(1,2,3-cd)pyrene	50	51.7	103
Isophorone	50	47.1	94.3
2-Methylnaphthalene	50	44.7	89.4
2-Methylphenol	50	41.7	83.4
4-Methylphenol	50	42.6	85.2
Naphthalene	50	43.4	86.8
2-Nitroaniline	50	48.4	96.7
3-Nitroaniline	50	46.8	93.6
4-Nitroaniline	50	56.1	112
Nitrobenzene	50	47.1	94.1
2-Nitrophenol	50	47.3	94.6
4-Nitrophenol	50	55.4	111
N-Nitrosodiphenylamine	50	46.7	93.4
N-Nitroso-di-propylamine	50	44.6	89.3
Pentachlorophenol	50	56.9	114
Phenanthrene	50	49.7	99.4
Phenol	50	40.9	81.8
Pyrene	50	49.2	98.4
1,2,4-Trichlorobenzene	50	39.1	78.2
2,4,5-Trichlorophenol	50	47.7	95.4
2,4,6-Trichlorophenol	50	49.2	98.4

x• = Average recovery for five initial demonstration of capability measurements, in µg/L

[a] Extraction using acidic pH only with a modified continuous liquid-liquid extractor with hydrophobic membrane according to Method 3520. These values are for guidance only. Appropriate derivation of acceptance criteria for similar extraction conditions may result in much different recovery ranges. See Method 8000 for information on developing and updating acceptance criteria for method performance.

TABLE 7

EXTRACTION EFFICIENCY AND AQUEOUS STABILITY RESULTS

	Percent Recovery, Day 0		Percent Recovery, Day 7	
Compound	Mean	RSD	Mean	RSD
3-Amino-9-ethylcarbazole	80	8	73	3
4-Chloro-1,2-phenylenediamine	91	1	108	4
4-Chloro-1,3-phenylenediamine	84	3	70	3
1,2-Dibromo-3-chloropropane	97	2	98	5
Dinoseb	99	3	97	6
Parathion	100	2	103	4
4,4'-Methylenebis(N,N-dimethylaniline)	108	4	90	4
5-Nitro-o-toluidine	99	10	93	4
2-Picoline	80	4	83	4
Tetraethyl dithiopyrophosphate	92	7	70	1

Data taken from Reference 6.

TABLE 8

MEAN PERCENT RECOVERIES AND PERCENT RSD VALUES FOR SEMIVOLATILE ORGANIC FROM SPIKED CLAY SOIL AND TOPSOIL BY AUTOMATED SOXHLET EXTRACTION (METHOD 3541) WITH HEXANE-ACETONE (1:1)[a]

	Clay Soil		Topsoil	
Compound	Mean Recovery	RSD	Mean Recovery	RSD
1,3-Dichlorobenzene	0	--	0	--
1,2-Dichlorobenzene	0	--	0	--
Nitrobenzene	0	--	0	--
Benzal chloride	0	--	0	--
Benzotrichloride	0	--	0	--
4-Chloro-2-nitrotoluene	0	--	0	--
Hexachlorocyclopentadiene	4.1	15	7.8	23
2,4-Dichloronitrobenzene	35.2	7.6	21.2	15
3,4-Dichloronitrobenzene	34.9	15	20.4	11
Pentachlorobenzene	13.7	7.3	14.8	13
2,3,4,5-Tetrachloronitrobenzene	55.9	6.7	50.4	6.0
Benefin	62.6	4.8	62.7	2.9
alpha-BHC	58.2	7.3	54.8	4.8
Hexachlorobenzene	26.9	13	25.1	5.7
delta-BHC	95.8	4.6	99.2	1.3
Heptachlor	46.9	9.2	49.1	6.3
Aldrin	97.7	12	102	7.4
Isopropalin	102	4.3	105	2.3
Heptachlor epoxide	90.4	4.4	93.6	2.4
trans-Chlordane	90.1	4.5	95.0	2.3
Endosulfan I	96.3	4.4	101	2.2
Dieldrin	129	4.7	104	1.9
2,5-Dichlorophenyl-4-nitrophenyl ether	110	4.1	112	2.1
Endrin	102	4.5	106	3.7
Endosulfan II	104	4.1	105	0.4
p,p'-DDT	134	2.1	111	2.0
2,3,6-Trichlorophenyl-4'-nitrophenyl ether	110	4.8	110	2.8
2,3,4-Trichlorophenyl-4'-nitrophenyl ether	112	4.4	112	3.3
Mirex	104	5.3	108	2.2

[a] The operating conditions for the Soxtec apparatus were as follows: immersion time 45 min; extraction time 45 min; the sample size was 10 g; the spiking concentration was 500 ng/g, except for the surrogate compounds at 1000 ng/g, 2,5-Dichlorophenyl-4-nitrophenyl ether, 2,3,6-Trichlorophenyl-4-nitrophenyl ether, and 2,3,4-Trichlorophenyl-4-nitrophenyl ether at 1500 ng/g, Nitrobenzene at 2000 ng/g, and 1,3-Dichlorobenzene and 1,2-Dichlorobenzene at 5000 ng/g.

TABLE 9

SINGLE LABORATORY ACCURACY AND PRECISION DATA FOR THE EXTRACTION OF SEMIVOLATILE ORGANICS FROM SPIKED CLAY BY AUTOMATED SOXHLET (METHOD 3541)[a]

Compound	Mean Recovery	RSD
Phenol	47.8	5.6
Bis(2-chloroethyl)ether	25.4	13
2-Chlorophenol	42.7	4.3
Benzyl alcohol	55.9	7.2
2-Methylphenol	17.6	6.6
Bis(2-chloroisopropyl)ether	15.0	15
4-Methylphenol	23.4	6.7
N-Nitroso-di-n-propylamine	41.4	6.2
Nitrobenzene	28.2	7.7
Isophorone	56.1	4.2
2-Nitrophenol	36.0	6.5
2,4-Dimethylphenol	50.1	5.7
Benzoic acid	40.6	7.7
Bis(2-chloroethoxy)methane	44.1	3.0
2,4-Dichlorophenol	55.6	4.6
1,2,4-Trichlorobenzene	18.1	31
Naphthalene	26.2	15
4-Chloroaniline	55.7	12
4-Chloro-3-methylphenol	65.1	5.1
2-Methylnaphthalene	47.0	8.6
Hexachlorocyclopentadiene	19.3	19
2,4,6-Trichlorophenol	70.2	6.3
2,4,5-Trichlorophenol	26.8	2.9
2-Chloronaphthalene	61.2	6.0
2-Nitroaniline	73.8	6.0
Dimethyl phthalate	74.6	5.2
Acenaphthylene	71.6	5.7
3-Nitroaniline	77.6	5.3
Acenaphthene	79.2	4.0
2,4-Dinitrophenol	91.9	8.9
4-Nitrophenol	62.9	16
Dibenzofuran	82.1	5.9
2,4-Dinitrotoluene	84.2	5.4
2,6-Dinitrotoluene	68.3	5.8

Compound	Mean Recovery	RSD
Diethyl phthalate	74.9	5.4
4-Chlorophenyl-phenyl ether	67.2	3.2
Fluorene	82.1	3.4
4-Nitroaniline	79.0	7.9
4,6-Dinitro-2-methylphenol	63.4	6.8
N-Nitrosodiphenylamine	77.0	3.4
4-Bromophenyl-phenyl ether	62.4	3.0
Hexachlorobenzene	72.6	3.7
Pentachlorophenol	62.7	6.1
Phenanthrene	83.9	5.4
Anthracene	96.3	3.9
Di-n-butyl phthalate	78.3	40
Fluoranthene	87.7	6.9
Pyrene	102	0.8
Butyl benzyl phthalate	66.3	5.2
3,3'-Dichlorobenzidine	25.2	11
Benzo(a)anthracene	73.4	3.8
Bis(2-ethylhexyl) phthalate	77.2	4.8
Chrysene	76.2	4.4
Di-n-octyl phthalate	83.1	4.8
Benzo(b)fluoranthene	82.7	5.0
Benzo(k)fluoranthene	71.7	4.1
Benzo(a)pyrene	71.7	4.1
Indeno(1,2,3-cd)pyrene	72.2	4.3
Dibenz(a,h)anthracene	66.7	6.3
Benzo(g,h,i)perylene	63.9	8.0
1,2-Dichlorobenzene	0	--
1,3-Dichlorobenzene	0	--
1,4-Dichlorobenzene	0	--
Hexachloroethane	0	--
Hexachlorobutadiene	0	--

[a] Number of determinations was three. The operating conditions for the Soxtec apparatus were as follows: immersion time 45 min; extraction time 45 min; the sample size was 10 g clay soil; the spike concentration was 6 mg/kg per compound. The sample was allowed to equilibrate 1 hour after spiking.

Data taken from Reference 7.

TABLE 10

PRECISION AND BIAS VALUES FOR METHOD 3542[1]

Compound	Mean Recovery	Standard Deviation	% RSD
2-Fluorophenol	74.6	28.6	38.3
Phenol-d_5	77.8	27.7	35.6
Nitrobenzene-d_5	65.6	32.5	49.6
2-Fluorobiphenyl	75.9	30.3	39.9
2,4,6-Tribromophenol	67.0	34.0	50.7
Terphenyl-d_{14}	78.6	32.4	41.3

[1] The surrogate values shown in Table 10 represent mean recoveries for surrogates in all Method 0010 matrices in a field dynamic spiking study.

TABLE 11

PRESSURIZED FLUID EXTRACTION (METHOD 3545) RECOVERY VALUES
AS PERCENT OF SOXTEC™

	Clay			Loam			Sand			Mean
Compound	Low	Mid	High	Low	Mid	High	Low	Mid	High	Rec.
Phenol	93.3	78.7	135.9	73.9	82.8	124.6	108.8	130.6	89.7	102.0
Bis(2-chloroethyl) ether	102.1	85.1	109.1	96.0	88.0	103.6	122.3	119.9	90.8	101.9
2-Chlorophenol	100.8	82.6	115.0	93.8	88.9	111.1	115.0	115.3	91.9	101.6
1,3-Dichlorobenzene	127.7	129.7	110.0	*364.2	129.9	119.0	*241.3	*163.7	107.1	120.6
1,4-Dichlorobenzene	127.9	127.0	110.5	*365.9	127.8	116.4	*309.6	*164.1	105.8	119.2
1,2-Dichlorobenzene	116.8	115.8	101.3	*159.2	113.4	105.5	*189.3	134.0	100.4	112.5
2-Methylphenol	98.9	82.1	119.7	87.6	89.4	111.0	133.2	128.0	92.1	104.7
Bis(2-chloroisopropyl)ether	109.4	71.5	108.0	81.8	81.0	88.6	118.1	148.3	94.8	100.2
o-Toluidine	100.0	89.7	117.2	100.0	*152.5	120.3	100.0	*199.5	102.7	110.3
N-Nitroso-di-n-propylamine	103.0	79.1	107.7	83.9	88.1	96.2	109.9	123.3	91.4	98.1
Hexachloroethane	97.1	125.1	111.0	*245.4	117.1	128.1	*566.7	147.9	103.7	118.6
Nitrobenzene	104.8	82.4	106.6	86.8	84.6	101.7	119.7	122.1	93.3	100.2
Isophorone	100.0	86.4	98.2	87.1	87.5	109.7	135.5	118.4	92.7	101.7
2,4-Dimethylphenol	100.0	104.5	140.0	100.0	114.4	123.1	100.0	*180.6	96.3	109.8
2-Nitrophenol	80.7	80.5	107.9	91.4	86.7	103.2	122.1	107.1	87.0	96.3
Bis(chloroethoxy)methane	94.4	80.6	94.7	86.5	84.4	99.6	130.6	110.7	93.2	97.2
2,4-Dichlorophenol	88.9	87.8	111.4	85.9	87.6	103.5	123.3	107.0	92.1	98.6
1,2,4-Trichlorobenzene	98.0	97.8	98.8	123.0	93.7	94.5	137.0	99.4	95.3	104.2
Naphthalene	101.7	97.2	123.6	113.2	102.9	129.5	*174.5	114.0	89.8	106.1
4-Chloroaniline	100.0	*150.2	*162.4	100.0	125.5	*263.6	100.0	*250.8	114.9	108.1
Hexachlorobutadiene	101.1	98.7	102.2	124.1	90.3	98.0	134.9	96.1	96.8	104.7
4-Chloro-3-methylphenol	90.4	80.2	114.7	79.0	85.2	109.8	131.6	116.2	90.1	99.7
2-Methylnaphthalene	93.2	89.9	94.6	104.1	92.2	105.9	146.2	99.1	93.3	102.1
Hexachlorocyclopentadiene	100.0	100.0	0.0	100.0	100.0	6.8	100.0	100.0	*238.3	75.8
2,4,6-Trichlorophenol	94.6	90.0	112.0	84.2	91.2	103.6	101.6	95.9	89.8	95.9
2,4,5-Trichlorophenol	84.4	91.9	109.6	96.1	80.7	103.6	108.9	83.9	87.9	94.1
2-Chloronaphthalene	100.0	91.3	93.6	97.6	93.4	98.3	106.8	93.0	92.0	96.2
2-Nitroaniline	90.0	83.4	97.4	71.3	88.4	89.9	112.1	113.3	87.7	92.6
2,6-Dinitrotoluene	83.1	90.6	91.6	86.4	90.6	90.3	104.3	84.7	90.9	90.3
Acenaphthylene	104.9	95.9	100.5	99.0	97.9	108.8	118.5	97.8	92.0	101.7
3-Nitroaniline	*224.0	115.6	97.6	100.0	111.8	107.8	0.0	111.7	99.0	92.9
Acenaphthene	102.1	92.6	97.6	97.2	96.9	104.4	114.2	92.0	89.0	98.4
4-Nitrophenol	0.0	93.2	121.5	18.1	87.1	116.6	69.1	90.5	84.5	75.6
2,4-Dinitrotoluene	73.9	91.9	100.2	84.7	93.8	98.9	100.9	84.3	87.3	90.7

TABLE 11
(continued)

Compound	Clay			Loam			Sand			Mean
	Low	Mid	High	Low	Mid	High	Low	Mid	High	Rec.
Dibenzofuran	89.5	91.7	109.3	98.5	92.2	111.4	113.8	92.7	90.4	98.8
4-Chlorophenyl phenyl ether	83.0	94.5	98.7	95.7	94.3	94.2	111.4	87.7	90.3	94.4
Fluorene	85.2	94.9	89.2	102.0	95.5	93.8	121.3	85.7	90.9	95.4
4-Nitroaniline	77.8	114.8	94.5	129.6	103.6	95.4	*154.1	89.3	87.5	99.1
N-Nitrosodiphenylamine	82.6	96.7	93.8	92.9	93.4	116.4	97.5	110.9	86.7	96.8
4-Bromophenyl phenyl ether	85.6	92.9	92.8	91.1	107.6	89.4	118.0	97.5	87.1	95.8
Hexachlorobenzene	95.4	91.7	92.3	95.4	93.6	83.7	106.8	94.3	90.0	93.7
Pentachlorophenol	68.2	85.9	107.7	53.2	89.8	88.1	96.6	59.8	81.3	81.2
Phenanthrene	92.1	93.7	93.3	100.0	97.8	113.3	124.4	101.0	89.9	100.6
Anthracene	101.6	95.0	93.5	92.5	101.8	118.4	123.0	94.5	90.6	101.2
Carbazole	94.4	99.3	96.6	105.5	96.7	111.4	115.7	83.2	88.9	99.1
Fluoranthene	109.9	101.4	94.3	111.6	96.6	109.6	123.2	85.4	92.7	102.7
Pyrene	106.5	105.8	107.6	116.7	90.7	127.5	103.4	95.5	93.2	105.2
3,3'-Dichlorobenzidine	100.0	*492.3	131.4	100.0	*217.6	*167.6	100.0	*748.8	100.0	116.5
Benzo(a)anthracene	98.1	107.0	98.4	119.3	98.6	104.0	105.0	93.4	89.3	101.5
Chrysene	100.0	108.5	100.2	116.8	93.0	117.0	106.7	93.6	90.2	102.9
Benzo(b)fluoranthene	106.6	109.9	75.6	121.7	100.7	93.9	106.9	81.9	93.6	99.0
Benzo(k)fluoranthene	102.4	105.2	88.4	125.5	99.4	95.1	144.7	89.2	78.1	103.1
Benzo(a)pyrene	107.9	105.5	80.8	122.3	97.7	104.6	101.7	86.2	92.0	99.9
Indeno(1,2,3-cd)pyrene	95.1	105.7	93.8	126.0	105.2	90.4	133.6	82.6	91.9	102.7
Dibenz(a,h)anthracene	85.0	102.6	82.0	118.8	100.7	91.9	142.3	71.0	93.1	98.6
Benzo(g,h,i)perylene	98.0	0.0	81.2	0.0	33.6	78.6	128.7	83.0	94.2	66.4
Mean	95.1	94.3	101.0	95.5	96.5	104.1	113.0	100.9	92.5	

* Values greater than 150% were not used to determine the averages, but the 0% values were used.

TABLE 12

SINGLE LABORATORY ACCURACY AND PRECISION FOR THE EXTRACTION OF PAHs FROM A CERTIFIED REFERENCE SEDIMENT EC-1, USING METHOD 3561 (SFE - SOLID TRAP)

Compound	Certified Value (mg/kg)	SFE Value[a] (mg/kg)	Percent of Certified Value	SFE RSD
Naphthalene	(27.9)[b]	41.3 ± 3.6	(148)	8.7
Acenaphthylene	(0.8)	0.9 ± 0.1	(112)	11.1
Acenaphthene	(0.2)	0.2 ± 0.01	(100)	0.05
Fluorene	(15.3)	15.6 ± 1.8	(102)	11.5
Phenanthrene	15.8 ± 1.2	16.1 ± 1.8	102	11.2
Anthracene	(1.3)	1.1 ± 0.2	(88)	18.2
Fluoranthene	23.2 ± 2.0	24.1 ± 2.1	104	8.7
Pyrene	16.7 ± 2.0	17.2 ± 1.9	103	11.0
Benz(a)anthracene	8.7 ± 0.8	8.8 ± 1.0	101	11.4
Chrysene	(9.2)	7.9 ± 0.9	(86)	11.4
Benzo(b)fluoranthene	7.9 ± 0.9	8.5 ± 1.1	108	12.9
Benzo(k)fluoranthene	4.4 ± 0.5	4.1 ± 0.5	91	12.2
Benzo(a)pyrene	5.3 ± 0.7	5.1 ± 0.6	96	11.8
Indeno(1,2,3-cd)pyrene	5.7 ± 0.6	5.2 ± 0.6	91	11.5
Benzo(g,h,i)perylene	4.9 ± 0.7	4.3 ± 0.5	88	11.6
Dibenz(a,h)anthracene	(1.3)	1.1 ± 0.2	(85)	18.2

[a] Relative standard deviations for the SFE values are based on six replicate extractions.

[b] Values in parentheses were obtained from, or compared to, Soxhlet extraction results which were not certified.

Data are taken from Reference 10.

TABLE 13

SINGLE LABORATORY ACCURACY AND PRECISION FOR THE EXTRACTION OF PAHs FROM A CERTIFIED REFERENCE SEDIMENT HS-3, USING METHOD 3561 (SFE - SOLID TRAP)

Compound	Certified Value (mg/kg)	SFE Value[a] (mg/kg)	Percent of Certified Value	SFE RSD
Naphthalene	9.0 ± 0.7	7.4 ± 0.6	82	8.1
Acenaphthylene	0.3 ± 0.1	0.4 ± 0.1	133	25.0
Acenaphthene	4.5 ± 1.5	3.3 ± 0.3	73	9.0
Fluorene	13.6 ± 3.1	10.4 ± 1.3	77	12.5
Phenanthrene	85.0 ± 20.0	86.2 ± 9.5	101	11.0
Anthracene	13.4 ± 0.5	12.1 ± 1.5	90	12.4
Fluoranthene	60.0 ± 9.0	54.0 ± 6.1	90	11.3
Pyrene	39.0 ± 9.0	32.7 ± 3.7	84	11.3
Benz(a)anthracene	14.6 ± 2.0	12.1 ± 1.3	83	10.7
Chrysene	14.1 ± 2.0	12.0 ± 1.3	85	10.8
Benzo(b)fluoranthene	7.7 ± 1.2	8.4 ± 0.9	109	10.7
Benzo(k)fluoranthene	2.8 ± 2.0	3.2 ± 0.5	114	15.6
Benzo(a)pyrene	7.4 ± 3.6	6.6 ± 0.8	89	12.1
Indeno(1,2,3-cd)pyrene	5.0 ± 2.0	4.5 ± 0.6	90	13.3
Benzo(g,h,i)perylene	5.4 ± 1.3	4.4 ± 0.6	82	13.6
Dibenz(a,h)anthracene	1.3 ± 0.5	1.1 ± 0.3	85	27.3

[a] Relative standard deviations for the SFE values are based on three replicate extractions.

Data are taken from Reference 10.

TABLE 14

SINGLE LABORATORY ACCURACY AND PRECISION FOR THE EXTRACTION OF PAHs FROM A CERTIFIED REFERENCE SOIL SRS103-100, USING METHOD 3561 (SFE - LIQUID TRAP)

Compound	Certified Value (mg/kg)	SFE Value[a] (mg/kg)	Percent of Certified Value	SFE RSD
Naphthalene	32.4 ± 8.2	29.55	91	10.5
2-Methylnaphthalene	62.1 ± 11.5	76.13	122	2.0
Acenaphthene	632 ± 105	577.28	91	2.9
Dibenzofuran	307 ± 49	302.25	98	4.1
Fluorene	492 ± 78	427.15	87	3.0
Phenanthrene	1618 ± 340	1278.03	79	3.4
Anthracene	422 ± 49	400.80	95	2.6
Fluoranthene	1280 ± 220	1019.13	80	4.5
Pyrene	1033 ± 285	911.82	88	3.1
Benz(a)anthracene	252 ± 8	225.50	89	4.8
Chrysene	297 ± 26	283.00	95	3.8
Benzo(a)pyrene	97.2 ± 17.1	58.28	60	6.5
Benzo(b)fluoranthene + Benzo(k)fluoranthene	153 ± 22	130.88	86	10.7

[a] Relative standard deviations for the SFE values are based on four replicate extractions.

Data are taken from Reference 11.

TABLE 15

SINGLE LABORATORY RECOVERY DATA FOR SOLID-PHASE EXTRACTION (METHOD 3535) OF BASE/NEUTRAL/ACID EXTRACTABLES FROM SPIKED TCLP BUFFERS LOW SPIKE LEVEL

Analyte	Spike Level (µg/L)	Buffer 1 (pH = 2.886)		Buffer 2 (pH = 4.937)	
		Recovery (%)	RSD	Recovery (%)	RSD
1,4-Dichlorobenzene	3,750	63	10	63	9
Hexachloroethane	1,500	55	6	77	4
Nitrobenzene	1,000	82	10	100	5
Hexachlorobutadiene	250	65	3	56	4
2,4-Dinitrotoluene	65	89	4	101	5
Hexachlorobenzene	65	98	5	95	6
o-Cresol	100,000	83	10	85	5
m-Cresol*	100,000	86	8	85	3
p-Cresol*	100,000	*	*	*	*
2,4,6-Trichlorophenol	1,000	84	12	95	12
2,4,5-Trichlorophenol	200,000	83	11	88	3
Pentachlorophenol	50,000	82	9	78	9

Results from seven replicate spiked buffer samples.

* In this study, m-cresol and p-cresol co-eluted and were quantitated as a mixture of both isomers.

Data from Reference 12.

TABLE 16

SINGLE LABORATORY RECOVERY DATA FOR SOLID-PHASE EXTRACTION (METHOD 3535) OF BASE/NEUTRAL/ACID EXTRACTABLES FROM SPIKED TCLP BUFFERS HIGH SPIKE LEVEL

Analyte	Spike Level (µg/L)	Buffer 1 (pH = 2.886)		Buffer 2 (pH = 4.937)	
		Recovery (%)	RSD	Recovery (%)	RSD
1,4-Dichlorobenzene	15,000	63	10	63	9
Hexachloroethane	6,000	54	7	46	7
Nitrobenzene	4,000	81	4	81	13
Hexachlorobutadiene	1,000	81	5	70	11
2,4-Dinitrotoluene	260	99	8	98	3
Hexachlorobenzene	260	89	8	91	9
o-Cresol*	400,000	92	15	90	4
m-Cresol*	400,000	95	8	82	6
p-Cresol*	400,000	82	14	84	7
2,4,6-Trichlorophenol	4,000	93	12	104	12
2,4,5-Trichlorophenol	800,000	93	14	97	23
Pentachlorophenol	200,000	84	9	73	8

Results from seven replicate spiked buffer samples.

* In this study, recoveries of these compounds were determined from triplicate spikes of the individual compounds into separate buffer solutions.

Data from Reference 12.

TABLE 17

RECOVERY DATA FROM THREE LABORATORIES FOR SOLID-PHASE EXTRACTION (METHOD 3535) OF BASE/NEUTRAL/ACID EXTRACTABLES FROM SPIKED TCLP LEACHATES FROM SOIL SAMPLES

Buffer 1 pH = 2.886		Lab 1			Lab 2			Lab 3		
Analyte	Spike Level (µg/L)*	%R	RSD	n	%R	RSD	n	%R	RSD	n
o-Cresol	200,000	86	8	7	35.3	0.7	3	7.6	6	3
m-Cresol**	--	77	8	7	--	--	--	--	--	--
p-Cresol**	--	--	--	--	--	--	--	7.7	11	3
2,4,6-Trichlorophenol	2,000	106	6	7	96.3	3.9	3	44.8	5	3
2,4,5-Trichlorophenol	400,000	93	3	7	80.5	4.5	3	63.3	11	3
Pentachlorophenol	100,000	79	2	7	33.8	12.2	3	29.2	13	3
1,4-Dichlorobenzene	7,500	51	5	7	81.3	5.3	3	19.2	7	3
Hexachloroethane	3,000	50	5	7	66.2	2.1	3	12.6	11	3
Nitrobenzene	2,000	80	8	7	76.3	5.3	3	63.9	12	3
Hexachlorobutadiene	500	53	8	7	63.3	4.8	3	9.6	9	3
2,4-Dinitrotoluene	130	89	8	7	35.7	2.6	3	58.2	17	3
Hexachlorobenzene	130	84	21	7	92.3	1.6	3	71.7	9	3

(continued)

TABLE 17
(continued)

Buffer 2 pH = 4.937		Lab 1			Lab 2			Lab 3		
Analyte	Spike Level (µg/L)*	%R	RSD	n	%R	RSD	n	%R	RSD	n
o-Cresol	200,00	97	13	7	37.8	4.5	3	6.1	24	3
m-Cresol**	--	83	4	7	--	--	--	6.0	25	3
p-Cresol**	--	--	--	--	--	--	--	--	--	--
2,4,6-Trichlorophenol	2,000	104	4	7	91.7	8.0	3	37.7	25	3
2,4,5-Trichlorophenol	400,000	94	4	7	85.2	0.4	3	64.4	10	3
Pentachlorophenol	100,000	109	11	7	41.9	28.2	3	36.6	32	3
1,4-Dichlorobenzene	7,500	50	5	7	79.7	1.0	3	26.5	68	3
Hexachloroethane	3,000	51	3	7	64.9	2.0	3	20.3	90	3
Nitrobenzene	2,000	80	4	7	79.0	2.3	3	59.4	6	3
Hexachlorobutadiene	500	57	5	7	60	3.3	3	16.6	107	3
2,4-Dinitrotoluene	130	86	6	7	38.5	5.2	3	62.2	6	3
Hexachlorobenzene	130	86	7	7	91.3	0.9	3	75.5	5	3

* 250-mL aliquots of leachate were spiked. Lab 1 spiked at one-half these levels.

** m-Cresol and p-Cresol coelute. Lab 1 and Lab 3 reported o-Cresol and the sum of — and p-Cresol. Lab 2 reported the sum of all three isomers of Cresol.

Data from Reference 12.

TABLE 18

SINGLE-LABORATORY PAH ANALYSIS DATA FROM A REAL SOIL CONTAMINATED WITH CREOSOTE, USING METHOD 3546
(MICROWAVE EXTRACTION)

Compound	Concentration (µg/kg)	RSD (%)	REAC values (µg/kg)
Naphthalene	2,170	12.4	710,000
2-Methylnaphthalene	28,710	3.1	N/R
1-Methylnaphthalene	33,180	2.4	N/R
Biphenyl	13,440	6.0	N/R
2,6-Dimethylnaphthalene	52,990	3.8	N/R
Acenaphthylene	16,320	3.1	21,000
Acenaphthene	801,210	6.0	1,700,000
Fluorene	789,980	3.4	990,000
Phenanthrene	1,627,480	0.7	3,300,000
Anthracene	346,010	4.0	360,000
Benzo(a)anthracene	300,380	2.7	310,000
Fluoranthene	1,331,690	1.6	1,600,000
Pyrene	1,037,710	3.0	1,100,000
Chrysene	293,200	3.4	320,000
Benzo(b)fluoranthene	152,000	3.8	140,000
Benzo(k)fluoranthene	127,740	3.6	130,000
Benzo(e)pyrene	87,610	3.9	N/R
Benzo(a)pyrene	128,330	3.9	110,000
Perylene	35,260	4.3	N/R
Indeno(123-cd)pyrene	63,900	5.0	25,000
Dibenz(a,h)anthracene	17,290	6.9	N/R
Benzo(ghi)perylene	42,720	6.9	20,000

*n = 4

Soil samples obtained from US EPA Emergency Response Center archive bank through their contract laboratory REAC (Edison, NJ). The standard Soxhlet extraction procedures were performed by REAC three years earlier; this long storage period is believed to account for the low naphthalene recovery data in the present study

REAC data labeled N/R = not reported

TABLE 19

SINGLE-LABORATORY PAH RECOVERY DATA FROM HS-5 MARINE SEDIMENT MATERIALS, USING METHOD 3546 (MICROWAVE EXTRACTION)

Compound	Certified Value (µg/kg)	Confidence Interval (µg/kg)	Recovery (%)
Naphthalene	250	180 - 320	76
Acenaphthylene	150	*	107
Acenaphthene	230	130 - 330	61
Fluorene	400	300 - 500	63
Phenanthrene	5,200	4,200 - 6,200	72
Anthracene	380	230 - 530	84
Fluoranthene	8,400	5,800 - 10,000	81
Pyrene	5,800	4,000 - 7,600	69
Benzo(a)anthracene	2,900	1,700 - 4,100	53
Chrysene	2,800	1,900 - 3,700	76
Benzo(b)fluoranthene	2,000	1,000 - 3,000	84
Benzo(k)fluoranthene	1,000	600 - 1,400	137
Benzo(a)pyrene	1,700	900 - 2,500	52
Indeno(123-cd) pyrene	1,300	600 - 2,000	63
Dibenz(a,h)anthracene	200	100 - 300	125
Benzo(ghi)perylene	1,300	1000 - 1600	64

n = 3

* values not certified

The uncertainties represent 90% confidence intervals

TABLE 20

SINGLE-LABORATORY PAH RECOVERY DATA FROM HS-4 MARINE SEDIMENT MATERIALS, USING METHOD 3546 (MICROWAVE EXTRACTION)

Compound	Certified Value (µg/kg)	Confidence Interval (µg/kg)	Recovery (%)
Naphthalene	150	*	54
Acenaphthylene	150	*	82
Acenaphthene	150	*	63
Fluorene	150	*	81
Phenanthrene	680	600 - 760	81
Anthracene	140	70 - 210	108
Fluoranthene	1250	1,150 - 1,350	84
Pyrene	940	820 - 1,060	85
Benzo(a)anthracene	530	470 - 580	78
Chrysene	650	570 - 730	84
Benzo(b)fluoranthene	700	550 - 850	84
Benzo(k)fluoranthene	360	310 - 410	156
Benzo(a)pyrene	650	570 - 730	73
Indeno(123-cd) pyrene	510	360 - 660	88
Dibenz(a,h)anthracene	120	70 - 170	117
Benzo(ghi)perylene	580	360 - 800	91

n = 3

* values not certified

The uncertainties represent 90% confidence intervals

TABLE 21

SINGLE-LABORATORY PAH RECOVERY DATA FROM HS-3 MARINE SEDIMENT MATERIALS, USING METHOD 3546 (MICROWAVE EXTRACTION)

Compound	Certified Value (µg/kg)	Confidence Interval (µg/kg)	Recovery (%)
Naphthalene	9,000	8300 - 9,700	61
Acenaphthylene	300	200 - 400	199
Acenaphthene	4,500	3,000 - 6,000	80
Fluorene	13,300	10,200 -16,400	58
Phenanthrene	85,000	65000 -105,000	87
Anthracene	13,400	12,900 -13,900	48
Fluoranthene	60,000	51,000-69,000	91
Pyrene	39,000	30,000-48,000	86
Benzo(a)anthracene	14,600	12,600-16,600	78
Chrysene	14,100	12,100-16,100	91
Benzo(b)fluoranthene	7,700	6,500-8,900	101
Benzo(k)fluoranthene	2,800	800-4,800	275
Benzo(a)pyrene	7,400	3,000-7,000	74
Indeno(123-cd)pyrene	5,400	4,100-6,700	100
Dibenz(a,h)anthracene	1,300	800-1,800	118
Benzo(ghi)perylene	5,000	3,000-7,000	99

n = 3

* values not certified

The uncertainties represent 90% confidence intervals

TABLE 22

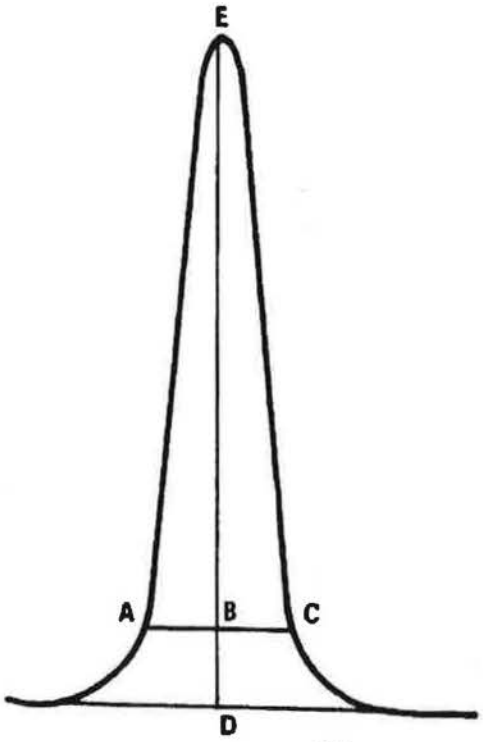
SINGLE-LABORATORY PAH RECOVERY DATA FROM SRM 1941 MARINE SEDIMENT, USING METHOD 3546 (MICROWAVE EXTRACTION)

Compound	Certified Value (µg/kg)	Recovery (%)
Naphthalene	1010	97.4
Fluorene	100	100.0
Phenanthrene	490	102.0
Fluoranthene	980	116.7
Pyrene	810	97.3
Benz(a)anthracene	430	89.8
Chrysene	380	130.3
Benzo(b)fluoranthene	740	95.8
Benzo(k)fluoranthene	360	130.2
Benz(e)pyrene	550	81.0
Benzo(a)pyrene	630	76.0
Perylene	450	72.4
Indeno(123-cd)pyrene	500	126.0
Dibenz(a,h)anthracene	110	78.7
Benz(ghi)perylene	530	85.2

n = 3

All RSDs < 10%

FIGURE 1
TAILING FACTOR CALCULATION

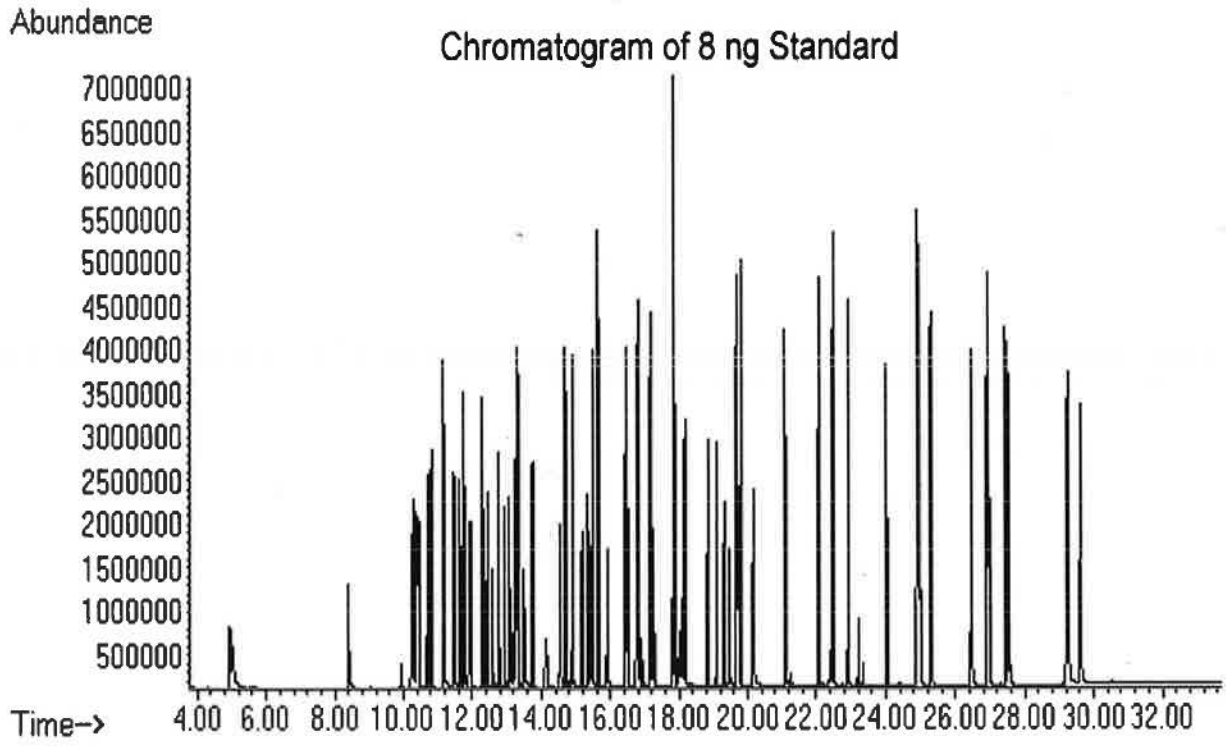


TAILING FACTOR $= \frac{BC}{AB}$

Example calculation: Peak Height = DE = 100 mm
10% Peak Height = BD = 10 mm
Peak Width at 10% Peak Height = AC = 23 mm
AB = 11 mm
BC = 12 mm

Therefore: Tailing Factor $= \frac{12}{11} = 1.1$

FIGURE 2
GAS CHROMATOGRAM OF BASE/NEUTRAL AND ACID CALIBRATION STANDARD



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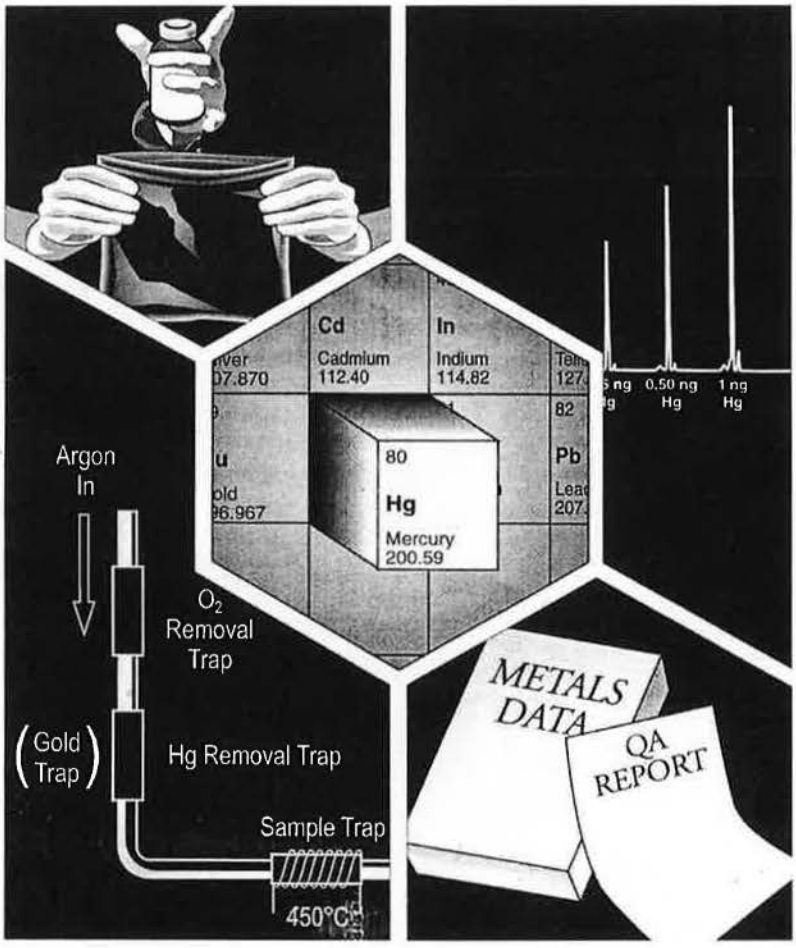
August 2002



United States
Environmental Protection
Agency

Office of Water
4303

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Disclaimer

This Method has been reviewed and approved for publication by the Statistics and Analytical Support Branch within EPA's Engineering and Analysis Division. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Table of Contents

Introduction

Method 1631 (the "Method") supports technology-based and water quality-based monitoring programs authorized under the Clean Water Act (CWA; the "Act").

CWA Sections 301 and 306 require EPA to publish effluent standards that restrict the direct discharge of pollutants to the nations waters, and CWA Sections 307(b) and (c) require EPA to promulgate nationally applicable pretreatement standards which restrict pollutant discharges into sewers flowing to publicly owned treatment works (POTWs). The effluent limitations guidelines are published at CFR parts 401-503.

CWA Section 303 requires each State to set a water quality standard for each body of water within its boundaries. A State water quality standard consists of a designated use or uses of a water body or a segment of a water body, the water quality criteria that are necessary to protect the designated use or uses, and an antidegradation policy. CWA Section 304(a) requires EPA to publish water quality criteria that reflect the latest scientific knowledge concerning the physical fate of pollutants, the effects of pollutants on ecological and human health, and the effect of pollutants on biological community diversity, productivity, and stability. These water quality standards serve two purposes: (1) they establish the water quality goals for a specific water body, and (2) they are the basis for establishing water quality-based treatment controls and strategies beyond the technology-based controls required by CWA Sections 301(b) and 306.

In 1987, amendments to the CWA required States to adopt numeric criteria for toxic pollutants (designated in Section 307(a) of the Act) based on EPA Section 304(a) criteria or other scientific data, when the discharge or presence of those toxic pollutants could reasonably be expected to interfere with designated uses. Method 1631 was specifically developed to provide reliable measurements of mercury at EPA WQC levels.

In developing methods for determination of trace metals, EPA found that one of the greatest difficulties was precluding sample contamination during collection, transport, and analysis. The degree of difficulty, however, is highly dependent on the metal and site-specific conditions. Method 1631 is designed to preclude contamination in nearly all situations. It also contains procedures necessary to produce reliable results at the lowest WQC levels published by EPA. In recognition of the variety of situations to which this Method may be applied, and in recognition of continuing technological advances, Method 1631 is performance based. Alternative procedures may be used so long as those procedures are demonstrated to yield reliable results.

Requests for additional copies of this draft Method should be directed to:

U.S. EPA Sample Control Center
6101 Stevenson Avenue
Alexandria, VA 22304-3540
703/461–2100

Note: This Method is performance based. The laboratory is permitted to omit steps or modify procedures provided that all performance requirements in this Method are met. The laboratory must not omit or modify any procedure defined by the term "shall" or "must" and must perform all quality control tests.

Method 1631, Revision E

Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry

1.0 Scope and Application

1.1 Method 1631, Revision E (the "Method") is for determination of mercury (Hg) in filtered and unfiltered water by oxidation, purge and trap, desorption, and cold-vapor atomic fluorescence spectrometry (CVAFS). This Method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act. The Method is based on a contractor-developed procedure (Reference 16.1) and on peer-reviewed, published procedures for the determination of mercury in aqueous samples, ranging from sea water to sewage effluent (References 16.2–16.5).

1.2 This Method is accompanied by Method 1669: *Sampling Ambient Water for Determination of Trace Metals at EPA Water Quality Criteria Levels* (Sampling Method). The Sampling Method guidance document is recommended to preclude contamination during the sampling process.

1.3 This Method is for determination of Hg in the range of 0.5–100 ng/L. Application may be extended to higher levels by selection of a smaller sample size or by calibration of the analytical system across a higher range. For measurement of blank samples, the Method may be extended to a lower level by calibration to a lower calibration point. Section 10.4 gives requirements for extension of the calibration range.

1.4 The ease of contaminating ambient water samples with mercury and interfering substances cannot be overemphasized. This Method includes suggestions for improvements in facilities and analytical techniques that should minimize contamination and maximize the ability of the laboratory to make reliable trace metals determinations. Certain sections of this Method contain suggestions and other sections contain requirements to minimize contamination.

1.5 The detection limit and minimum level of quantitation in this Method usually are dependent on the level of interferences rather than instrument limitations. The method detection limit (MDL; 40 CFR 136, Appendix B) for Hg has been determined to be 0.2 ng/L when no interferences are present. The minimum level of quantitation (ML) has been established as 0.5 ng/L. An MDL as low as 0.05 ng/L can be achieved for low Hg samples by using a larger sample volume, a lower BrCl level (0.2%), and extra caution in sample handling.

1.6 Clean and ultraclean—The terms "clean" and "ultraclean" have been applied to the techniques needed to reduce or eliminate contamination in trace metals determinations. These terms are not used in this Method because they lack an exact definition. However, the information provided in this Method is consistent with the summary guidance on clean and ultraclean techniques (References 16.6-16.7).

1.7 This Method follows the EPA Environmental Methods Management Council's "Guidelines and Format for Methods to Be Proposed at 40 CFR, part 136 or part 141."

1.8 This Method is "performance based." The laboratory is permitted to modify the Method to overcome interferences or lower the cost of measurements if all performance criteria are met. Section 9.1.2.1 gives the requirements for establishing method equivalency.

1.9 Any modification of this Method, beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.10 This Method should be used only by analysts experienced in the use of CVAFS techniques and who are trained thoroughly in the sample handling and instrument techniques described in this Method. Each laboratory that uses this Method must demonstrate the ability to generate acceptable results using the procedures in Section 9.2.

1.11 This Method is accompanied by a data verification and validation guidance document, *Guidance on the Documentation and Evaluation of Trace Metals Data Collected for CWA Compliance Monitoring* (Reference 16.8), that can be used for verification and validation of the data obtained.

1.12 This Method uses either a bubbler or flow-injection system for determination of mercury in water. Separate calibration, analysis, and calculation procedures are provided for a bubbler system (Sections 10.2, 11.2.1, and 12.2) and for a flow-injection system (Sections 10.3, 11.2.2, and 12.3).

2.0 Summary of Method

2.1 A 100- to 2000-mL sample is collected directly into a cleaned, pretested, fluoropolymer or glass bottle using sample handling techniques designed for collection of mercury at trace levels (Reference 16.9).

2.2 For dissolved Hg, the sample is filtered through a 0.45-µm capsule filter prior to preservation.

2.3 The sample is preserved by adding either pretested 12N hydrochloric acid (HCl) or bromine monochloride (BrCl) solution. If a sample will also be used for the determination of methyl mercury, it should be preserved according to procedures in the method that will be used for determination of methylmercury.

2.4 Prior to analysis, all Hg in a 100-mL sample aliquot is oxidized to Hg(II) with BrCl.

2.5 After oxidation, the sample is sequentially reduced with $NH_2OH{\cdot}HCl$ to destroy the free halogens, then reduced with stannous chloride ($SnCl_2$) to convert Hg(II) to volatile Hg(0).

2.6 The Hg(0) is separated from solution either by purging with nitrogen, helium, or argon, or by vapor/liquid separation. The Hg(0) is collected onto a gold trap (Figures 1, 2, and 3).

2.7 The Hg is thermally desorbed from the gold trap into an inert gas stream that carries the released Hg(0) to a second gold (analytical) trap. The Hg is desorbed from the analytical trap into a gas stream that carries the Hg into the cell of a cold-vapor atomic fluorescence spectrometer (CVAFS) for detection (Figures 2 and 3).

2.8 Quality is assured through calibration and testing of the oxidation, purging, and detection systems.

3.0 Definitions

3.1 Total mercury—all BrCl-oxidizable mercury forms and species found in an unfiltered aqueous solution. This includes, but is not limited to, Hg(II), Hg(0), strongly organo-complexed Hg(II) compounds, adsorbed particulate Hg, and several tested covalently bound organo-mercurials (e.g., CH_3HgCl, $(CH_3)_2Hg$, and $C_6H_5HgOOCCH_3$). The recovery of Hg bound within microbial cells may require the additional step of UV photo-oxidation. In this Method, total mercury and total recoverable mercury are synonymous.

3.2 Dissolved mercury—all BrCl-oxidizable mercury forms and species found in the filtrate of an aqueous solution that has been filtered through a 0.45-µm filter.

3.3 Apparatus—Throughout this Method, the sample containers, sampling devices, instrumentation, and all other materials and devices used in sample collection, sample processing, and sample analysis that come in contact with the sample and therefore require careful cleaning will be referred to collectively as the Apparatus.

3.4 Definitions of other terms used in this Method are given in the glossary (Section 17.0).

4.0 Contamination and Interferences

4.1 Preventing samples from becoming contaminated during the sampling and analysis process constitutes one of the greatest difficulties encountered in trace metals determinations. Over the last two decades, marine chemists have come to recognize that much of the historical data on the concentrations of dissolved trace metals in seawater are erroneously high because the concentrations reflect contamination from sampling and analysis rather than ambient levels. Therefore, it is imperative that extreme care be taken to avoid contamination when collecting and analyzing samples for trace metals.

4.2 Samples may become contaminated by numerous routes. Potential sources of trace metals contamination during sampling include: metallic or metal-containing labware (e.g., talc gloves that contain high levels of zinc), containers, sampling equipment, reagents, and reagent water; improperly cleaned or stored equipment, labware, and reagents; and atmospheric inputs such as dirt and dust. Even human contact can be a source of trace metals contamination. For example, it has been demonstrated that dental work (e.g., mercury amalgam fillings) in the mouths of laboratory personnel can contaminate samples directly exposed to exhalation (Reference 16.9).

4.3 Contamination Control

4.3.1 Philosophy—The philosophy behind contamination control is to ensure that any object or substance that contacts the sample is metal free and free from any material that may contain mercury.

4.3.1.1 The integrity of the results produced cannot be compromised by contamination of samples. This Method and the Sampling Method give requirements and suggestions for control of sample contamination.

4.3.1.2 Substances in a sample cannot be allowed to contaminate the laboratory work area or instrumentation used for trace metals measurements. This Method gives requirements and suggestions for protecting the laboratory.

4.3.1.3 Although contamination control is essential, personnel health and safety remain the highest priority. The Sampling Method and Section 5 of this Method give suggestions and requirements for personnel safety.

4.3.2 Avoiding contamination—The best way to control contamination is to completely avoid exposure of the sample to contamination in the first place. Avoiding exposure means performing operations in an area known to be free from contamination. Two of the most important factors in avoiding/reducing sample contamination are (1) an awareness of potential sources of contamination and (2) strict attention to work being done. Therefore, it is imperative that the procedures described in this Method be carried out by well-trained, experienced personnel.

4.3.3 Use a clean environment—The ideal environment for processing samples is a class-100 clean room. If a clean room is not available, all sample preparation should be performed in a class-100 clean bench or a nonmetal glove box fed by mercury-and particle-free air or nitrogen. Digestion should be performed in a nonmetal fume hood equipped with HEPA filtration and ideally situated in a clean room.

4.3.4 Minimize exposure—The Apparatus that will contact samples, blanks, or standard solutions should be opened or exposed only in a clean room, clean bench, or glove box so that exposure to an uncontrolled atmosphere is minimized. When not being used, the Apparatus should be covered with clean plastic wrap, stored in the clean bench or in a plastic box or glove box, or bagged in clean zip-type bags. Minimizing the time between cleaning and use will also minimize contamination.

4.3.5 Clean work surfaces—Before a given batch of samples is processed, all work surfaces in the hood, clean bench, or glove box in which the samples will be processed should be cleaned by wiping with a lint-free cloth or wipe soaked with reagent water.

4.3.6 Wear gloves—Sampling personnel must wear clean, non-talc gloves during all operations involving handling of the Apparatus, samples, and blanks. Only clean gloves may touch the Apparatus. If another object or substance is touched, the glove(s) must be changed before again handling the Apparatus. If it is even suspected that gloves have become contaminated, work must be halted, the contaminated gloves removed, and a new pair of clean gloves put on. Wearing multiple layers of clean gloves will allow the old pair to be quickly stripped with minimal disruption to the work activity.

4.3.7 Use metal-free Apparatus—All Apparatus used for determination of mercury at ambient water quality criteria levels must be nonmetallic, free of material that may contain metals, or both.

4.3.7.1 Construction materials—Only fluoropolymer or glass containers must be used for collection of samples that will be analyzed for mercury because mercury vapors can diffuse in or out of other materials, leading to results that are biased low or high. Polyethylene and/or polypropylene labware may be used for digestion and other purposes because the time of sample exposure to these materials is relatively short. All materials, regardless of construction, that will directly or

indirectly contact the sample, must be known to be clean and free of Hg at the levels specified in this Method before proceeding.

4.3.7.2 Serialization—It is recommended that serial numbers be indelibly marked or etched on each piece of reusable Apparatus so that contamination can be traced, and logbooks should be maintained to track the sample from the container through the labware to introduction into the instrument. It may be useful to dedicate separate sets of labware to different sample types; e.g., receiving waters vs. effluents. However, the Apparatus used for processing blanks and standards must be mixed with the Apparatus used to process samples so that contamination of all labware can be detected.

4.3.7.3 The laboratory or cleaning facility is responsible for cleaning the Apparatus used by the sampling team. If there are any indications that the Apparatus is not clean when received by the sampling team (e.g., ripped storage bags), an assessment of the likelihood of contamination must be made. Sampling must not proceed if it is possible that the Apparatus is contaminated. If the Apparatus is contaminated, it must be returned to the laboratory or cleaning facility for proper cleaning before any sampling activity resumes.

4.3.8 Avoid sources of contamination—Avoid contamination by being aware of potential sources and routes of contamination.

4.3.8.1 Contamination by carryover—Contamination may occur when a sample containing a low concentration of mercury is processed immediately after a sample containing a relatively high concentration of mercury. The Hg concentration at which the analytical system (purge, traps, detector) will carry greater than 0.5 ng/L of Hg into a succeeding bubbler or system blank must be determined by analyzing calibration solutions containing successively larger concentrations of Hg. This test must be run prior to first use of the analytical system and whenever a change is made that would increase the amount of carryover. When a sample contains ½ or greater of this determined Hg concentration, a bubbler blank (bubbler system) or system blank (flow injection system) must be analyzed to demonstrate no carryover at the blank criteria level. For the bubbler system, the blank must be run using the same bubbler and sample trap used to run the high concentration sample. Samples analyzed following a sample that has been determined to result in carryover must be reanalyzed. Samples that are known or suspected to contain the lowest concentration of mercury should be analyzed first followed by samples containing higher levels.

4.3.8.2 Contamination by samples—Significant laboratory or instrument contamination may result when untreated effluents, in-process waters, landfill leachates, and other undiluted samples containing concentrations of mercury greater than 100 ng/L are processed and analyzed. Samples known or suspected to contain Hg concentrations greater than 100 ng/L should be diluted prior to bringing them into the clean room or laboratory dedicated for processing trace metals samples.

4.3.8.3 Contamination by indirect contact—Apparatus that may not directly come in contact with the samples may still be a source of contamination. For example, clean tubing placed in a dirty plastic bag may pick up contamination from the bag and subsequently transfer the contamination to the sample. It is imperative that every piece of the Apparatus that is directly or indirectly used in the collection, processing, and analysis of water samples be thoroughly cleaned (Section 6.1.2).

4.3.8.4 Contamination by airborne particulate matter—Less obvious substances capable of contaminating samples include airborne particles. Samples may be contaminated by airborne dust, dirt, particles, or vapors from unfiltered air supplies; nearby corroded or rusted pipes, wires, or other fixtures; or metal-containing paint. Whenever possible, sample processing and analysis should occur as far as possible from sources of airborne contamination.

4.3.8.5 Contamination from reagents— Contamination can be introduced into samples from method reagents used during processing and analysis. Reagent blanks must be analyzed for contamination prior to use (see Section 9.4.3). If reagent blanks are contaminated, a new batch of reagents must be prepared (see Section 9.4.3.2).

4.4 Interferences

4.4.1 At the time of promulgation of this Method, gold and iodide were known interferences. At a mercury concentration of 2.5 ng/L and at increasing iodide concentrations from 30 to 100 mg/L, test data have shown that mercury recovery will be reduced from 100 to 0 percent. At iodide concentrations greater than 3 mg/L, the sample should be pre-reduced with $SnCl_2$ (to remove the brown color) and additional or more concentrated $SnCl_2$ should be added. To preclude loss of Hg, the additional $SnCl_2$ should be added in a closed vessel or analysis should proceed immediately. If samples containing iodide concentrations greater than 30 mg/L are analyzed, it may be necessary to clean the analytical system with 4N HCl after the analysis (Reference 16.10).

4.4.2 The potential exists for destruction of the gold traps if free halogens are purged onto them, or if they are overheated (>500 °C). When the instructions in this Method are followed, neither of these outcomes is likely.

4.4.3 Water vapor may collect in the gold traps and subsequently condense in the fluorescence cell upon desorption, giving a false peak due to scattering of the excitation radiation. Condensation can be avoided by predrying the gold trap. Traps that tend to absorb large quantities of water vapor should not be used.

4.4.4 The fluorescent intensity is strongly dependent upon the presence of molecular species in the carrier gas that can cause "quenching" of the excited atoms. The dual amalgamation technique eliminates quenching due to trace gases, but it remains the laboratory's responsibility to ensure high purity inert carrier gas and a leak-free analytical train.

5.0 Safety

5.1 The toxicity or carcinogenicity of each chemical used in this Method has not been precisely determined; however, each compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

5.1.1 Chronic mercury exposure may cause kidney damage, muscle tremors, spasms, personality changes, depression, irritability and nervousness. Organo-mercurials may cause permanent brain damage. Because of the toxicological and physical properties of Hg, pure standards should be handled only by highly trained personnel thoroughly familiar with handling and cautionary procedures and the associated risks.

5.1.2 It is recommended that the laboratory purchase a dilute standard solution of the Hg in this Method. If primary solutions are prepared, they shall be prepared in a hood, and a NIOSH/MESA-approved toxic gas respirator shall be worn.

5.2 This Method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a current file of OSHA regulations for safe handling of the chemicals specified in this Method. OSHA rules require that a reference file of material safety data sheets (MSDSs) must be made available to all personnel involved in these analyses (29 CFR 1917.28, Appendix E). It also is suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this Method and that the results of this monitoring be made available to the analyst. Personal hygiene monitoring should be performed using OSHA or NIOSH approved personal hygiene monitoring methods. Additional information on laboratory safety can be found in References 16.11-16.14. The references and bibliography included in Reference 16.14 are particularly comprehensive in dealing with the general subject of laboratory safety.

5.3 Samples suspected to contain concentrations of Hg at µg/L or higher levels are handled using essentially the same techniques employed in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. Assistance in evaluating the health hazards of particular laboratory conditions may be obtained from certain consulting laboratories and from State Departments of Health or Labor, many of which have an industrial health service. Each laboratory must develop a safety program for handling Hg.

5.3.1 Facility—When samples known or suspected of containing high concentrations of mercury are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leak-tight or in a fume hood demonstrated to have adequate airflow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in an accident.

5.3.2 Protective equipment—Disposable plastic gloves, apron or lab coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be used. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters.

5.3.3 Training—Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

5.3.4 Personal hygiene—Hands and forearms should be washed thoroughly after each manipulation and before breaks (coffee, lunch, and shift).

5.3.5 Confinement—Isolated work areas posted with signs, segregated glassware and tools, and plastic absorbent paper on bench tops will aid in confining contamination.

5.3.6 Effluent vapors—The effluent from the CVAFS should pass through either a column of activated charcoal or a trap containing gold or sulfur to amalgamate or react mercury vapors.

5.3.7 Waste handling—Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel must be trained in the safe handling of waste.

5.3.8 Decontamination

5.3.8.1 Decontamination of personnel—Use any mild soap with plenty of scrubbing action.

5.3.8.2 Glassware, tools, and surfaces—Sulfur powder will react with Hg to produce mercuric sulfide, thereby eliminating the possible volatilization of Hg. Satisfactory cleaning may be accomplished by dusting a surface lightly with sulfur powder, then washing with any detergent and water.

5.3.9 Laundry—Clothing known to be contaminated should be collected in plastic bags. Persons that convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. If the launderer knows of the potential problem, the clothing may be put into a washer without contact. The washer should be run through a cycle before being used again for other clothing.

5.3.10 Wipe tests—A useful method of determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper. Extraction and analysis by this Method can achieve a limit of detection of less than 1 ng per wipe. Less than 0.1 µg per wipe indicates acceptable cleanliness; anything higher warrants further cleaning. More than 10 µg on a wipe constitutes an acute hazard and requires prompt cleaning before further use of the equipment or work space, and indicates that unacceptable work practices have been employed.

6.0 Apparatus and Materials

Disclaimer: The mention of trade names or commercial products in this Method is for illustrative purposes only and does not constitute endorsement or recommendation for use by the Environmental Protection Agency. Equivalent performance may be achievable using apparatus, materials, or cleaning procedures other than those suggested here. The laboratory is responsible for demonstrating equivalent performance.

6.1 Sampling equipment

6.1.1 Sample collection bottles-fluoropolymer or glass, 125- to 1000-mL, with fluoropolymer or fluoropolymer-lined cap.

6.1.2 Cleaning

6.1.2.1 New bottles are cleaned by heating to 65–75 °C in 4 N HCl or concentrated HNO_3 for at least 48 h. The bottles are cooled, rinsed three times with reagent water, and filled with reagent water containing 1% HCl. These bottles are capped and placed in a clean oven at 60-70°C overnight. After cooling, they are rinsed three more times with reagent water, filled with reagent water containing 0.4% (v/v) HCl, and placed in a mercury-free Class-100 clean bench until the outside surfaces are dry. The bottles are tightly capped (with a wrench), double-bagged in new polyethylene zip-type bags until needed, and stored in wooden or plastic boxes until use. The bottles may be shipped to the sampling site containing dilute HCl solution (e.g., 0.04%), containing reagent water, or empty.

6.1.2.2 Used bottles known not to have contained mercury at high (>100 ng/L) levels are cleaned as above, except for only 6–12 h in hot 4 N HCl.

6.1.2.3 Bottle blanks must be analyzed as described in Section 9.4.7. To verify the effectiveness of the cleaning procedures, bottle blanks must be demonstrated to be free of mercury at the ML of this Method.

6.1.2.4 As an alternative to cleaning by the laboratory, bottles may be purchased from a commercial supplier and each lot certified to be clean. Bottles from the lot must be tested as bottle blanks (Section 9.4.7) and demonstrated to be free of mercury at the ML of this Method. If mercury is present above this level in any bottle, either the lot must be rejected or the bottles must be re-cleaned.

6.1.3 Filtration Apparatus

6.1.3.1 Filter—0.45-µm, 15-mm diameter capsule filter (Gelman Supor 12175, or equivalent)

6.1.3.2 Peristaltic pump—115-V a.c., 12-V d.c., internal battery, variable-speed, single-head (Cole-Parmer, portable, "Masterflex L/S," Catalog No. 07570-10 drive with Quick Load pump head, Catalog No. 07021-24, or equivalent).

6.1.3.3 Tubing—styrene/ethylene/butylene/silicone (SEBS) resin for use with peristaltic pump, approx 3/8-in ID by approximately 3 ft (Cole-Parmer size 18, Catalog No. 06424-18, or approximately 1/4-in OD, Cole-Parmer size 17, Catalog No. 06424-17, or equivalent). Tubing is cleaned by soaking in 5–10% HCl solution for 8–24 h, rinsing with reagent water in a clean bench in a clean room, and drying in the clean bench by purging with metal-free air or nitrogen. After drying, the tubing is double-bagged in clear polyethylene bags, serialized with a unique number, and stored until use.

6.2 Equipment for bottle and glassware cleaning

6.2.1 Vat, 100–200 L, high-density polyethylene (HDPE), half filled with 4 N HCl in reagent water.

6.2.2 Panel immersion heater, 500-W, all-fluoropolymer coated, 120 vac (Cole-Parmer H-03053-04, or equivalent)

WARNING: *Read instructions carefully!! The heater will maintain steady state, without temperature feedback control, of 60–75°C in a vat of the size described. However, the equilibrium temperature will be higher (up to boiling) in a smaller vat. Also, the heater plate MUST be maintained in a vertical position, completely submerged and away from the vat walls to avoid melting the vat or burning out!*

6.2.3 Laboratory sink—in Class-100 clean area, with high-flow reagent water (Section 7.1) for rinsing.

6.2.4 Clean bench—Class-100, for drying rinsed bottles.

6.2.5 Oven—stainless steel, in Class-100 clean area, capable of maintaining ± 5°C in the 60–70°C temperature range.

6.3 Cold vapor atomic fluorescence spectrometer (CVAFS): The CVAFS system used may either be purchased from a supplier, or built in the laboratory from commercially available components.

6.3.1 Commercially available CVAFS—Tekran (Toronto, ON) Series 2600 CVAFS, Brooks-Rand (Seattle, WA) Model III CVAFS, Leeman Labs Hydra AF Gold*plus* CVAFS, or equivalent

6.3.2 Custom-built CVAFS (Reference 16.15). Figure 2 shows the schematic diagram. The system consists of the following:

6.3.2.1 Low-pressure 4-W mercury vapor lamp

6.3.2.2 Far UV quartz flow-through fluorescence cell—12 mm x 12 mm x 45 mm, with a 10-mm path length (NSG Cells, or equivalent).

6.3.2.3 UV-visible photomultiplier (PMT)—sensitive to < 230 nm. This PMT is isolated from outside light with a 253.7-nm interference filter (Oriel Corp., Stamford, CT, or equivalent).

6.3.2.4 Photometer and PMT power supply (Oriel Corp. or equivalent), to convert PMT output (nanoamp) to millivolts

6.3.2.5 Black anodized aluminum optical block—holds fluorescence cell, PMT, and light source at perpendicular angles, and provides collimation of incident and fluorescent beams (Frontier Geosciences Inc., Seattle, WA, or equivalent).

6.3.2.6 Flowmeter—with needle valve capable of reproducibly keeping the carrier gas flow rate at 30 mL/min

6.4 Hg purging system—Figure 2 shows the schematic diagram for the purging system. The system consists of the following:

6.4.1 Flow meter/needle valve—capable of controlling and measuring gas flow rate to the purge vessel at 350 ± 50 mL/min.

6.4.2 Fluoropolymer fittings—connections between components and columns are made using 6.4-mm OD fluoropolymer tubing and fluoropolymer friction-fit or threaded tubing connectors. Connections between components requiring mobility are made with 3.2-mm OD fluoropolymer tubing because of its greater flexibility.

6.4.3 Acid fume pretrap—10-cm long x 0.9-cm ID fluoropolymer tube containing 2–3 g of reagent grade, nonindicating, 8–14 mesh soda lime chunks, packed between wads of silanized glass wool. This trap is cleaned of Hg by placing on the output of a clean cold vapor generator (bubbler) and purging for 1 h with N_2 at 350 mL/min.

6.4.4 Cold vapor generator (bubbler)—200-mL borosilicate glass (15 cm high x 5.0 cm diameter) with standard taper 24/40 neck, fitted with a sparging stopper having a coarse glass frit that extends to within 0.2 cm of the bubbler bottom (Frontier Geosciences, Inc. or equivalent).

6.5 The dual-trap Hg(0) preconcentrating system

6.5.1 Figures 2 and 3 show the dual-trap amalgamation system (Reference 16.5).

6.5.2 Gold-coated sand traps—10-cm long x 6.5-mm OD x 4-mm ID quartz tubing. The tube is filled with 3.4 cm of gold-coated 45/60 mesh quartz sand (Frontier Geosciences Inc., Seattle, WA, or equivalent). The ends are plugged with quartz wool.

6.5.2.1 Traps are fitted with 6.5-mm ID fluoropolymer friction-fit sleeves for making connection to the system. When traps are not in use, fluoropolymer end plugs are inserted in trap ends to eliminate contamination.

6.5.2.2 At least six traps are needed for efficient operation, one as the "analytical" trap, and the others to sequentially collect samples.

6.5.3 Heating of gold-coated sand traps—To desorb Hg collected on a trap, heat for 3.0 min to 450–500 °C (a barely visible red glow when the room is darkened) with a coil consisting of 75 cm of 24-gauge Nichrome wire at a potential of 10-14 vac. Potential is applied and finely adjusted with an autotransformer.

6.5.4 Timers—The heating interval is controlled by a timer-activated 120-V outlet (Gralab, or equivalent), into which the heating coil autotransformer is plugged. Two timers are required, one each for the "sample" trap and the "analytical" trap.

6.5.5 Air blowers—After heating, traps are cooled by blowing air from a small squirrel-cage blower positioned immediately above the trap. Two blowers are required, one each for the "sample" trap and the "analytical" trap.

6.6 Recorder—Any multi-range millivolt chart recorder or integrator with a range compatible with the CVAFS is acceptable. By using a two-pen recorder with pen sensitivity offset by a factor of 10, the dynamic range of the system is extended to 10^3.

6.7 Pipettors—All-plastic pneumatic fixed-volume and variable pipettors in the range of 10 µL to 5.0 mL.

6.8 Analytical balance capable of weighing to the nearest 0.01 g

7.0 Reagents and Standards

Note: The quantities of reagents and the preparation procedures in this section are for illustrative purposes. Equivalent performance may be achievable using quantities of reagents and procedures other than those suggested here. The laboratory is responsible for demonstrating equivalent performance.

7.1 Reagent water—18-MΩ minimum, ultrapure deionized water starting from a prepurified (distilled, reverse osmosis, etc.) source. Water should be monitored for Hg, especially after ion exchange beds are changed.

7.2 Air—It is very important that the laboratory air be low in both particulate and gaseous mercury. Ideally, mercury work should be conducted in a new laboratory with mercury-free paint on the walls. A source of air that is very low in Hg should be brought directly into the Class-100 clean bench air intake. If this is not possible, air coming into the clean bench can be cleaned for mercury by placing a gold-coated cloth prefilter over the intake. Gold-coated cloth filter: Soak 2 m^2 of cotton gauze in 500 mL of 2% gold chloride solution at pH 7. In a hood, add 100 mL of 30% $NH_2OH{\cdot}HCl$ solution, and homogenize into the cloth with gloved hands. The material will turn black as colloidal gold is precipitated. Allow the mixture to set for several hours, then rinse

with copious amounts of deionized water. Squeeze-dry the rinsed cloth, and spread flat on newspapers to air-dry. When dry, fold and place over the intake prefilter of the laminar flow hood.

CAUTION: Great care should be taken to avoid contaminating the laboratory with gold dust. This could cause interferences with the analysis if gold becomes incorporated into the samples or equipment. The gilding procedure should be done in a remote laboratory if at all possible.

7.3 Hydrochloric acid—trace-metal purified reagent-grade HCl containing less than 5 pg/mL Hg. The HCl should be analyzed for Hg before use.

7.4 Hydroxylamine hydrochloride—Dissolve 300 g of $NH_2OH{\cdot}HCl$ in reagent water and bring to 1.0 L. This solution may be purified by the addition of 1.0 mL of $SnCl_2$ solution and purging overnight at 500 mL/min with Hg-free N_2. Flow injection systems may require the use of less $SnCl_2$ for purification of this solution.

7.5 Stannous chloride—Bring 200 g of $SnCl_2{\cdot}2H_2O$ and 100 mL concentrated HCl to 1.0 L with reagent water. Purge overnight with mercury-free N_2 at 500 mL/min to remove all traces of Hg. Store tightly capped.

7.6 Bromine monochloride (BrCl)—In a fume hood, dissolve 27 g of reagent grade KBr in 2.5 L of low-Hg HCl. Place a clean magnetic stir bar in the bottle and stir for approximately 1 h in the fume hood. Slowly add 38 g reagent grade $KBrO_3$ to the acid while stirring. When all of the $KBrO_3$ has been added, the solution color should change from yellow to red to orange. Loosely cap the bottle, and allow to stir another hour before tightening the lid.

WARNING: *This process generates copious quantities of free halogens (Cl_2, Br_2, BrCl), which are released from the bottle. Add the $KBrO_3$ slowly in a fume hood!*

7.7 Stock mercury standard—NIST-certified 10,000-ppm aqueous Hg solution (NIST-3133). This solution is stable at least until the NIST expiration date.

7.8 Secondary Hg standard—Add approx 0.5 L of reagent water and 5 mL of BrCl solution (Section 7.6) to a 1.00-L Class A volumetric flask. Add 0.100 mL of the stock mercury standard (Section 7.7) to the flask and dilute to 1.00 L with reagent water. This solution contains 1.00 µg/mL (1.00 ppm) Hg. Transfer the solution to a fluoropolymer bottle and cap tightly. This solution is considered stable until the NIST expiration date.

7.9 Working Hg Standard A—Dilute 1.00 mL of the secondary Hg standard (Section 7.8) to 100 mL in a Class A volumetric flask with reagent water containing 0.5% by volume BrCl solution (Section 7.6). This solution contains 10.0 ng/mL and should be replaced monthly, or longer if extended stability is demonstrated.

7.10 Working Hg Standard B—Dilute 0.10 mL of the secondary Hg standard (Section 7.8) to 1000 mL in a Class A volumetric flask with reagent water containing 0.5% by volume BrCl solution (Section 7.6). This solution contains 0.10 ng/mL and should be replaced monthly, or longer if extended stability is demonstrated.

7.11 Initial Precision and Recovery (IPR) and Ongoing Precision and Recovery (OPR) solutions—Using the working Hg standard A (Section 7.9), prepare IPR and OPR solutions at a

concentration of 5 ng/L Hg in reagent water. IPR/OPR solutions are prepared using the same amounts of reagents used for preparation of the calibration standards.

7.12 Nitrogen—Grade 4.5 (standard laboratory grade) nitrogen that has been further purified by the removal of Hg using a gold-coated sand trap.

7.13 Argon—Grade 5.0 (ultra high-purity, GC grade) argon that has been further purified by the removal of Hg using a gold-coated sand trap.

8.0 Sample Collection, Preservation, and Storage

8.1 Before samples are collected, consideration should be given to the type of data required (i.e., dissolved or total), so that appropriate preservation and pretreatment steps can be taken. An excess of BrCl should be confirmed either visually (presence of a yellow color) or with starch iodide indicating paper, using a separate sample aliquot, prior to sample processing or direct analysis to ensure the sample has been properly preserved.

8.2 Samples are collected into rigorously cleaned fluoropolymer bottles with fluoropolymer or fluoropolymer-lined caps. Glass bottles may be used if Hg is the only target analyte. It is critical that the bottles have tightly sealing caps to avoid diffusion of atmospheric Hg through the threads (Reference 16.4). Polyethylene sample bottles must not be used (Reference 16.15).

8.3 Collect samples using guidance provided in the Sampling Method (Reference 16.9). Procedures in the Sampling Method are based on rigorous protocols for collection of samples for mercury (References 16.4 and 16.15).

NOTE: *Discrete samplers have been found to contaminate samples with Hg at the ng/L level. Therefore, great care should be exercised if this type of sampler is used. It may be necessary for the sampling team to use other means of sample collection if samples are found to be contaminated using the discrete sampler.*

8.4 Sample filtration—For dissolved Hg, a sample is filtered through a 0.45-µm capsule filter (Section 6.1.3.1) in a mercury-free clean area prior to preservation. If the sample is filtered, it must be accompanied by a blank that has been filtered under the same conditions. The Sampling Method describes sample filtration procedures.

8.5 Preservation—Samples are preserved by adding either 5 mL/L of pretested 12N HCl or 5 mL/L BrCl solution to the sample bottle. If a sample will be used also for the determination of methyl mercury, it should be collected and preserved according to procedures in the method that will be used for determination of methyl mercury (e.g., HCl or H_2SO_4 solution). Preserved samples are stable for up to 90 days of the date of collection.

8.5.1 Samples to be analyzed for total or dissolved Hg only may be shipped to the laboratory unpreserved and unrefrigerated if they are collected in fluoropolymer or glass bottles and capped tightly. Samples must be either preserved or analyzed within 48 hours of collection. If a sample is oxidized in the sample bottle, the time to preservation can be extended to 28 days.

8.5.2 Samples that are acid-preserved may lose Hg to coagulated organic materials in the water or condensed on the walls (Reference 16.16). The best approach is to add BrCl directly to the sample bottle at least 24 hours before analysis. If other Hg species are to be analyzed, these aliquots must be removed prior to the addition of BrCl. If BrCl

cannot be added directly to the sample bottle, the bottle must be shaken vigorously prior to sub-sampling.

8.5.3 Handling of the samples in the laboratory should be undertaken in a mercury-free clean bench, after rinsing the outside of the bottles with reagent water and drying in the clean air hood.

NOTE: *Because of the potential for contamination, it is recommended that filtration and preservation of samples be performed in the clean room in the laboratory. However, if circumstances prevent overnight shipment of samples, samples should be filtered and preserved in a designated clean area in the field in accordance with the procedures given in Method 1669 (Reference 16.9). If filtered in the field, samples ideally should be filtered into the sample bottle.*

8.6 Storage—Sample bottles should be stored in clean (new) polyethylene bags until sample analysis.

8.7 Sample preservation, storage, and holding time requirements also are given at 40 CFR part 136.3(e) Table II.

9.0 Quality Control

9.1 Each laboratory that uses this Method is required to operate a formal quality assurance program (Reference 16.17). The minimum requirements of this program consist of an initial demonstration of laboratory capability, ongoing analysis of standards and blanks as a test of continued performance, and the analysis of matrix spikes (MS) and matrix spike duplicates (MSD) to assess precision and recovery. Laboratory performance is compared to established performance criteria to determine that the results of analyses meet the performance characteristics of the Method.

9.1.1 The laboratory shall make an initial demonstration of the ability to generate acceptable accuracy and precision. This ability is established as described in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, the laboratory is permitted certain options to improve results or lower the cost of measurements. These options include automation of the dual-amalgamation system, single-trap amalgamation (Reference 16.18), direct electronic data acquisition, calibration using gas-phase elemental Hg standards, use of the bubbler or flow-injection systems, or changes in the detector (i.e., CVAAS) when less sensitivity is acceptable or desired. Changes in the determinative technique, such as the use of colorimetry, are not allowed. If an analytical technique other than the CVAFS technique specified in this Method is used, that technique must have a specificity for mercury equal to or better than the specificity of the technique in this Method.

9.1.2.1 Each time this Method is modified, the laboratory is required to repeat the procedure in Section 9.2 to demonstrate that an MDL (40 CFR part 136, Appendix B) less than or equal to one-third the regulatory compliance limit or less than or equal to the MDL of this Method (Table 1), whichever is greater, can be achieved. If the change will affect calibration, the instrument must be recalibrated according to Section 10.

Note: If the compliance limit is greater than the concentration of Hg in the OPR/OPR (5 ng/L), the acceptance criteria for blanks and the concentrations of mercury spiked into quality control samples may be increased to support measurements at the compliance limit. For example, if the compliance limit is 12

ng/L (National Toxics Rule, 40 CFR 131.36), the MDL must be less than or equal to 4 ng/L; concentrations of the calibration standards may be 5, 10, 20, 50 , and 100 ng/L; concentrations of the IPR/OPR samples may be 10 ng/L; spike concentrations and acceptance criteria for MS/MSD samples would remain as specified in Section 9.3; and an appropriate blank acceptance criterion would be 5 ng/L.

9.1.2.2 The laboratory is required to maintain records of modifications made to this Method. These records include the following, at a minimum:

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and the quality control officer who witnessed and will verify the analyses and modification

9.1.2.2.2 A narrative stating the reason(s) for the modification(s)

9.1.2.2.3 Results from all quality control (QC) tests demonstrating the performance of the modified method, including the following:

(a) Calibration (Section 10)
(b) Initial precision and recovery (Section 9.2.2)
(c) Analysis of blanks (Section 9.4)
(d) Matrix spike/matrix spike duplicate analysis (Section 9.3)
(e) Ongoing precision and recovery (Section 9.5)
(f) Quality control sample (Section 9.6)
(g) Method detection limit (Section 9.2.1)

9.1.2.2.4 Data that will allow an independent reviewer to validate each determination by tracking the instrument output to the final result. These data are to include the following:

(a) Sample numbers and other identifiers
(b) Processing dates
(c) Analysis dates
(d) Analysis sequence/run chronology
(e) Sample weight or volume
(f) Copies of logbooks, chart recorder, or other raw data output
(g) Calculations linking raw data to the results reported

9.1.3 Analyses of MS and MSD samples are required to demonstrate the accuracy and precision and to monitor matrix interferences. Section 9.3 describes the procedure and QC criteria for spiking.

9.1.4 Analyses of blanks are required to demonstrate acceptable levels of contamination. Section 9.4 describes the procedures and criteria for analyzing blanks.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through analysis of the ongoing precision and recovery (OPR) sample and the quality control sample (QCS) that the system is in control. Sections 9.5 and 9.6 describe these procedures, respectively.

9.1.6 The laboratory shall maintain records to define the quality of the data that are generated. Sections 9.3.7 and 9.5.3 describe the development of accuracy statements.

9.1.7 Quality of the analyses is controlled by an analytical batch. An analytical batch is a set of samples oxidized with the same batch of reagents, and analyzed during the same 12-hour shift. A batch may be from 1 to as many as 20 samples. Each batch must be accompanied by 3 system blanks (Section 9.4.2 for the flow-injection system), a

minimum of 3 bubbler blanks (Section 9.4.1 for the bubbler system), 1 OPR sample at the beginning and end of the batch (Section 9.5), a QCS (Section 9.6), and at least 3 method blanks (Section 9.4.4). In addition, there must be 1 MS and 1 MSD sample for every 10 samples (a frequency of 10%). A typical analytical sequence would be:

(a) Three system blanks (Section 9.4.2) or a minimum of 3 bubbler blanks (Section 9.4.1)
(b) A minimum of five, non-zero calibration standards (Section 10.2.2.1)
(c) On-going precision and recovery (Section 9.5)
(d) Quality control sample (Section 9.6)
(e) Method blank (Section 9.4.4)
(f) Seven samples
(g) Method blank (Section 9.4.4)
(h) Three samples
(i) Matrix spike (Section 9.3)
(j) Matrix spike duplicate (Section 9.3)
(k) Four samples
(l) Method blank (Section 9.4.4)
(m) Six samples
(n) Matrix spike (Section 9.3)
(o) Matrix spike duplicate (Section 9.3)
(p) Ongoing precision and recovery (Section 9.5)

The above sequence includes calibration. If system performance is verified at the end of the sequence using the OPR, analysis of samples and blanks may proceed without recalibration (i.e., the analytical sequence would be entered at Step (d) above), unless more than 12 hours has elapsed since verification of system performance. If more than 12 hours has elapsed, the sequence would be initiated at Step (c) above.

9.2 Initial demonstration of laboratory capability

9.2.1 Method detection limit—To establish the ability to detect Hg, the laboratory shall achieve an MDL that is less than or equal to the MDL listed in Section 1.5 or one-third the regulatory compliance limit, whichever is greater. The MDL shall be determined according to the procedure at 40 CFR 136, Appendix B using the apparatus, reagents, and standards that will be used in the practice of this Method. This MDL shall be used for determination of laboratory capability only, and should be determined when a new operator begins work or whenever, in the judgment of the laboratory, a change in instrument hardware or operating conditions would dictate reevaluation of capability.

9.2.2 Initial precision and recovery (IPR)—To establish the ability to generate acceptable precision and recovery, the laboratory shall perform the following operations:

9.2.2.1 Analyze four replicates of the IPR solution (5 ng/L, Section 7.11) according to the procedure beginning in Section 11.

9.2.2.2 Using the results of the set of four analyses, compute the average percent recovery (X), and the standard deviation of the percent recovery (s) for Hg.

9.2.2.3 Compare s and X with the corresponding limits for initial precision and recovery in Table 2. If s and X meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If, however, s exceeds the

precision limit or X falls outside the acceptance range, system performance is unacceptable. Correct the problem and repeat the test (Section 9.2.2.1).

9.3 Matrix spike (MS) and matrix spike duplicate (MSD)—To assess the performance of the Method on a given sample matrix, the laboratory must spike, in duplicate, a minimum of 10% (1 sample in 10) from a given sampling site or, if for compliance monitoring, from a given discharge. Therefore, an analytical batch of 20 samples would require two pairs of MS/MSD samples (four spiked samples total).

9.3.1 The concentration of the spike in the sample shall be determined as follows:

9.3.1.1 If, as in compliance monitoring, the concentration of Hg in the sample is being checked against a regulatory compliance limit, the spiking level shall be at that limit or at 1–5 times the background concentration of the sample (as determined in Section 9.3.2), whichever is greater.

9.3.1.2 If the concentration of Hg in a sample is not being checked against a limit, the spike shall be at 1–5 times the background concentration or at 1-5 times the ML in Table 1, whichever is greater.

9.3.2 To determine the background concentration (B), analyze one sample aliquot from each set of 10 samples from each site or discharge according to the procedure in Section 11. If the expected background concentration is known from previous experience or other knowledge, the spiking level may be established *a priori*.

9.3.2.1 If necessary, prepare a standard solution to produce an appropriate level in the sample (Section 9.3.1).

9.3.2.2 Spike two additional sample aliquots with identical amounts of the spiking solution and analyze these aliquots as described in Section 11.1.2 to determine the concentration after spiking (A).

9.3.3 Calculate the percent recovery (R) in each aliquot using the following equation:

$$\%\ R = 100\ \frac{(A-B)}{T}$$

where:

A = Measured concentration of analyte after spiking
B = Measured concentration of analyte before spiking
T = True concentration of the spike

9.3.4 Compare percent recovery (R) with the QC acceptance criteria in Table 2.

9.3.4.1 If results of the MS/MSD are similar and fail the acceptance criteria, and recovery for the OPR standard (Section 9.5) for the analytical batch is within the acceptance criteria in Table 2, an interference is present and the results may not be reported or otherwise used for permitting or regulatory compliance purposes. If the interference can be attributed to sampling, the site or discharge should be resampled. If the interference can be attributed to a method deficiency, the laboratory must modify the method, repeat the test required in Section 9.1.2, and repeat analysis of the sample and MS/MSD. However, during the development

of Method 1631, very few interferences have been noted in the determination of Hg using this Method. (See Section 4.4 for information on interferences.)

9.3.4.2 If the results of both the spike and the OPR test fall outside the acceptance criteria, the analytical system is judged to be not in control, and the results may not be reported or used for permitting or regulatory compliance purposes. The laboratory must identify and correct the problem and reanalyze all samples in the sample batch.

9.3.5 Relative percent difference (RPD)—Compute the RPD between the MS and MSD results according to the following equation using the concentrations found in the MS and MSD. Do not use the recoveries calculated in Section 9.3.3 for this calculation because the RPD is inflated when the background concentration is near the spike concentration.

$$RPD = 200 \times \frac{(|D1-D2|)}{(D1+D2)}$$

Where:
D1 = concentration of Hg in the MS sample
D2 = concentration of Hg in the MSD sample

9.3.6 The RPD for the MS/MSD pair must not exceed the acceptance criterion in Table 2. If the criterion is not met, the system is judged to be out of control. The problem must be identified and corrected, and the MS/MSD and corresponding samples reanalyzed.

9.3.7 As part of the QC program for the laboratory, method precision and recovery for samples should be assessed and records maintained. After analyzing five samples in which the recovery passes the test in Section 9.3.4, compute the average percent recovery (R_a) and the standard deviation of the percent recovery (s_r). Express the accuracy assessment as a percent recovery interval from $R_a - 2s_r$ to $R_a + 2s_r$. For example, if $R_a = 90\%$ and $s_r = 10\%$ for five analyses, the accuracy interval is expressed as 70–110%. Update the accuracy assessment regularly (e.g., after every five to ten new accuracy measurements).

9.4 Blanks—Blanks are critical to the reliable determination of Hg at low levels. The sections below give the minimum requirements for analysis of blanks. Analysis of additional blanks is recommended as necessary to pinpoint sources of contamination in, and external to, the laboratory.

9.4.1 Bubbler blanks—Bubbler blanks are analyzed to demonstrate that bubbler systems are free from contamination at levels that could affect data quality. At least three bubbler blanks must be run during calibration and with each analytical batch.

9.4.1.1 To analyze a bubbler blank, place a clean gold trap on the bubbler. Purge and analyze previously purged water using the procedure in Section 11, and determine the amount of Hg remaining in the system.

9.4.1.2 If the bubbler blank is found to contain more than 50 pg Hg, the system is out of control. The problem must be investigated and remedied, and the samples run on that bubbler must be reanalyzed. If the blanks from other bubblers contain less than 50 pg Hg, the data associated with those bubblers remain valid, provided that all other criteria in Section 9 also are met.

9.4.1.3 The mean result for all bubbler blanks (from bubblers passing the specification in Section 9.4.1.2) must be < 25 pg (0.25 ng/L) Hg with a standard deviation (n-1) of <10 pg (0.10 ng/L). If the mean is < 25 pg, the average peak area or height is subtracted from all raw data before results are calculated (Section 12.2).

9.4.1.4 If Hg in the bubbler blank exceeds the acceptance criteria in Section 9.4.1.3, the system is out of control. The problem must be resolved and the system recalibrated. Usually, the bubbler blank is too high for one of the following reasons:

(a) Bubblers need rigorous cleaning;
(b) Soda-lime is contaminated; or
(c) Carrier gas is contaminated.

9.4.2 System blanks— System blanks are analyzed to demonstrate that flow injection systems are free from contamination at levels that could affect data quality. Three system blanks must be run during calibration and with each analytical batch.

9.4.2.1 To analyze a system blank, analyze reagent water containing the same amount of reagents used to prepare the calibration standards.

9.4.2.2 If a system blank is found to contain ≥ 0.50 ng/L Hg, the system is out of control. The problem must be investigated and remedied, and the system recalibrated. If the blanks contain < 0.50 ng/L Hg, the data associated with the blanks remain valid, provided that all other criteria in Section 9 also are met.

9.4.2.3 The mean result for the three system blanks must be <0.5 ng/L Hg with a standard deviation (n-1) <0.1 ng/L. If the mean exceeds these criteria, the system is out of control, and the problem must be resolved and the system recalibrated. If the mean is <0.5 ng/L, the average peak height or area is subtracted from all raw data before results are calculated (Section 12.3).

9.4.3 Reagent blanks—Reagent blanks are used to demonstrate that the reagents used to prepare samples for Hg analyses are free from contamination. The Hg concentration in reagent blanks is determined by analyzing the reagent solutions using either the bubbler or flow-injection system. For the bubbler system, reagent may be added directly to previously purged water in the bubbler.

9.4.3.1 Reagent blanks are required when the batch of reagents (bromine monochloride plus hydroxylamine hydrochloride) are prepared. The amount of Hg in a reagent blank containing 0.5% (v/v) BrCl solution (Section 7.6) and 0.2% (v/v) hydroxylamine hydrochloride solution (Section 7.4) must be < 20 pg (0.2 ng/L).

9.4.3.2 The presence of more than 20 pg (0.2 ng/L) of Hg indicates a problem with the reagent solution. The purging of certain reagent solutions, such as $SnCl_2$ or NH_2OH, with mercury-free nitrogen or argon can reduce Hg to acceptable levels. Because BrCl cannot be purified, a new batch must be prepared and tested if the BrCl is contaminated.

9.4.4 Method blanks— Method blanks are used to demonstrate that the analytical system is free from contamination that could otherwise compromise sample results. Method blanks are prepared and analyzed using sample containers, labware, reagents, and analytical procedures identical to those used to prepare and analyze the samples.

9.4.4.1 A minimum of three method blanks per analytical batch are required for both the bubbler and flow-injection systems.

9.4.4.2 If the result for any method blank containing the nominal amount of reagent used to prepare a sample (Section 11.1.1) is found to contain ≥ 0.50 ng/L (50 pg) Hg, the system is out of control. Mercury in the analytical system must be reduced until a method blank is free from contamination at the 0.50 ng/L level. Samples associated with a contaminated method blank must be reanalyzed.

9.4.4.3 Because method blanks are analyzed using procedures identical to those used to analyze samples, any sample requiring an increased amount of reagent must be accompanied by at least one method blank that includes an identical amount of reagent.

9.4.5 Field blanks–Field blanks are used to demonstrate that samples have not been contaminated by the sample collection and transport activities.

9.4.5.1 Analyze the field blank(s) shipped with each set of samples (samples collected from the same site at the same time, to a maximum of 10 samples). Analyze the blank immediately before analyzing the samples in the batch.

9.4.5.2 If Hg or any potentially interfering substance is found in the field blank at a concentration equal to or greater than the ML (Table 1), or greater than one-fifth the level in the associated sample, whichever is greater, results for associated samples may be the result of contamination and may not be reported or otherwise used for regulatory compliance purposes.

9.4.5.3 Alternatively, if sufficient multiple field blanks (a minimum of three) are collected, and the average concentration (of the multiple field blanks) plus two standard deviations is equal to or greater than the regulatory compliance limit or equal to or greater than one-half of the level in the associated sample, results for associated samples may be the result of contamination and may not be reported or otherwise used for regulatory compliance purposes.

9.4.5.4 If contamination of the field blanks and associated samples is known or suspected, the laboratory should communicate this to the sampling team so that the source of contamination can be identified and corrective measures taken before the next sampling event.

9.4.6 Equipment blanks—Before any sampling equipment is used at a given site, the laboratory or cleaning facility is required to generate equipment blanks on all sampling equipment that will be used to demonstrate that the sampling equipment is free from contamination.

9.4.6.1 Equipment blanks are generated in the laboratory or at the equipment cleaning facility by processing reagent water through the sampling devices using the same procedures that are used in the field (see Sampling Method). Therefore, the "clean hands/dirty hands" technique used during field sampling should be followed when preparing equipment blanks at the laboratory or cleaning facility for low level mercury measurements. If grab samples are to be collected using any ancillary equipment, e.g., an extension pole or a dipper, an equipment blank

is generated by submersing this equipment into the reagent water and analyzing the resulting reagent water collected.

9.4.6.2 The equipment blank must be analyzed using the procedures in this Method. If mercury or any potentially interfering substance is detected in the blank at or above the level specified for the field blank (Section 9.4.5), the source of contamination or interference must be identified, and the problem corrected. The equipment must be demonstrated to be free from mercury and interferences before the equipment may be used in the field.

9.4.7 Bottle blanks— Bottles must be subjected to conditions of use to verify the effectiveness of the cleaning procedures. A representative set of sample bottles (Section 6.1.2) should be filled with reagent water acidified to pH <2 and allowed to stand for a minimum of 24 h. At least 5% of the bottles from a given lot should be tested, and the time that the bottles are allowed to stand should be as close as possible to the actual time that the sample will be in contact with the bottle. After standing, the water must be analyzed for any signs of contamination. If a bottle shows contamination at or above the level specified for the field blank (Section 9.4.5), the problem must be identified, the cleaning procedures corrected or cleaning solutions changed, and all affected bottles re-cleaned.

9.5 Ongoing precision and recovery (OPR)—To demonstrate that the analytical system is within the performance criteria of this Method and that acceptable precision and recovery is being maintained within each analytical batch, the laboratory shall perform the following operations:

9.5.1 Analyze the OPR solution (5 ng/L, Section 7.11) prior to the analysis of each analytical batch according to the procedure beginning in Section 11. An OPR also must be analyzed at the end of an analytical sequence or at the end of each 12-hour shift.

9.5.2 Compare the recovery with the limits for ongoing precision and recovery in Table 2. If the recovery is in the range specified, the analytical system is in control and analysis of samples and blanks may proceed. If, however, the concentration is not in the specified range, the analytical process is not in control. Correct the problem and repeat the ongoing precision and recovery test. All reported results must be associated with an OPR that meets the Table 2 performance criteria at the beginning and end of each batch.

9.5.3 The laboratory should add results that pass the specification in Section 9.5.2 to IPR and previous OPR data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory also should develop a statement of laboratory data quality by calculating the average percent recovery (R_a) and the standard deviation of the percent recovery (s_r). Express the accuracy as a recovery interval from $R_a - 2s_r$ to $R_a + 2s_r$. For example, if $R_a = 95\%$ and $s_r = 5\%$, the accuracy is 85–105%.

9.6 Quality control sample (QCS) – The laboratory must obtain a QCS from a source different from the Hg used to produce the standards used routinely in this Method (Sections 7.7–7.10). The QCS should be analyzed as an independent check of system performance.

9.7 Depending on specific program requirements, the laboratory may be required to analyze field duplicates and field spikes collected to assess the precision and accuracy of the sampling, sample transportation, and storage techniques. The relative percent difference (RPD) between field duplicates should be less than 20%. If the RPD of the field duplicates exceeds 20%, the laboratory should communicate this to the sampling team so that the source of error can be identified and corrective measures taken before the next sampling event.

10.0 Calibration and Standardization

10.1 Calibration and standardization— Separate calibration procedures are provided for a bubbler system (Section 10.2) and flow-injection system (Section 10.3). Both systems are calibrated using standards traceable to NIST Standard Reference Materials. If system performance is verified at the end of an analytical batch using the OPR, analysis of samples and blanks may proceed without recalibration, unless more than 12 hours has elapsed since verification of system performance.

10.2 Bubbler system calibration

10.2.1 Establish the operating conditions necessary to purge Hg from the bubbler and to desorb Hg from the traps in a sharp peak. Further details for operation of the purge-and-trap, desorption, and analysis systems are given in Sections 11.2.1 and 11.2.2.

10.2.2 The calibration must contain a minimum of five non-zero points and the results of analysis of three bubbler blanks. The lowest calibration point must be at the Minimum Level (ML).

NOTE: *The purge efficiency of the bubbler system is 100% and is independent of volume at the volumes used in this Method. Calibration of this system is typically performed using units of mass. For purposes of working in concentration, the volume is assumed to be 100 mL.*

10.2.2.1 Standards are analyzed by the addition of aliquots of Hg working standard A (Section 7.9) and Hg working standard B (Section 7.10) directly into the bubblers. Add 0.50 mL of working standard B and 0.5 mL $SnCl_2$ to the bubbler. Swirl to produce a standard containing 50 pg of Hg (0.5 ng/L). Purge under the optimum operating conditions (Section 10.2.1). Sequentially follow with the addition of aliquots of 0.05, 0.25, 0.50 and 1.0 mL of working standard A to produce standards of 500, 2500, 5000, and 10,000 pg Hg (5.0, 25.0, 50.0 and 100.0 ng/L).

NOTE: *If calibration to the higher levels results in carryover (Section 4.3.8.1), calibrate the system across a narrower range (Section 10.4)*

10.2.2.2 Analyze the standards beginning with the lowest concentration and proceeding to the highest. Tabulate the height or area for each peak.

10.2.2.3 Prepare and analyze a minimum of 3 bubbler blanks. If multiple bubblers are used, there must be 1 bubbler blank per bubbler (to a maximum of 4 bubblers). Calculate the mean peak area or height for the bubbler blanks.

10.2.2.4 For each calibration point, subtract the mean peak height or area of the bubbler blanks from the peak height or area for each standard. Calculate the calibration factor (CF_x) for Hg in each of the five standards using the mean bubbler-blank-subtracted peak height or area and the following equation:

$$CF_x = \frac{(A_x) - (\bar{A}_{BB})}{(C_x)}$$

Where:

A_x = *peak height or area for Hg in standard*
$\bar{A}_{BB}$ = *mean peak height or area for Hg in bubbler blank*
C_x = *mass in standard analyzed (ng)*

10.2.2.5 Calculate the mean calibration factor (CF_m), the standard deviation of the calibration factor (SD; n-1), and the relative standard deviation (RSD) of the calibration factor, where RSD = 100 x SD/CF_m.

10.2.2.6 If RSD ≤ 15%, calculate the recovery for the lowest standard using CF_m. If the RSD ≤ 15% and the recovery of the lowest standard is in the range of 75-125%, the calibration is acceptable and CF_m may be used to calculate the concentration of Hg in samples. If RSD > 15% or if the recovery of the lowest standard is not in the range of 75-125%, recalibrate the analytical system and repeat the test.

10.2.2.7 Calculate the concentration of Hg in the bubbler blanks (Section 10.2.2.1) using CF_m. The bubbler blanks must meet the criteria in Section 9.4.1; otherwise, mercury in the system must be reduced and the calibration repeated until the bubbler blanks meet the criteria.

10.3 Flow-injection system calibration

10.3.1 Establish the operating conditions necessary to purge Hg from the gas-liquid separator and dryer tube and desorb Hg from the traps in a sharp peak. Further details for operating the analytical system are given in Section 11.2.1.

10.3.2 The calibration must contain a minimum of 5 non-zero points and the results of analysis of 3 system blanks. The lowest calibration point must be at the minimum level (ML).

10.3.2.1 Place 25-30 mL of reagent water and 250 µL of concentrated BrCl solution (Section 7.6) in each of 5 calibrated 50-mL autosampler vials. Prepare the 0.5 ng/L calibration standard by adding 250 µL of working standard B (Section 7.10) to the vial. Dilute to the mark with reagent water. Sequentially follow with the addition of aliquots of 25, 125, 250 and 500 µL of working standard A (Section 7.9) to produce standards of 5.0, 25.0, 50.0 and 100.0 ng/L, respectively. Cap the vials and invert once to mix.

10.3.2.2 Immediately prior to analysis, remove the caps and add 125 µL of NH_2OH solution (Section 7,4). Re-cap, invert once to mix, and allow to stand until the yellow color disappears. Remove all caps and place vials into the analysis rack.

10.3.2.3 Analyze the standards beginning with the lowest concentration and proceeding to the highest. Tabulate the height or area for the Hg peak.

10.3.2.4 Prepare and analyze a minimum of 3 system blanks and tabulate the peak heights or areas. Calculate the mean peak area or height for the system blanks.

10.3.2.5 For each calibration point, subtract the mean peak height or area of the system blanks (Section 9.4.2) from the peak height or area for each standard. Calculate

the calibration factor (CF_x) for Hg in each of the five standards using the mean reagent-blank-subtracted peak height or area and the following equation:

$$CF_x = \frac{(A_x) - (\overline{A}_{SB})}{(C_x)}$$

Where:

A_x = *peak height or area for Hg in standard*
$\overline{A}_{SB}$ = *mean peak height or area for Hg in calibration blanks*
C_x = *concentration of standard analyzed (ng/L)*

10.3.2.6 Calculate the mean calibration factor (CF_m), the standard deviation of the calibration factor (SD; n-1), and the relative standard deviation (RSD) of the calibration factor, where RSD = 100 x SD/CF_m.

10.3.2.7 If RSD ≤ 15%, calculate the recovery for the lowest standard (0.5 ng/L) using CF_m. If the RSD ≤ 15% and the recovery of the lowest standard is in the range of 75-125%, the calibration is acceptable and CF_m may be used to calculate the concentration of Hg in samples, blanks, and OPRs. If RSD > 15% or if the recovery of the lowest standard is not in the range of 75-125%, recalibrate the analytical system and repeat the test.

10.3.2.8 Calculate the concentration of Hg in the system blanks (Section 9.4.2) using CF_m. The system blanks must meet the criteria in Section 9.4.2; otherwise, mercury in the system must be reduced and the calibration repeated until the system blanks meet the criteria.

10.4 Calibration to a range other than 0.5 to 100 ng/L—This Method may be calibrated to a range other than 0.5 to 100 ng/L, provided that the following requirements are met:

(a) There must be a minimum of five non-zero calibration points.
(b) The difference between successive calibration points must be no greater than a factor of 10 and no less than a factor of 2 and should be approximately evenly spaced on a logarithmic scale over the calibration range.
(c) The relative standard deviation (RSD) of the calibration factors for all calibration points must be less than 15%.
(d) The calibration factor for any calibration point at a concentration greater than 100 ng/L must be within ±15% of the average calibration factor for the points at or below 100 ng/L.
(e) The calibration factor for any point <0.5 ng/L must be within 25% of the average calibration factor for all points.
(f) If calibration is to a higher range and this Method is used for regulatory compliance, the ML must be less than one-third the regulatory compliance limit

11.0 Procedure

NOTE: *The following procedures for analysis of samples are provided as guidelines. Laboratories may find it necessary to optimize the procedures, such as drying time or potential applied to the Nichrome wires, for the laboratory's specific instrument set-up.*

11.1 Sample Preparation

11.1.1 Pour a 100-mL aliquot from a thoroughly shaken, acidified sample, into a 125-mL fluoropolymer bottle. If BrCl was not added as a preservative (Section 8.5), add the amount of BrCl solution (Section 7.6) given below, cap the bottle, and digest at room temperature for a 12 h minimum.

11.1.1.1 For clear water and filtered samples, add 0.5 mL of BrCl; for brown water and turbid samples, add 1.0 mL of BrCl. If the yellow color disappears because of consumption by organic matter or sulfides, more BrCl should be added until a permanent (12-h) yellow color is obtained.

11.1.1.2 Some highly organic matrices, such as sewage effluent, will require high levels of BrCl (e.g., 5 mL/100 mL of sample) and longer oxidation times, or elevated temperatures (e.g., place sealed bottles in oven at 50 °C for 6 h). The oxidation must be continued until it is complete. Complete oxidation can be determined by either observation of a permanent yellow color remaining in the sample or the use of starch iodide indicating paper to test for residual free oxidizer. The sample also may be diluted to reduce the amount of BrCl required, provided that the resulting level of mercury is sufficient for reliable determination.

11.1.2 Matrix spikes and matrix spike duplicates—For every 10 or fewer samples, pour 2 additional 100-mL aliquots from a selected sample (see Section 9.3), spike at the level specified in Section 9.3, and process in the same manner as the samples. There must be a minimum of 2 MS/MSD pairs for each analytical batch of 20 samples.

11.2 Hg reduction and purging—Separate procedures are provided for the bubbler system (Section 11.2.1) and flow-injection (Section 11.2.2).

11.2.1 Hg reduction and purging for the bubbler system

11.2.1.1 Add 0.2-0.25 mL of NH_2OH solution to the BrCl-oxidized sample in the 125-mL sample bottle. Cap the bottle and swirl the sample. The yellow color will disappear, indicating the destruction of the BrCl. Allow the sample to react for 5 min with periodic swirling to be sure that no traces of halogens remain.

NOTE: *Purging of free halogens onto the gold trap will result in damage to the trap and low or irreproducible results.*

11.2.1.2 Connect a fresh trap to the bubbler, pour the reduced sample into the bubbler, add 0.5 mL of $SnCl_2$ solution, and purge the sample onto a gold trap with N_2 at 350 ± 50 mL/min for 20 min.

11.2.1.3 When analyzing Hg samples, the recovery is quantitative, and organic interferents are destroyed. Thus, standards, bubbler blanks, and small amounts of high-level samples may be run directly in previously purged water. After very high samples (Section 4.3.8.1), a small degree of carryover (<0.01%) may occur. Bubblers that contain such samples must be demonstrated to be clean prior to proceeding with low level samples. Samples run immediately following a sample that has been determined to result in carryover must be reanalyzed using a bubbler that is demonstrated to be clean as per Section 4.3.8.1.

11.2.2 Hg reduction and purging for the flow-injection system

11.2.2.1 Add 0.2-0.25 mL of NH_2OH solution (Section 7.4) to the BrCl-oxidized sample in the 125-mL sample bottle or in the autosampler tube (the amount of NH_2OH required will be approximately 30 percent of the BrCl volume). Cap the bottle and swirl the sample. The yellow color will disappear, indicating the destruction of the BrCl. Allow the sample to react for 5 minutes with periodic swirling to be sure that no traces of halogens remain.

NOTE: *Purging of free halogens onto the gold trap will result in damage to the trap and low or irreproducible results.*

11.2.2.2 Pour the sample solution into an autosampler vial and place the vial in the rack.

11.2.2.3 Carryover may occur after analysis of a sample containing a high level of mercury. Samples run immediately following a sample that has been determined to result in carryover (Section 4.3.8.1) must be reanalyzed using a system demonstrated to be clean as per Section 4.3.8.1.

11.3 Desorption of Hg from the gold trap

11.3.1 Remove the sample trap from the bubbler, place the Nichrome wire coil around the trap and connect the trap into the analyzer train between the incoming Hg-free argon and the second gold-coated (analytical) sand trap (Figure 2).

11.3.2 Pass argon through the sample and analytical traps at a flow rate of approximately 30 mL/min for approximately 2 min to drive off condensed water vapor.

11.3.3 Apply power to the coil around the sample trap for 3 minutes to thermally desorb the Hg (as Hg(0)) from the sample trap onto the analytical trap.

11.3.4 After the 3-min desorption time, turn off the power to the Nichrome coil, and cool the sample trap using the cooling fan.

11.3.5 Turn on the chart recorder or other data acquisition device to start data collection, and apply power to the Nichrome wire coil around the analytical trap. Heat the analytical trap for 3 min (1 min beyond the point at which the peak returns to baseline).

11.3.6 Stop data collection, turn off the power to the Nichrome coil, and cool the analytical trap to room temperature using the cooling fan.

11.3.7 Place the next sample trap in line and proceed with analysis of the next sample.

NOTE: *Do not heat a sample trap while the analytical trap is still warm; otherwise, the analyte may be lost by passing through the analytical trap.*

11.4 Peaks generated using this technique should be very sharp and almost symmetrical. Mercury elutes at approximately 1 minute and has a width at half-height of about 5 seconds.

11.4.1 Broad or asymmetrical peaks indicate a problem with the desorption train, such as improper gas flow rate, water vapor on the trap(s), or an analytical trap damaged by chemical fumes or overheating.

11.4.2 Damage to an analytical trap is also indicated by a sharp peak, followed by a small, broad peak.

11.4.3 If the analytical trap has been damaged, the trap and the fluoropolymer tubing downstream from it should be discarded because of the possibility of gold migration onto downstream surfaces.

11.4.4 Gold-coated sand traps should be tracked by unique identifiers so that any trap producing poor results can be quickly recognized and discarded.

12.0 Data Analysis and Calculations

12.1 Separate procedures are provided for calculation of sample results using the bubbler system (Section 12.2) and the flow-injection system (Section 12.3), and for method blanks (Section 12.4).

12.2 Calculations for the bubbler system

12.2.1 Calculate the mean peak height or area for Hg in the bubbler blanks measured during system calibration or with the analytical batch (A_{BB}; n = 3 minimum).

12.2.2 Calculate the concentration of Hg in ng/L (parts-per-trillion; ppt) in each sample according to the following equation:

$$[Hg]\ (ng/L) = \frac{A_s - \overline{A}_{BB}}{CF_m \times V}$$

where:
A_s = *peak height (or area) for Hg in sample*
$\overline{A}_{BB}$ = *peak height (or area) for Hg in bubbler blank*
CF_m = *mean calibration factor (Section* 10.2.2.5)
V = *Volume of sample (L)*

12.3 Calculations for the flow-injection system

12.3.1 Calculate the mean peak height or area for Hg in the system blanks measured during system calibration or with each analytical batch (A_{SB}; n = 3)

12.3.2 Calculate the concentration of Hg in ng/L in each sample according to the following equation:

$$[Hg]\ (ng/L) = \frac{(A_s - \overline{A}_{SB})}{CF_m} \times \frac{V_{std}}{V_{sample}}$$

where:
A_s = *peak height (or area) for Hg in sample*
$\overline{A}_{SB}$ = *mean peak height (or area) for Hg in system blanks*
CF_m = *mean calibration factor (Section* 10.3.2.6)
V_{std} = *volume (mL) used for standards - volume (mL) reagent used in standards*
V_{sample} = *volume (mL) of sample - volume (mL) reagent used in sample*

12.4 Calculations for concentration of Hg in method blanks, field blanks, and reagent blanks.

12.4.1 Calculate the concentration of Hg in the method blanks (C_{MB}), field blanks (C_{FB}), or reagent blanks (C_{RB}) in ng/L, using the equation in Section 12.2.2 (if bubbler system is used) or Section 12.3.2 (if flow injection system is used) and substituting the peak height or area resulting from the method blank, field blank, or reagent blank for A_s.

12.4.2 Determine the mean concentration of Hg in the method blanks associated with the analytical batch (a minimum of three). If a sample requires additional reagent(s) (e.g., BrCl), a corresponding method blank containing an identical amount of reagent must be analyzed (Section 9.4.4.3). The concentration of Hg in the corresponding method blank may be subtracted from the concentration of Hg in the sample per Section 12.5.2.

12.5 Reporting

12.5.1 Report results for Hg at or above the ML, in ng/L, to three significant figures. Report results for Hg in samples below the ML as <0.5 ng/L, or as required by the regulatory authority or in the permit. Report results for Hg in reagent blanks and field blanks at or above the ML, in ng/L, to three significant figures. Report results for Hg in reagent blanks, method blanks, or field blanks below the ML but at or above the MDL to two significant figures. Report results for Hg not detected in reagent blanks, method blanks, or field blanks as <0.2 ng/L, or as required by the regulatory authority or in the permit.

12.5.2 Report results for Hg in samples, method blanks and field blanks separately. In addition to reporting results for the samples and blank(s) separately, the concentration of Hg in the method blanks or field blanks associated with the sample may be subtracted from the results for that sample, or must be subtracted if requested or required by a regulatory authority or in a permit.

12.5.3 Results from tests performed with an analytical system that is not in control must not be reported or otherwise used for permitting or regulatory compliance purposes, but do not relieve a discharger or permittee of reporting timely results.

13.0 Method Performance

13.1 This Method was tested in 12 laboratories using reagent water, freshwater, marine water and effluent (Reference 16.19). The quality control acceptance criteria listed in Table 2 were verified by data gathered in the interlaboratory study, and the method detection limit (MDL) given in Section 1.5 was verified in all 12 laboratories. In addition, the techniques in this Method have been compared with other techniques for low-level mercury determination in water in a variety of studies, including ICES-5 (Reference 16.20) and the International Mercury Speciation Intercomparison Exercise (Reference 16.21).

13.2 Precision and recovery data for reagent water, freshwater, marine water, and secondary effluent are given in Table 3.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address waste generation. When it is not feasible to reduce wastes at the source, the Agency recommends recycling as the next best option. The acids used in this Method should be reused as practicable by purifying by electrochemical techniques. The only other chemicals used in this Method are the neat materials used in preparing standards. These standards are used in extremely small amounts and pose little threat to the environment when managed properly. Standards should be prepared in volumes consistent with laboratory use to minimize the disposal of excess volumes of expired standards.

14.2 For information about pollution prevention that may be applied to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Governmental Relations and Science Policy, 1155 16th Street NW, Washington DC 20036, 202/872–4477.

15.0 Waste Management

15.1 The laboratory is responsible for complying with all Federal, State, and local regulations governing waste management, particularly hazardous waste identification rules and land disposal restrictions, and for protecting the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required. An overview of requirements can be found in *Environmental Management Guide for Small Laboratories* (EPA 233-B-98-001).

15.2 Acids, samples at pH <2, and BrCl solutions must be neutralized before being disposed of, or must be handled as hazardous waste.

15.3 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better: Laboratory Chemical Management for Waste Reduction*, both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

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17.0 Glossary

The definitions and purposes below are specific to this Method, but have been conformed to common usage as much as possible.

17.1 **Ambient Water**—Waters in the natural environment (e.g., rivers, lakes, streams, and other receiving waters), as opposed to effluent discharges.

17.2 **Analytical Batch**—A batch of up to 20 samples that are oxidized with the same batch of reagents and analyzed during the same 12-hour shift. Each analytical batch must also include at least three bubbler blanks, an OPR, and a QCS. In addition, MS/MSD samples must be prepared at a frequency of 10% per analytical batch (one MS/MSD for every 10 samples).

17.3 **Bottle Blank**—The bottle blank is used to demonstrate that the bottle is free from contamination prior to use. Reagent water known to be free of mercury at the MDL of this Method is added to a bottle, acidified to pH <2 with BrCl or HCl, and allowed to stand for a minimum of 24 hours. The time that the bottle is allowed to stand should be as close as possible to the actual time that the sample will be in contact with the bottle. After standing, the water is analyzed.

17.4 **Bubbler Blank**—For this Method, the bubbler blank is specific to the bubbler system and is used to determine that the analytical system is free from contamination. After analysis of a standard, blank, or sample, the solution in the bubbler is purged and analyzed. A minimum of three bubbler blanks is required for system calibration.

17.5 **Equipment Blank**—Reagent water that has been processed through the sampling device at a laboratory or other equipment cleaning facility prior to shipment of the sampling equipment to the sampling site. The equipment blank is used to demonstrate that the sampling equipment is free from contamination prior to use. Where appropriate, the "clean hands/dirty hands" technique used during field sampling should be followed when preparing equipment blanks at the laboratory or cleaning facility.

17.6 **Field Blank**—Reagent water that has been transported to the sampling site and exposed to the same equipment and operations as a sample at the sampling site. The field blank is used to demonstrate that the sample has not been contaminated by the sampling and sample transport systems.

17.7 **Intercomparison Study**—An exercise in which samples are prepared and split by a reference laboratory, then analyzed by one or more testing laboratories and the reference laboratory. The intercomparison, with a reputable laboratory as the reference laboratory, serves as the best test of the precision and accuracy of the analyses at natural environmental levels.

17.8 **Matrix Spike (MS) and Matrix Spike Duplicate (MSD)**—Aliquots of an environmental sample to which known quantities of the analyte(s) of interest is added in the laboratory. The MS and MSD are analyzed exactly like a sample. Their purpose is to quantify the bias and precision caused by the sample matrix. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the MS and MSD corrected for these background concentrations.

17.9 **May**—This action, activity, or procedural step is allowed but not required.

17.10 **May not**—This action, activity, or procedural step is prohibited.

17.11 **Method blank**— Method blanks are used to determine the concentration of mercury in the analytical system during sample preparation and analysis, and consist of a volume of reagent water that is carried through the entire sample preparation and analysis. Method blanks are prepared by placing reagent water in a sample bottle and analyzing the water using reagents and procedures identical to those used to prepare and analyze the corresponding samples. A minimum of three method blanks is required with each analytical batch.

17.12 **Minimum Level (ML)**—The lowest level at which the entire analytical system must give a recognizable signal and acceptable calibration point for the analyte. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed. The ML is calculated by multiplying the MDL by 3.18 and rounding the result to the number nearest to (1, 2, or 5) x 10^n, where n is an integer (See Section 1.5).

17.13 **Must**—This action, activity, or procedural step is required.

17.14 **Quality Control Sample (QCS)**—A sample containing Hg at known concentrations. The QCS is obtained from a source external to the laboratory, or is prepared from a source of standards different from the source of calibration standards. It is used as an independent check of instrument calibration.

17.15 **Reagent blank**—Reagent blanks are used to determine the concentration of mercury in the reagents (BrCl, $NH_2OH{\cdot}HCl$, and $SnCl_2$) that are used to prepare and analyze the samples. In this Method, reagent blanks are required when each new batch of reagents is prepared.

17.16 **Reagent Water**—Water demonstrated to be free of mercury at the MDL of this Method. It is prepared from 18 MΩ ultrapure deionized water starting from a prepurified source. Reagent water is used to wash bottles, as trip and field blanks, and in the preparation of standards and reagents.

17.17 **Regulatory Compliance Limit**—A limit on the concentration or amount of a pollutant or contaminant specified in a nationwide standard, in a permit, or otherwise established by a regulatory authority.

17.18 **Shall**—This action, activity, or procedure is required.

17.19 **Should**—This action, activity, or procedure is suggested, but not required.

17.20 **Stock Solution**— A solution containing an analyte that is prepared from a reference material traceable to NIST, or a source that will attest to the purity and authenticity of the reference material.

17.21 **System Blank**— For this Method, the system blank is specific for the flow-injection system and is used to determine contamination in the analytical system and in the reagents used to prepare the calibration standards. A minimum of three system blanks is required during system calibration.

17.22 **Ultraclean Handling**— A series of established procedures designed to ensure that samples are not contaminated during sample collection, storage, or analysis.

18.0 Tables and Figures

Table 1

Lowest Ambient Water Quality Criterion for Mercury and the Method Detection Limit and Minimum Level of Quantitation for EPA Method 1631

Metal	Lowest Ambient Water Quality Criterion[1]	Method Detection Limit (MDL) and Minimum Level (ML)	
		MDL[2]	ML[3]
Mercury (Hg)	1.3 ng/L	0.2 ng/L	0.5 ng/L

1. Lowest water quality criterion for the Great Lakes System (Table 4, 40 CFR 132.6). The lowest Nationwide criterion is 12 ng/L (40 CFR 131.36).
2. Method detection limit (40 CFR 136, Appendix B)
3. Minimum level of quantitation (see Glossary)

Table 2

Quality Control Acceptance Criteria for Performance Tests in EPA Method 1631

Acceptance Criteria	Section	Limit (%)
Initial Precision and Recovery (IPR)	9.2.2	
Precision (RSD)	9.2.2.3	21
Recovery (X)	9.2.2.3	79-121
Ongoing Precision and Recovery (OPR)	9.5.2	77-123
Matrix Spike/Matrix Spike Duplicate (MS/MSD)	9.3	
Recovery	9.3.4	71-125
Relative Percent Difference (RPD)	9.3.5	24

Table 3

Precision and Recovery for Reagent Water, Fresh Water, Marine Water, and Effluent Water Using Method 1631

Matrix	*Mean Recovery (%)	*Precision (% RSD)
Reagent Water	98.0	5.6
Fresh Water (Filtered)	90.4	8.3
Marine Water (Filtered)	92.3	4.7
Marine Water (Unfiltered)	88.9	5.0
Secondary Effluent (Filtered)	90.7	3.0
Secondary Effluent (Unfiltered)	92.8	4.5

*Mean percent recoveries and RSDs are based on expected Hg concentrations.

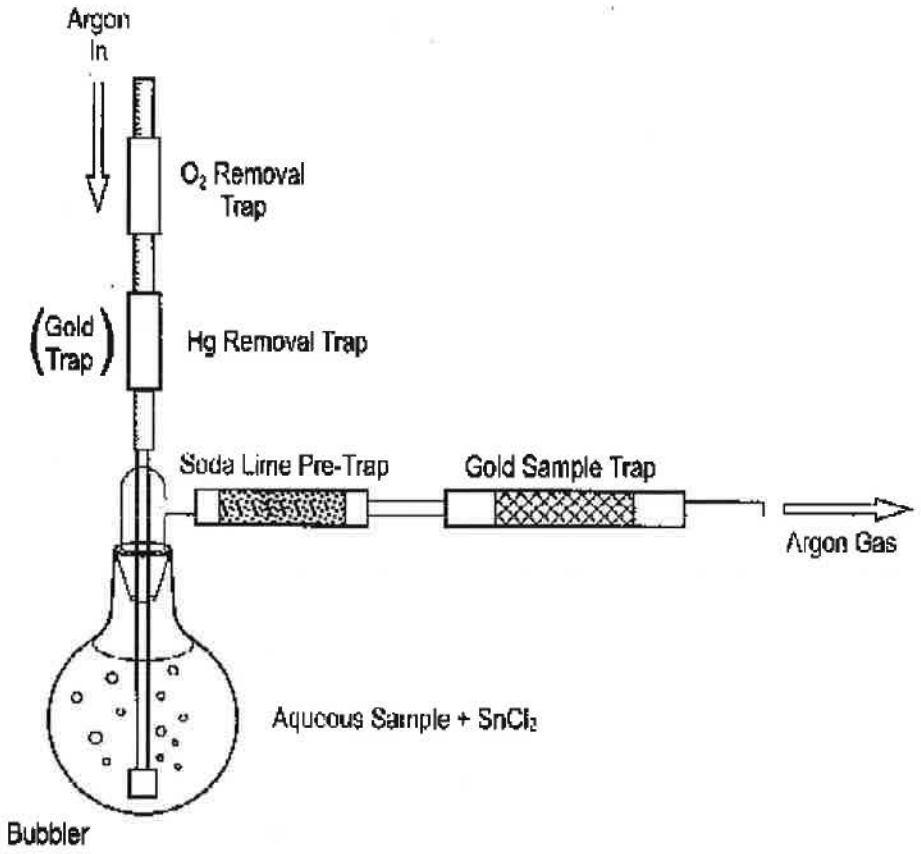

Figure 1. Schematic Diagram of Bubbler Setup

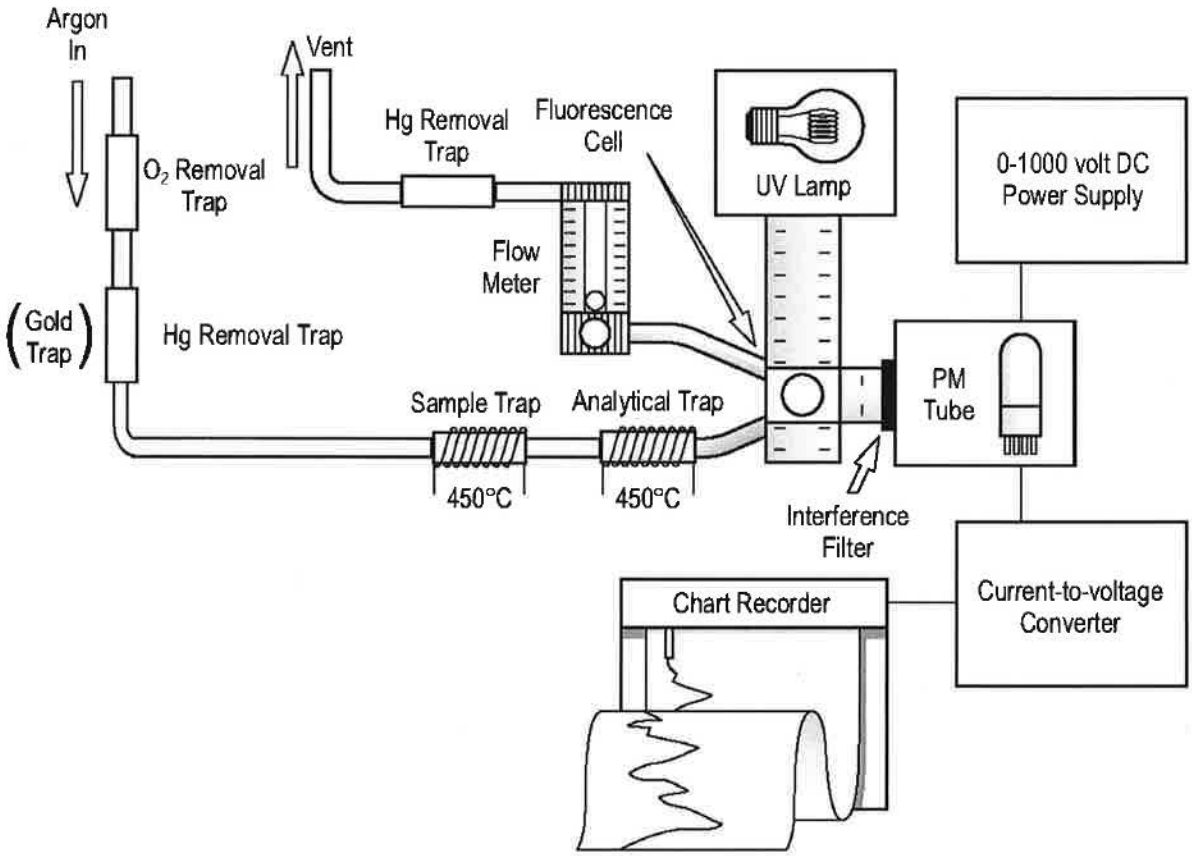

Figure 2. Schematic Diagram of the Bubbler, Purge and Trap, Cold Vapor Atomic Fluorescence Spectrometer (CVAFS) System

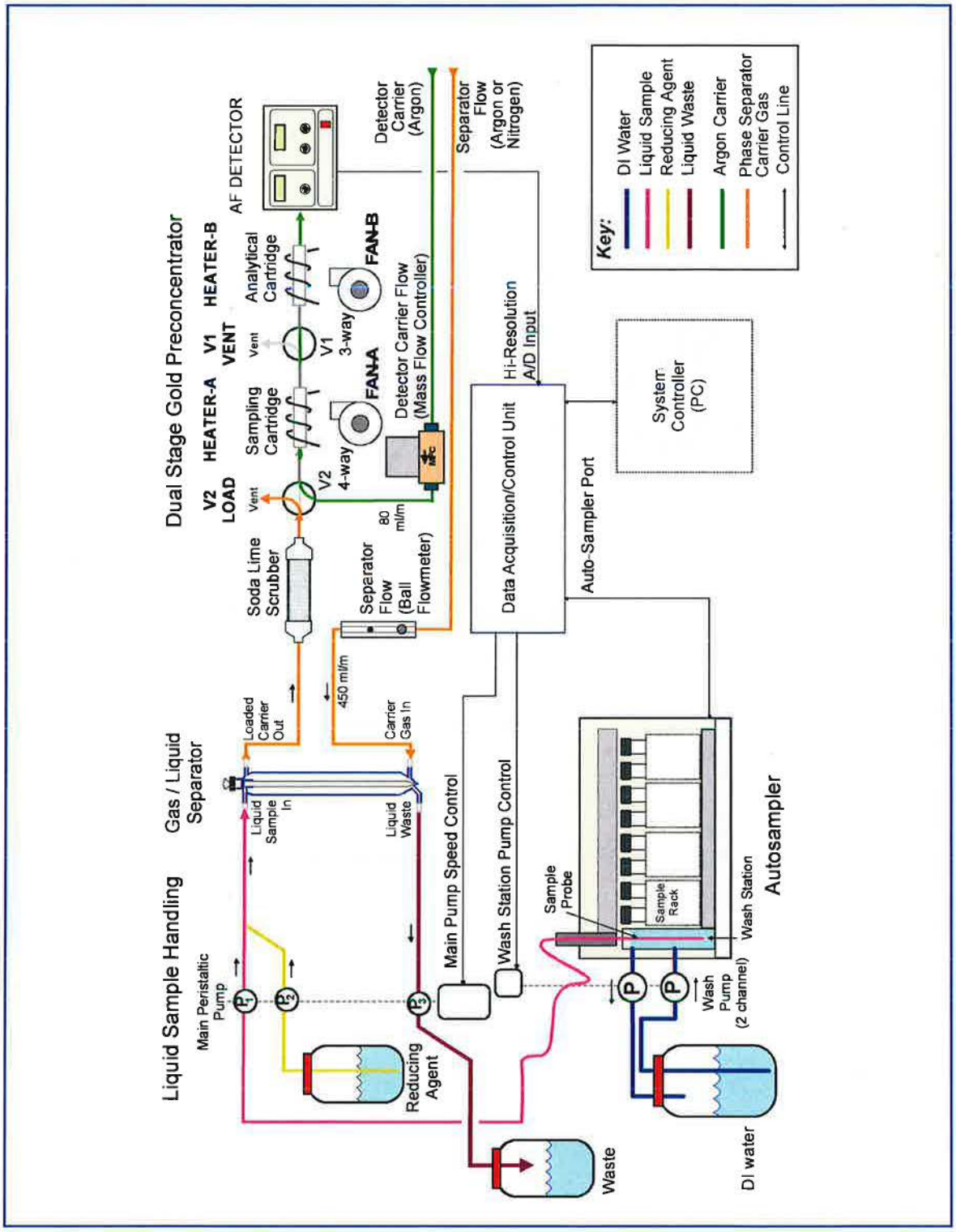

Figure 3. Schematic Diagram of the Flow-Injection, Cold Vapor Atomic Fluorescence Spectrometer (CVAFS) System

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ORIGINAL PAPER

Paleolimnology of a freshwater estuary to inform Area of Concern nutrient delisting efforts

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Abstract The St. Louis River Estuary (SLRE), a freshwater estuary bordering Duluth, Minnesota, Superior, Wisconsin, and the most western point of Lake Superior (46.74°, − 92.13°), has a long history of human development since Euro-American settlement ~ 200 years ago. Due to degradation from logging, hydrologic modification, industrial practices, and untreated sewage, the SLRE was designated an Area of Concern in 1987. Action has been taken to restore water quality including the installation of the Western Lake Superior Sanitary District in 1978 to help remove beneficial use impairments. A better understanding of historical impacts and remediation is necessary to help document progress and knowledge gaps related to water quality, so a paleolimnological study of the SLRE was initiated. Various paleolimnological indicators (pigments, diatom communities, and diatom-inferred phosphorus) were analyzed from six cores taken throughout the SLRE and another from western Lake Superior. Reductions in eutrophic diatom taxa such as *Cyclotella meneghiniana* and *Stephanodiscus* after 1970 in certain cores suggest an improvement in water quality over the last 40 years. However, in cores taken from estuarine bay

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environments, persistence of eutrophic taxa such as *Cyclostephanos dubius* and *Stephanodiscus binderanus* indicate ongoing nutrient problems. Sedimentary pigments also indicate cyanobacteria increases in bays over the last two decades. Diatom model-inferred phosphorus and contemporary monitoring data suggest some of the problems associated with excess nutrient loads have been remediated, but modern conditions (internal phosphorus loading, changing climate) may be contributing to ongoing water quality impairments in some locations. The integrated record of biological, chemical, and physical indicators preserved in the sediments will aid state and federal agencies in determining where to target their resources.

Keywords Diatoms · Great lakes · Lake Superior · Beneficial use impairment · St. Louis River · Minnesota · Wisconsin

Introduction

The Laurentian Great Lakes' capacity for shipping, expansive timber stands, and iron-rich rock drew settlers to the region, leading to water quality impairments due to lack of infrastructure and regulation, especially in tributaries and estuaries where populations were condensed. Since European settlement the St. Louis River Estuary (SLRE) became one of the most impacted ecosystems in the Laurentian Great Lakes, beginning with the construction of the Duluth Shipping Canal in 1871, granting access to the eastern United States and beyond (MPCA and WDNR 1992). Approximately 1600 ha of open water and shoreline were filled with dredged material since 1861; dredging is still routine to maintain the port for commercial shipping (Devore 1978).

In the late 1800s to early 1900s, the St. Louis River watershed was almost entirely clear-cut by loggers, and land cover transitioned from forested to shrubland and agricultural land, leading to increased runoff of both water and soil. In order to control the transportation of timber downstream, 50–100 dams were installed on the river. Dams contribute to large changes in hydrologic regime and aquatic ecosystems (Baxter 1977; Bunn and Arthington 2002). Additionally, the SLRE was home to many sawmills, pulp mills, and paper mills. These were a major source of pollution to the estuary, discharging large quantities of sawdust and slab wood (MPCA and WDNR 1992).

To accommodate these growing industries the population of Duluth and Superior grew from an estimated 600 in 1865, to 100,000 by 1930 (MPCA and WDNR 1992). However, the region lacked proper stormwater and wastewater infrastructure, so untreated wastewater (e.g. human waste and high-phosphorus detergents) introduced nutrients and organic matter into the estuary, leading to episodic hypoxia (Hargis 1983; Carlson and Thomas 1984).

In 1966, the Federal Water Pollution Control Administration Great Lakes Regional Office made recommendations on strategies to reduce pollution, including installing wastewater and stormwater treatment facilities to reduce nutrient loading (FWPCA 1966). In response, the Western Lake Superior Sanitary District opened in 1978 to treat wastewater from a 1375 km^2 region including Duluth and surrounding communities (MPCA and WDNR 1992). A study comparing concentrations of various metals, nutrients, and physical parameters before and after 1978 showed water quality improvement in the SLRE including a decrease in total phosphorus, turbidity, total coliform, lead, and copper and an increase in dissolved oxygen (McCollor 1990).

To further address the polluted state of the estuary, in 1987 the St. Louis River Estuary was designated an Area of Concern, a program designed by the International Joint Commission, a governing body regulating waters shared by Canada and the United States, as a part of the Great Lakes Water Quality Agreement (IJC 1987). Each Area of Concern is defined by various beneficial use impairments that define the extent of degradation; of these the SLRE received 9 including one for excessive loading of sediment and nutrients (impairment 6) (MPCA and WDNR 1992). In 1992, a remedial action plan was released outlining the degradation of the SLRE and included a path for improvement (MPCA and WDNR 1992).

Agencies hope to remove all impairments and delist the Area of Concern by 2025. To achieve this, there has been a large effort to remediate the estuary and agencies have developed removal targets for impairment 6. Best management practices within the watershed and the installation of wastewater treatment facilities helped to reduce the flux of nutrients to the SLRE (MPCA and WDNR 2013), but the extent of

nutrient reduction is poorly understood. A phosphorus dataset from an upstream and downstream location (beginning in 1958 and 1973 respectively) exists for the SLRE and consists of monthly measurements of surface water total phosphorus. These data show a reduction in phosphorus concentrations in the main estuary channel (Bellinger et al. 2016). Still, measurements of nutrient concentrations and sediment loading, and biological responses to these changes, are absent for most of the SLRE's past, especially pre-European settlement conditions.

A paleolimnological study of the SLRE was initiated to help to provide insight on long-term environmental impacts and remediation while considering the known human history of the watershed. We hypothesized that paleolimnology would provide valuable retrospective data in support of the Area of Concern delisting process. To date, paleoecological studies of such hydrologically complex, moving water systems have been uncommon, so we applied some additional considerations outside more traditional, lake-based paleolimnological approaches. Subfossil diatoms (Bacillariophyceae) and algal pigments were the primary indicators used. To examine the historical environmental conditions of the SLRE, relative abundances of diatom taxa before, during, and after European settlement were characterized. Changes in diatom assemblages and inferred environmental information were correlated with anthropogenic activities and validated with additional data, including fossil pigments (to provide quantitative information about nutrient effects and less desirable taxa such as cyanobacteria) and sediment organic content. The extent of degradation and recent Area of Concern remediation was clarified, and management recommendations relevant to Area of Concern beneficial use impairment removal are provided herein.

Study area

The St. Louis River (Fig. 1) flows 288 km through northeastern Minnesota draining an area of 9412 km^2. On average it delivers 73.3 $m^3\ s^{-1}$ of water to western Lake Superior, making it the largest tributary to Lake Superior in the United States. The drainage basin land cover consists of forests (61%), wetlands (24%), and grasslands (7%); the remaining 8% is developed (MPCA 2013). The farthest downstream portion (after Minnesota Highway 23) of the river before it joins Lake Superior is the St. Louis River Estuary (SLRE). In contrast to a mostly rural upstream, the dominant land use surrounding the SLRE is urban. The estuary is bordered by two major cities—Duluth, Minnesota and Superior, Wisconsin—which have a combined population of 113,000 people (United States Census Bureau 2010).

Methods

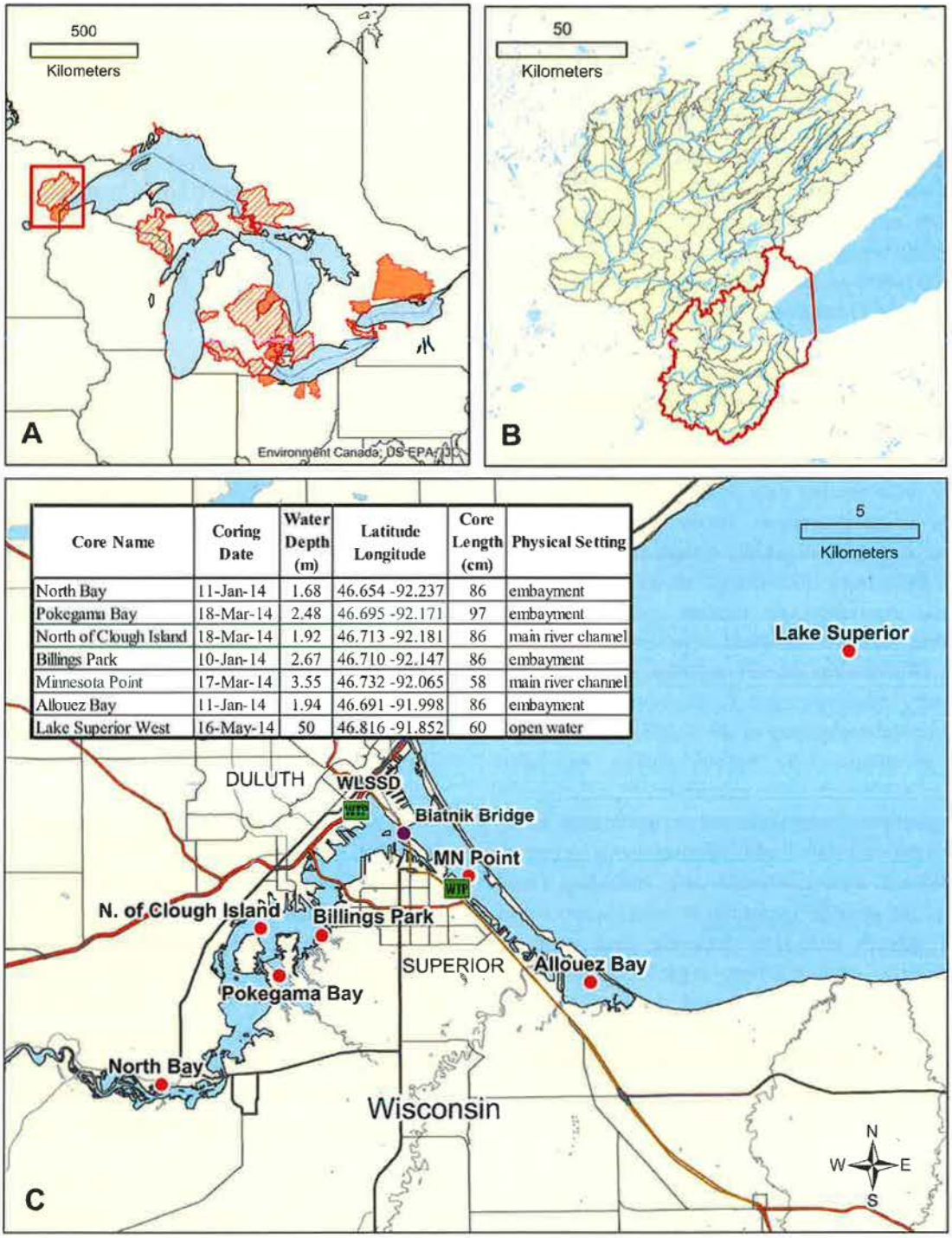
Site selection and coring

Sediment cores were taken from areas predicted to have undisturbed sediments and continuous depositional environments. Maps and hydrological data were used along with consultation with the US Army Corps of Engineers in order to avoid areas previously impacted by dredging or shipping activities. Sites were chosen to represent a variety of environments including different hydrologic regimes (bays, harbor, Lake Superior) and varying anthropogenic impacts (formerly polluted versus purportedly less impacted). A total of seven cores were collected; six cores from discrete locations in the lower SLRE and one core from western Lake Superior (Fig. 1).

At each coring location, we attempted to collect at least 200 years of sediment in order to evaluate the SLRE's anthropogenic record related to European settlement. SLRE cores were collected in winter of 2014. A piston corer designed for sampling recent lake sediments was used (Glew et al. 2001). The core taken from western Lake Superior was collected in May 2014 from the US Environmental Protection Agency's research vessel *Lake Guardian* by use of a multicorer (methods described by Shaw Chraïbi et al. 2014). For each core, approximately one meter of sediment was collected and sectioned into 1-cm intervals (0.25-cm intervals for the first 10 cm and then 0.5-cm intervals for Lake Superior). Samples were extruded in intervals using a close-sectioning extruder and kept refrigerated for later analyses.

Sediment chronology and content

To determine age and sediment accumulation rates for the past 150 to 200 years, sediment cores were analyzed for ^{210}Pb activity. Lead-210 activity was



Core Name	Coring Date	Water Depth (m)	Latitude Longitude	Core Length (cm)	Physical Setting
North Bay	11-Jan-14	1.68	46.654 -92.237	86	embayment
Pokegama Bay	18-Mar-14	2.48	46.695 -92.171	97	embayment
North of Clough Island	18-Mar-14	1.92	46.713 -92.181	86	main river channel
Billings Park	10-Jan-14	2.67	46.710 -92.147	86	embayment
Minnesota Point	17-Mar-14	3.55	46.732 -92.065	58	main river channel
Allouez Bay	11-Jan-14	1.94	46.691 -91.998	86	embayment
Lake Superior West	16-May-14	50	46.816 -91.852	60	open water

Fig. 1 **a** Map indicating the location of the St. Louis River estuary (SLRE) Area of Concern relative to all Great Lakes Areas of Concerns (orange) and their associated watersheds (red hash) (Environment Canada, EPA, IJC 2013), **b** Map of the St. Louis River drainage basin and the boundary (red) of the Area of Concern, **c** Map and table of coring locations in the SLRE (red circles) and the surface water monitoring station (purple circle). WTP = locations of wastewater treatment plants

measured from its daughter product, ^{210}Po, which is considered to be in secular equilibrium with the parent isotope. Aliquots (0.5–3.0 g) of freeze-dried sediment were spiked with a known quantity of ^{209}Po ($\sim$ 4 pCi g^{-1}) as an internal yield tracer and the isotopes distilled at 550 °C after treatment with concentrated

HCl. Polonium isotopes were then directly plated onto Au planchets from a 0.5 N HCl solution. Activity was measured for 1–3 × 10^5 s using an Ortec alpha spectrometry system. Supported ^{210}Pb was estimated by mean activity in the lowest core samples and subtracted from upcore activity to calculate unsupported ^{210}Pb. Core dates and sedimentation rates were calculated using the constant rate of supply model (Appleby and Oldfield 1978; Appleby 2001). Dating and sedimentation errors represented first-order propagation of counting uncertainty (Binford 1990).

For one core with a problematic decay profile (North of Clough Island), gamma spectrometry was used to measure supported ^{210}Pb and identify the distribution of ^{137}Cs in the core (Ritchie and McHenry 1973). Activity was measured using an Ortec-EGG (Oak Ridge, TN) High-Purity, Germanium Crystal Well, Photon Detector (Well Detector) coupled to a Digital Gamma-Ray Spectrometer (Dspec).

Loss on ignition analysis to determine inorganic and organic content followed Dean (1974). Accumulation rates of inorganic and organic content were based on sediment chronology. Analysis of water content and carbonate content and accumulation are presented in Alexson (2016).

Pigments

On a subset of four cores, pigments (carotenoids and chlorins) were analyzed to examine historical algal communities according to methods in Alexson (2016) and based on Reuss (2005) and Ruess and Conley (2005). The pigments analyzed represented total algae (chlorophyll *a*, pheophytin *a*, and *β*-carotene), diatoms (diatoxanthin and fucoxanthin), and dinoflagellates (fucoxanthin), cryptophytes (alloxanthin), and cyanobacteria (aphanizophyll and myxoxanthophyll). Pigments were extracted from the freeze-dried sample using an acetone/water mixture (90/10 by volume) for 24 h at − 20° C in darkness. After extraction, the material was quantitatively analyzed using a Shimadzu High Performance Liquid Chromatographer equipped with a photodiode array detector (SPD-M10Avp). Aphanizophyll and myxoxanthophyll were analyzed at 508 nm (4 nm bandwidth) in the first chromatogram. In the second chromatogram fucoxanthin, alloxanthin, diatoxanthin, and *β*-carotene were analyzed at 449 nm (4 nm bandwidth) and chlorophyll *a*, pheophytin *a* were analyzed at 666 nm (4 nm bandwidth). Concentrations are reported as microgram of pigment per gram of organic matter.

Diatoms

For each core interval analyzed for diatoms, approximately 1 g of wet sediment was subsampled and digested with nitric acid and then hydrogen peroxide to remove all organic material and isolate siliceous microfossils. Samples were then rinsed with distilled water to neutralize acid and applied to coverslips quantitatively using the Battarbee (1986) method. Briefly, Battarbee's method involves drying diatom slurry on coverslips in a settling tray of known area, thereby allowing for quantitative assessment of diatom densities during microscopic assessment. Coverslips were mounted to microscope slides with Naphrax for identification and enumeration.

Diatoms were identified and enumerated by use of oil immersion on a light microscope (1250× magnification). Diatoms on each slide were identified along random transects until at least 400 diatom valves were enumerated. Each diatom was identified to species level according to Reavie and Kireta (2015), Krammer and Lange-Bertalot (1986–1991), Patrick and Reimer (1966–1975), and several other taxonomic works. For all core intervals, percent relative abundance and diatom accumulation (valves cm^{-2} $year^{-1}$) based on the quantitative Battarbee (1986) method were calculated.

Phytoliths, sponge spicules, chrysophyte scales and stomatocysts, and testate amoebae plates were also counted when observed, though only chrysophyte cysts were abundant enough for analysis. These siliceous remains can be used to infer environmental conditions and may provide additional insight on the ecological condition of the SLRE (Smol et al. 2001).

A cluster analysis was completed to characterize the stratigraphic zonation which may reflect historical events leading to reorganization of the diatom community. For common taxa (at least five occurrences with at least 5% abundance in one or more samples), a depth-constrained cluster analysis was done using the "chclust" function in R with the "rioja" package (Juggins 2014) to identify temporally constrained diatom assemblage zones. The CONISS algorithm (Grimm 1987) was used to perform clustering constrained to vertical stratigraphy based on dissimilarity in squared Euclidian distances among samples. The

embedded function "bstick" was used to perform a broken-stick analysis and determine the minimum number of significant zones (Bennett 1996). In addition to zones determined by the broken stick analysis, zones were delineated based on apparent changes in abundance of species which are characteristic of certain environments; for instance, nutrient-tolerant *Stephanodiscus parvus* Stoermer & Håkansson is often associated with eutrophic environments (Stoermer and Håkansson 1984).

Ordination

In order to better assess the similarities among cores and track temporal trajectories, non-metric multidimensional scaling (NMDS) analyses were performed. NMDS is an ordination technique allowing for visualization of highly dimensional data in lower dimensional space. Multidimensional scaling examines distances between observations (e.g. samples or species); shorter distances indicate similarity. The statistical software R with the vegan package (R Core Team 2014; Oksanen et al. 2015) was used to create an NMDS plot from diatom relative abundance data. Species with a maximum relative abundance less than 5% were omitted to reduce analytical artifacts from rare species.

Diatom-inferred modeling

Diatom-inferred (DI) modeling translates fossil diatom data into a reconstruction of an environmental variable (Hall and Smol 1992; Ponader et al. 2007; Saunders 2011). To develop the DI models, diatom species in a training set of samples were related to total phosphorus (TP) measurements and species coefficients (phosphorus optima) were calculated. These species-specific coefficients were applied to the diatom assemblages in cores, and TP was inferred based on the relative abundances of fossil diatom taxa. Models were developed from two Great Lakes training sets: (1) open water (Reavie et al. 2014; used for the Lake Superior core) and (2) coastal embayments, wetlands, and high-energy areas (Reavie et al. 2006; used for the six estuary cores).

A set of analyses verified the efficacy of both models' ability to reconstruct phosphorus. An analog analysis determined similarities between diatom assemblages in the models and fossil assemblages. Using the R package analogue (Simpson and Oksanen 2015) assemblages from the model were matched to fossil assemblages following Flower et al. (1997) and Simpson et al. (2005). Analogs were determined using Bray–Curtis dissimilarity (Bray and Curtis 1957). Dissimilarities between fossil and modern samples were examined to determine how well fossil assemblages were represented in model assemblages. A constrained canonical correspondence analysis (CCA) was done to examine the relationship between modern phosphorus and diatom assemblages, and then fossil samples were ordinated passively to determine goodness of fit. Using the R packages vegan and analogue (Oksanen et al. 2015; Simpson and Oksanen 2015) a CCA defined residual distances of fossil assemblages (i.e. sample scores) and TP gradient (i.e. constrained CCA axis 1). Fossil residual distances within the 95% confidence interval of the modern sample distances were considered to have good fit to TP.

To determine if TP was related to changes in fossil species assemblages, we applied statistical analyses according to Reavie et al. (2014). Using the R package vegan (R Core Team 2014; Oksanen et al. 2015), each set of fossil data in a given core was summarized using principal components analysis (PCA) to derive axis scores representing the primary gradient of variation in the diatom assemblage data (Juggins and Birks 2012). A correlation coefficient (r) was calculated for historical diatom inferred total phosphorus (DI-TP) versus the axis 1 PCA scores. If |r| was significant (t test, $p < 0.05$), it was likely changes in fossil diatom assemblages in cores were at least in part determined by TP, and so use of the DI-TP model was considered appropriate.

To further analyze DI-TP, results were compared to the historical measured TP dataset presented in Bellinger et al. (2016). A lowess smoothing was applied to the measurements from the lower estuary (Blatnik Bridge) due to its closer proximity to coring locations, and plotted alongside DI-TP results.

Results

Sediment chronology

Exponential decay of ^{210}Pb with sediment depth was used to determine the validity of chronological profiles. With the exception of North of Clough

Island, cores showed a consistent record of sediment accumulation and were dateable (Fig. 2; Alexson 2016). ^{210}Pb data from North of Clough Island suggested recent disturbance, likely due to increased sedimentation from a 500-year flood that affected the SLRE in 2012 (Czuba et al. 2012). Unsupported (excess) ^{210}Pb data were relatively monotonous with depth, aside from an uppermost section above ~ 35 cm depth with higher concentrations. Supplementary dating using ^{137}Cs characterized high concentrations of that isotope around 1963 due to nuclear weapons testing (Krishnaswami and Lal 1978). Based on a peak in ^{137}Cs at 60 cm depth, we assigned a rough, recent chronology based on knowing the 1963 interval, acknowledging dates since 1963 are highly uncertain due to flood disturbance.

With the exception of North of Clough Island, cores showed increased sedimentation rates in the early 1900s or just prior (Fig. 3, left-most panels). Several cores demonstrated a rise in sedimentation rates: Allouez Bay and Billings Park had peaks around 1920–1930 and subsequently fell to sedimentation rates of 0.35–0.15 and 2.0–0.2 g cm^{-2} year^{-1} respectively. In both cores, sedimentation rates remain higher than pre-European settlement conditions (0.02 g cm^{-2} year^{-1} in Allouez Bay and 0.15 g cm^{-2} year^{-1} in Billings Park). In western Lake Superior, sedimentation rates peaked around 1970 at 0.12 g cm^{-2} year^{-1} with a secondary peak at 0.11 g cm^{-2} year^{-1} in 1900; rates recovered to near pre-European settlement conditions around 2000. This trend is similar to North Bay where there was a secondary peak in 1930 (0.3 g cm^{-2} year^{-1}) and a peak in 1970 (0.4 g cm^{-2} year^{-1}). Rates declined to 0.2 g cm^{-2} year^{-1} by 2000, but rates remained higher than pre-European settlement. In contrast, sedimentation rates continued to increase in cores from Minnesota Point and Pokegama Bay. At Minnesota Point, accumulation rates rose from less than 0.02–0.14 g cm^{-2} year^{-1}, with the greatest rate of change occurring in the last 40 years. Sedimentation rates at Pokegama Bay increased from 0.05 to

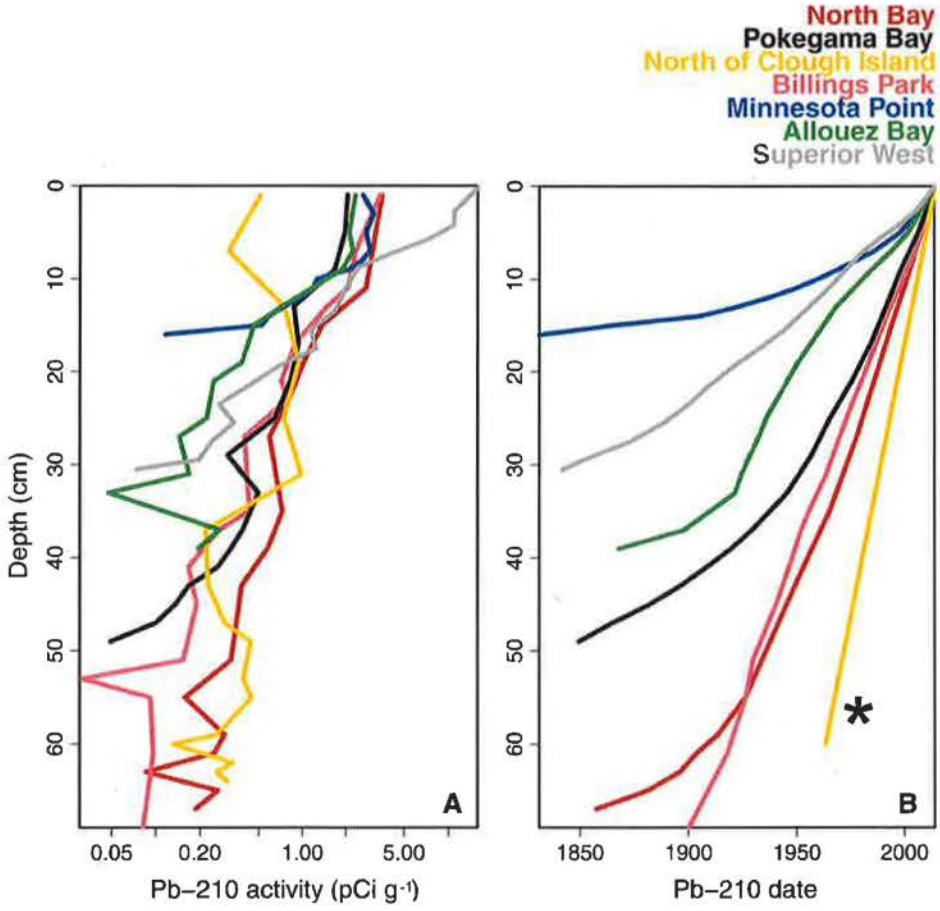


Fig. 2 **a** The magnitude of supported lead-210 (^{210}Pb) activity versus depth in the core. **b** ^{210}Pb inferred dates of the cores versus sediment depth. *The North of Clough core demonstrated a poor ^{210}Pb record, so we provide a very rough estimate of dates and accumulation rates based on ^{137}Cs data that indicated the ~ 1963 interval

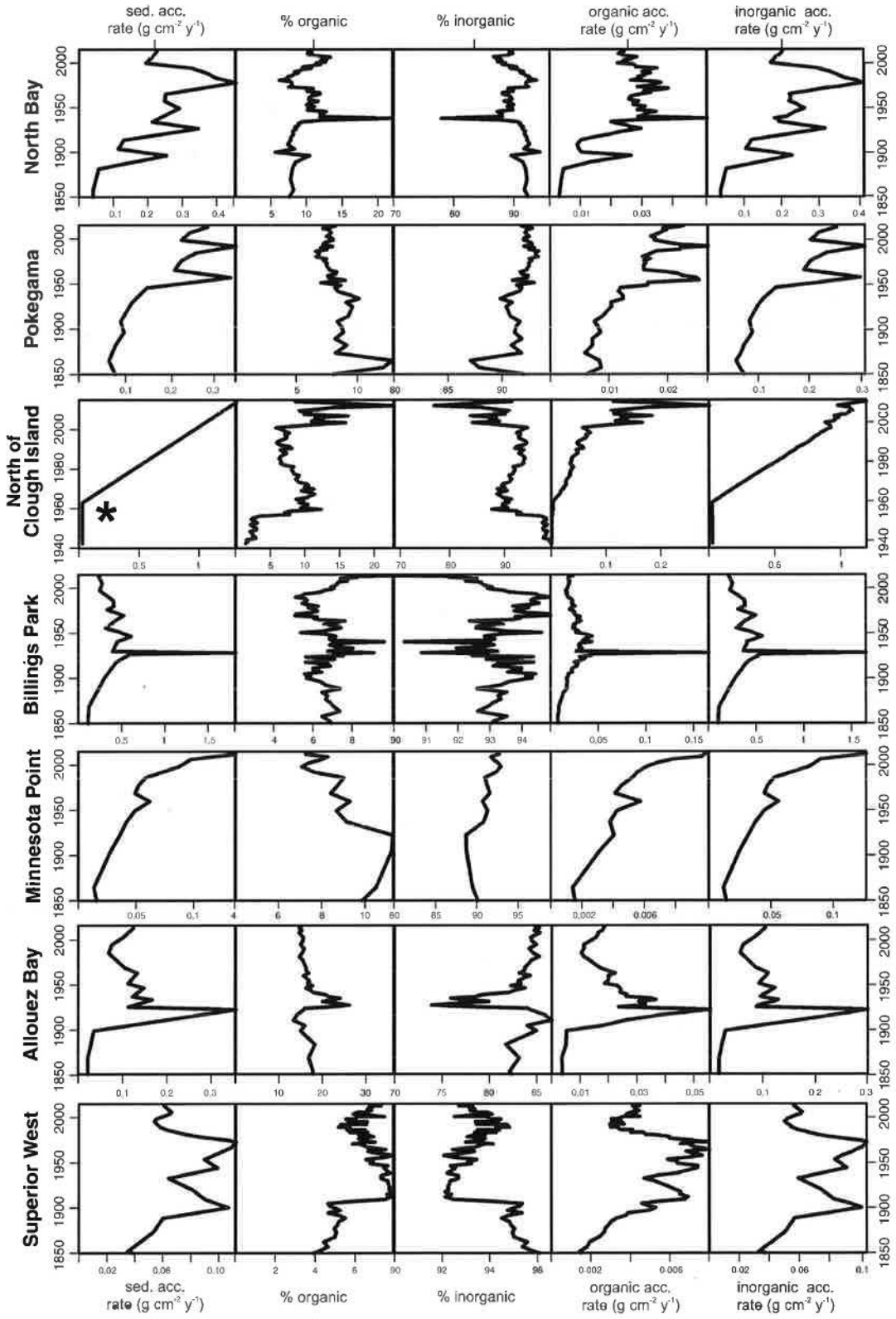


Fig. 3 Results of inorganic and organic content analyses of seven sediment cores from the SLRE and Lake Superior. *The North of Clough core demonstrated a poor ^{210}Pb record, so we provide a very rough estimate of dates and accumulation rates based on ^{137}Cs data that indicated the ~ 1963 interval. Note the x-axis (analyte) scales are different for each core

0.25 g cm^{-2} $year^{-1}$ with two peaks occurring at 1960 and 1990 (both around 0.35 g cm^{-2} $year^{-1}$). The accumulation profile for North of Clough Island was based on a single ^{137}Cs date, so we have great uncertainty about recent accumulation rates. Overall, differences in average sediment accumulation rates among cores reflected their physical settings, such as the lower rates in the more lacustrine areas (Minnesota Point and Lake Superior).

Sediment content

Cores from Allouez Bay and North Bay had the most distinct changes in organic content with a peak in the 1930s and a concomitant increase in % inorganic material (Fig. 3). An increase in organic content was also seen at North of Clough Island since 2000, and due to uncertainty in dating may reflect a depositional layer from the 2012 flood (Czuba et al. 2012).

Accumulation rates of organic and inorganic components largely followed total sedimentation rates, although there were some anomalies. In North of Clough Island, there was lower accumulation of organic material from 1970 to 2000 and increased accumulation from 2000 until present.

Pigments

Pigments in four estuary cores (North Bay, Billings Park, North of Clough Island, and Minnesota Point; Fig. 4) tracked historical shifts in algal groups. Pigments representative of total algae (chlorophyll *a*, pheophytin *a*, and *β*-carotene) showed increased algal production in recent sediments since ~ 1990 in North Bay and Billings Park. Fucoxanthin and diatoxanthin were higher in North Bay and Billings Park. Fucoxanthin (diatoms and dinoflagellates) increased rapidly in more recent intervals, since 1990 in North Bay and 2005 in Billings Park, whereas diatoxanthin (diatoms) showed a gradual increase since 1980. Pigments from cyanobacteria (aphanizophyll and myxoxanthophyll) have increased in both North Bay and Billings Park in the last 20 years. Although there was a strong peak in alloxanthin (representing cryptophytes) around 1970, since ~ 1980 pigment concentrations in Minnesota Point and North of Clough Island cores remained relatively low and steady. Because some pigments degrade with time (e.g. chlorophyll *a* tends to have low stability; Leavitt and Hodgson 2001), we note recent increases occur in pigments with known reliability in long-term preservation in sedimentary records (pheophytin *a*, fucoxanthin, diatoxanthin, aphanizophyll, and myxoxanthophyll, and especially *β*-carotene).

Diatoms

A total of 654 diatom taxa were observed from 88 genera. In SLRE cores, both benthic and planktonic diatoms were common whereas the species composition in Lake Superior was mostly planktonic. Diatom accumulation rates in North Bay, Pokegama Bay, Billings Park, and Allouez Bay peaked in the mid-twentieth century (Fig. 5) whereas accumulation rates were highest around 2000 in Minnesota Point and North of Clough Island. North Bay and Allouez Bay shifted to centric-dominated (i.e. planktonic) assemblages (~ 1900 and ~ 1940 respectively) and had mostly consistent proportions of pennates to centrics. Chrysophyte stomatocyst to diatom ratio was higher in earlier intervals of the North Bay, Pokegama Bay, Billings Park, Minnesota Point, and Allouez Bay cores. Chrysophytes are more competitive in oligotrophic environments; therefore, higher ratios of chrysophyte cysts to diatoms are associated with lower nutrients (Smol 1985). Long-term trends showed a decrease in chrysophyte stomatocyst to diatom ratio indicating increased nutrient loading. In North Bay, Allouez Bay, and Billings Park this ratio continued to decline, while it stabilized in Minnesota Point and Pokegama Bay (around 1970 and 1950 respectively). Accumulation rates of stomatocysts generally had similar stratigraphies to those of diatoms.

Several estuary cores (North Bay, Pokegama Bay, Minnesota Point, and Allouez Bay) transitioned (~ 1850–1900) from benthic diatoms (*Staurosira* Ehrenberg and *Staurosirella* Williams and Round) to assemblages dominated by planktonic *Aulacoseira* Thwaites (Fig. 6).

North Bay

North Bay had two significant zones determined by the broken stick analysis, pre and post-1900. However, based on apparent shifts in diatom assemblages, we delineated three zones in the core: (A) pre-1900, (B) 1900–1945, and (C) post-1945 (Fig. 6a). The core was made up of mostly planktonic diatoms (*Aulacoseira*, *Fragilaria* Lyngbye, and *Stephanodiscus*

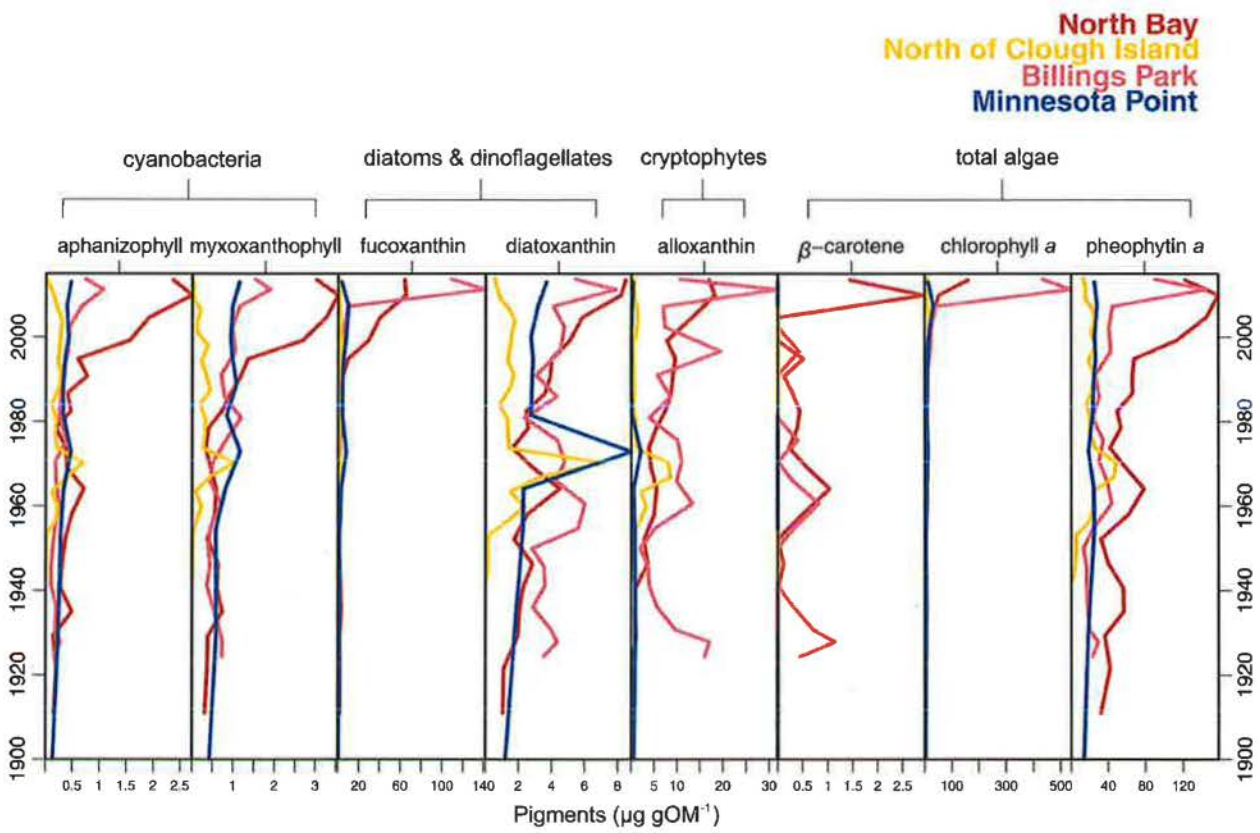


Fig. 4 Recent concentrations of various algal pigments determined by HPLC in four SLRE cores

Ehrenberg), but was also accompanied by some benthic genera (*Staurosira*, *Achnanthidium* Kützing, *Cocconeis* Ehrenberg, and *Navicula* Bory de Saint-Vincent). *Staurosira construens* var. *venter* (Ehrenberg) Hamilton and *Staurosirella pinnata* (Ehrenberg) Williams & Round dominated Zone A (combined ~ 20%). These epipsammic and epidemic diatoms indicate a low-nutrient, benthic-dominated community (Estepp and Reavie 2015; Morales 2010a). In the early 1900s (Zone B), species comprising the modern assemblage increased in abundance while *S. construens* var. *venter* and *S. pinnata* declined. Higher-nutrient indicators *Aulacoseira ambigua* (Grunow) Simonsen, *Stephanodiscus parvus*, *Cyclotella meneghiniana* Kützing, and *Stephanodiscus hantzschii* Grunow (Stoermer et al. 1985; Stoermer and Yang 1970; Stoermer and Håkansson 1984) appeared in greater abundance in Zone C. Since their initial increase, some species (*C. meneghiniana* and *S. hantzschii*) declined in the last decade, although a few high nutrient-tolerant taxa (*Aulacoseira granulata* (Ehrenberg) Simonsen and *S. parvus*) became more abundant. Also in Zone C, benthic and epiphytic taxa like *Cocconeis placentula* Ehrenberg (Round et al. 1990), and *Navicula gregaria* Donkin (Round et al. 1990) increased in abundance, reflecting a probable, local increase in macrophyte habitat. Benthic *Fragilaria vaucheriae* Petersen (Morales 2010b) and *Fragilaria mesolepta* Rabenhorst (Potapova and Spaulding 2013) were also higher in Zone C.

Pokegama Bay

Centric diatoms dominated the core from Pokegama Bay (Fig. 6b). While only four zones were determined to be significant by broken stick analysis, we interpreted five zones based on changes in characteristic species. The historical community in Zone A (pre-1830) consisted of *S. pinnata* (benthic; Estepp and Reavie 2015), *Achnanthidium minutissimum* (Kützing) Czarnecki (epiphytic; Potapova 2009), and *Aulacoseira pusilla* (Meister) Tuji & Houk (planktonic; Potapova 2010). In Zone B (1830–1910), *A. pusilla*, joined by *A. granulata* and *A. ambigua* increased in

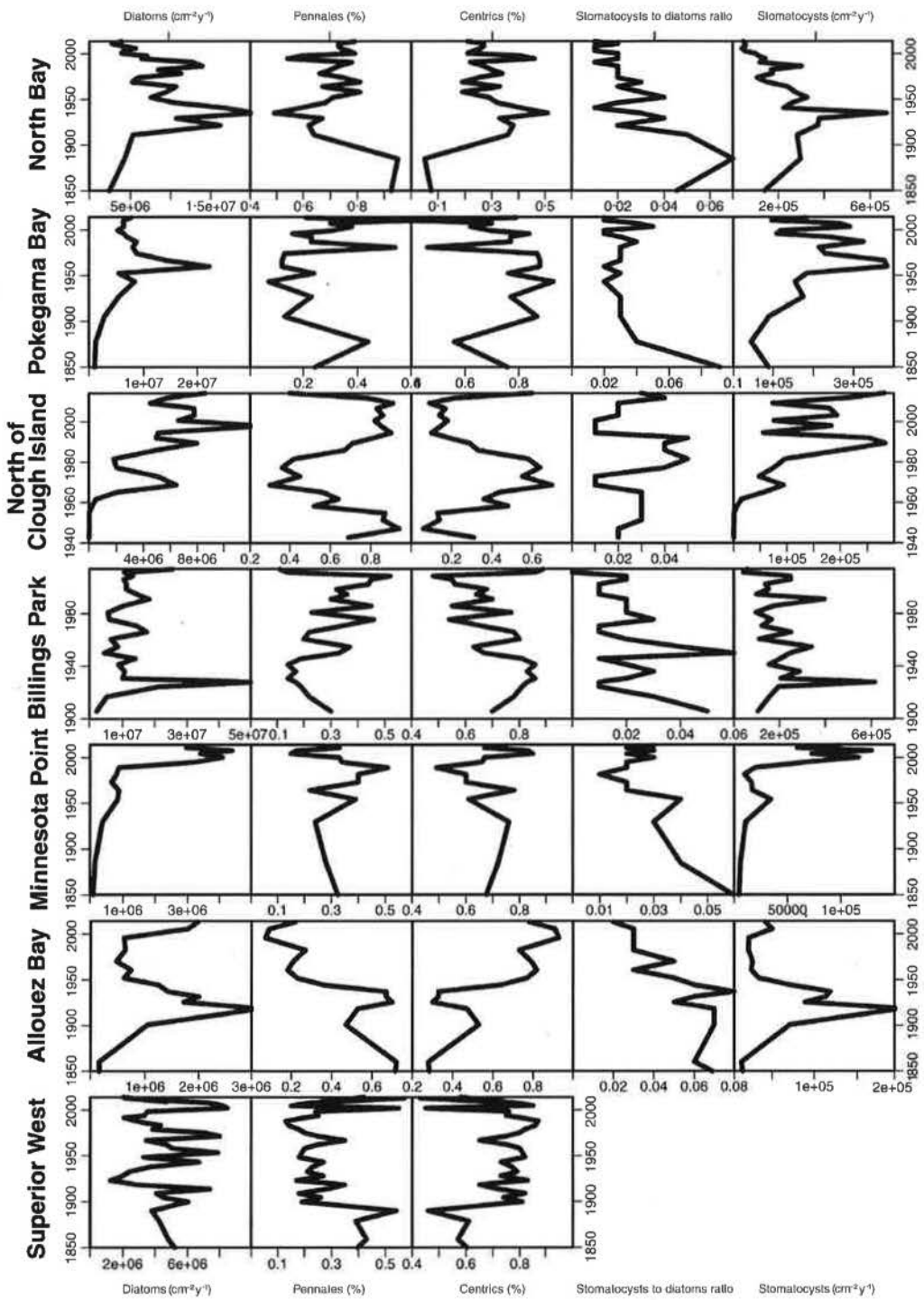


Fig. 5 Diatom accumulation rates, % pennates, % centrics, ratio of chrysophyte stomatocysts to diatoms, and chrysophyte stomatocyst accumulation rates of seven cores in the SLRE and Lake Superior. Chrysophyte stomatocysts were not in great enough abundance in Lake Superior to be plotted. Note that x-axis scales vary among cores to better illustrate temporal trends

abundance to dominate the assemblage. *A. ambigua* and *A. granulata* continued to increase in Zone C (1910–1970) until their peak (35% in 1940, 45% in 1960) after which they declined. Eutrophic *S. parvus* also increased in this period until it declined in Zone D (1970–1980), though its abundance was still higher

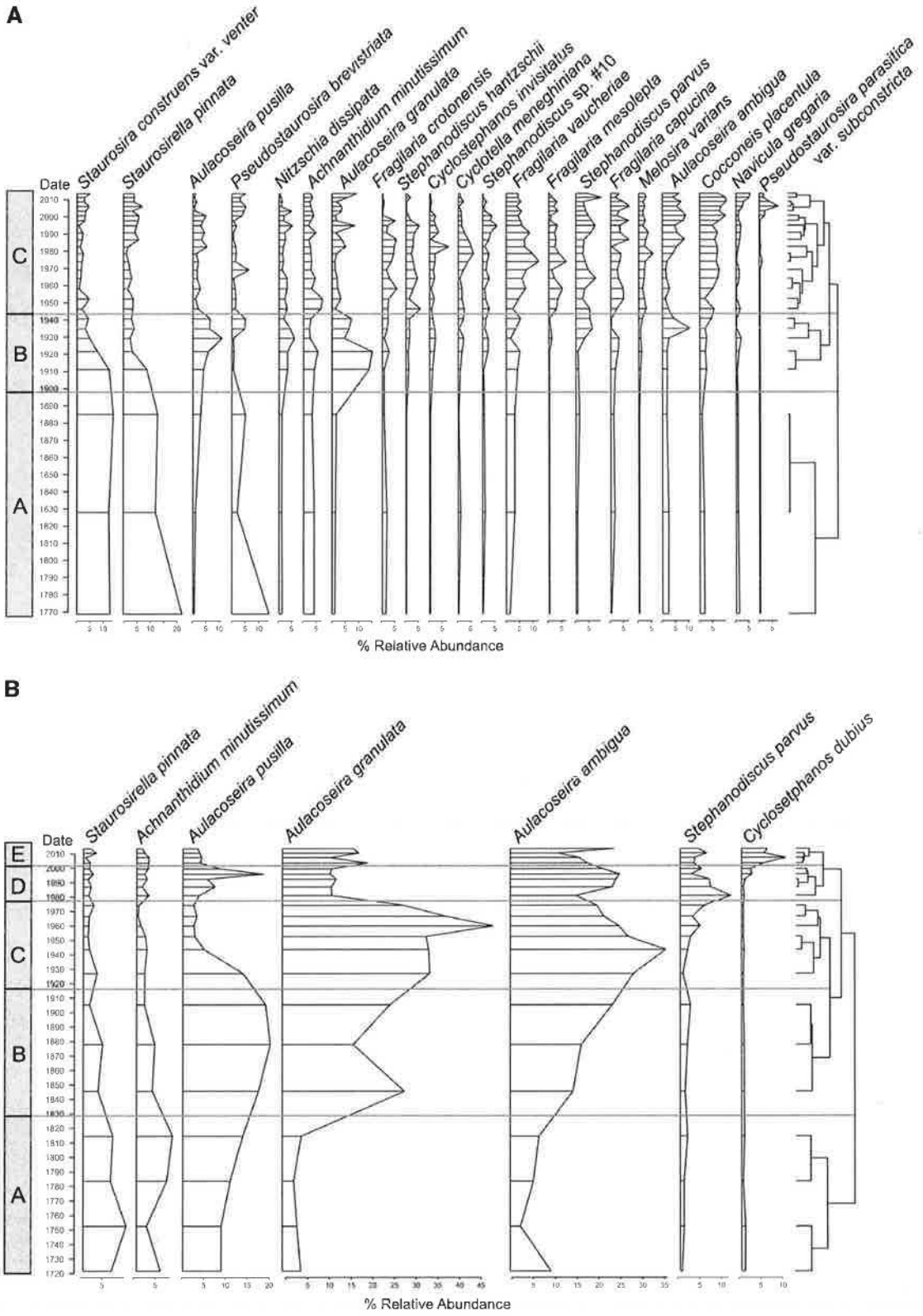


Fig. 6 Relative abundances of the most common taxa in the core taken from **a** North Bay, **b** Pokegama Bay, **c** North of Clough Island, **d** Billings Park, **e** Minnesota Point, **f** Allouez Bay, and **g** Western Lake Superior. The labeled zones represent changes in assemblages determined by cluster analysis

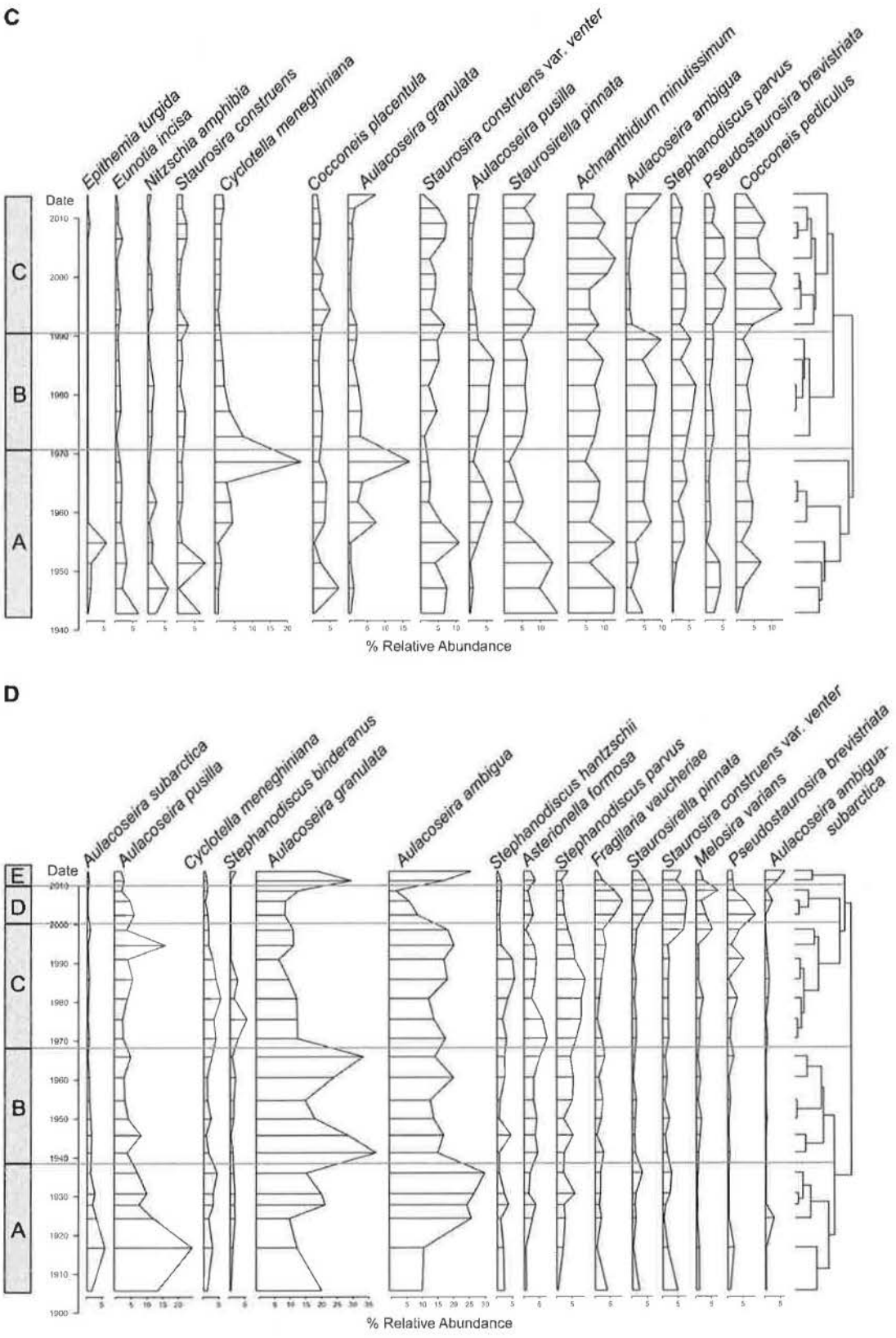
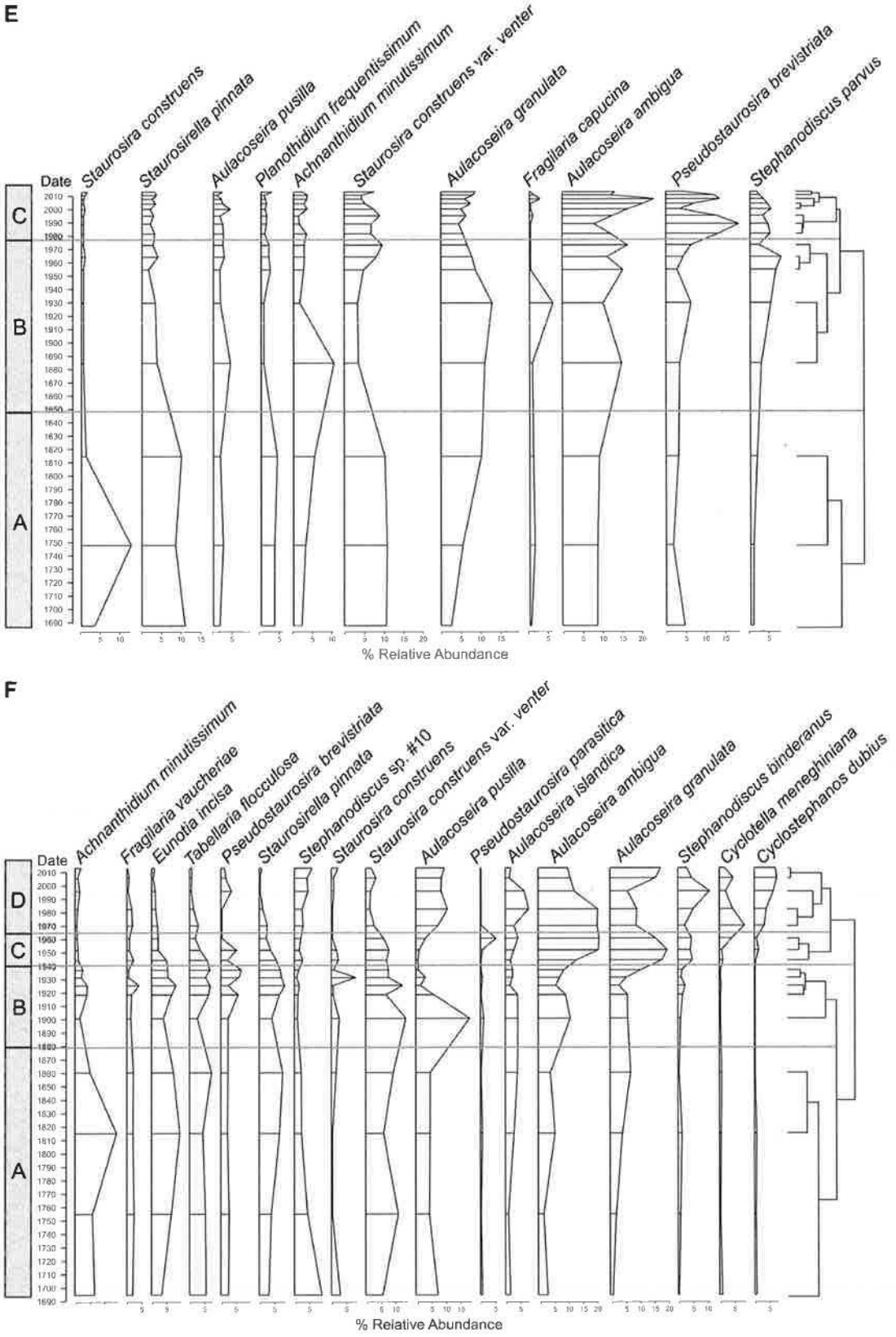


Fig. 6 continued



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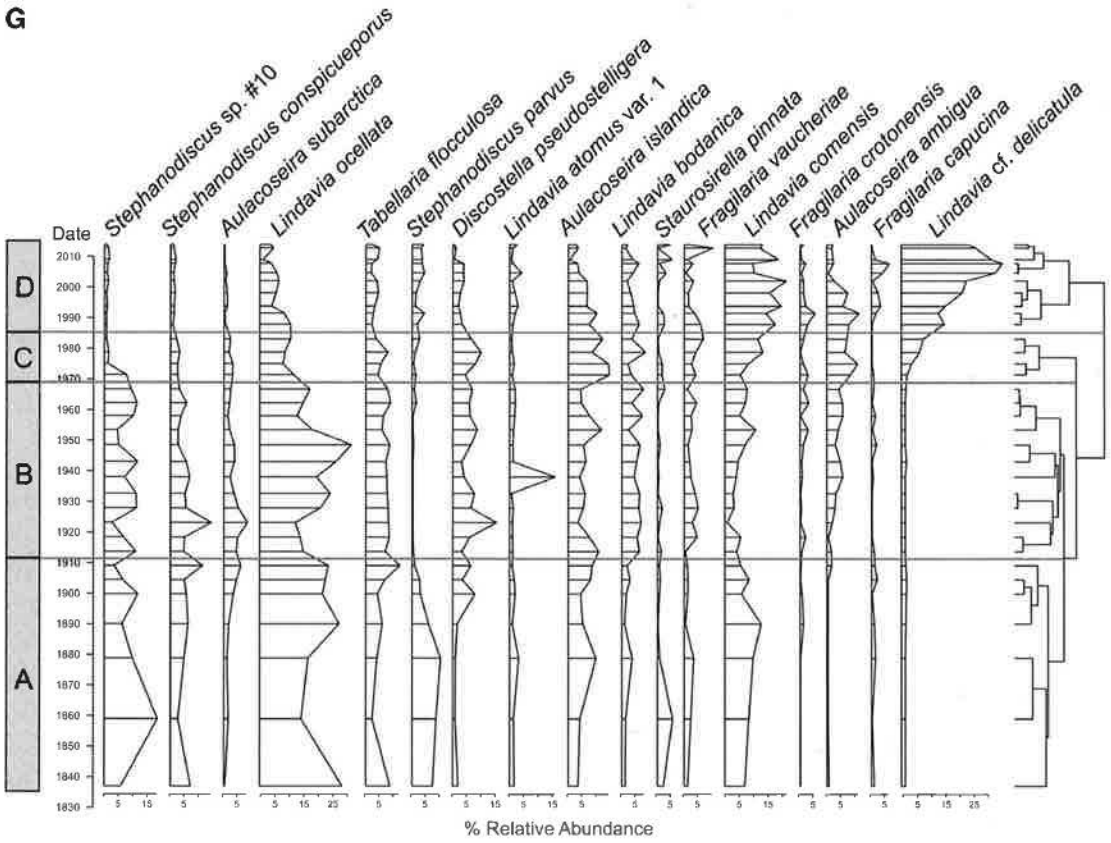

Fig. 6 continued

than pre-European settlement. In Zone E (post-2000), *Aulacoseira* still dominated (~ 40%) but was partly replaced by another eutrophic diatom, *Cyclostephanos dubius* (Fricke) Round (Hickel and Håkansson 1987), whose abundance grew to around 10% of the diatom assemblage.

North of Clough Island

The diatom record from North of Clough Island only extended back to 1940, so pre-impact conditions cannot be determined (Fig. 6c). The broken stick analysis determined at least three temporal zones were significant. In Zone A (pre-1970), eutrophic indicators *C. meneghiniana* and *A. granulata* peaked in the late 1960s (20% abundance) and then rapidly returned to earlier conditions (1–4%) in Zone B (1970–1990). In Zone B, *S. parvus* also increased, but abundance decreased around 1990 (the start of Zone C). Common genera were *Cyclotella* (Kützing) Brébisson, *Cocconeis*, *Aulacoseira*, *Achnanthidium*, *Staurosira*, and *Staurosirella*. We again note great uncertainty in the timing of changes in this core due to the recent flood, which may have deposited allochthonous material in an undetermined layer near the core surface. We have confidence the uppermost ~ 2 intervals represent post-flood deposition, and the assemblage (*S. pinnata*, *A. minutissimum*, and *A. ambigua*) indicates lower nutrients than pre-1970 taxa.

Billings Park

The core from Billings Park was dominated by planktonic diatoms, especially species from the genus *Aulacoseira* (Fig. 6d). The core had three significant zones; however, we determined five zones showed important changes in assemblages. The assemblage was made up of largely *A. pusilla*, A. *granulata*, and *A. ambigua* in Zone A (~ 1900–1940), but shifted to *A. granulata* and *A. ambigua* dominance in Zone B (1940–1970). Nutrient-tolerant diatoms *S. parvus*, *S. hantzschii*, *Stephanodiscus binderanus* (Kützing)

Krieger, and *C. meneghiniana* increased in abundance in Zone C (1970–2000), followed by a partial decline as they were replaced by small, benthic species (e.g. *S. construens* var. *venter*, *S. pinnata*, and *Pseudostaurosira brevistriata* (Grunow) Williams & Round) in Zone D (2000–2010). Zone E (post-2010) shifted back to an *Aulacoseira*-dominated assemblage similar to before ~ 1970.

Minnesota Point

The core from Minnesota Point was comprised of centric and araphid planktonic diatoms with a smaller proportion of benthic species (Fig. 6e). Three zones (two were determined significant by broken stick analysis) were identified: (A) pre-1850, (B) 1850–1980, and (C) post-1980. *Staurosira construens* Ehrenberg, *S. pinnata*, *S. construens* var. *venter*, *A. granulata*, and *A. ambigua* dominated Zone A, which existed as far back as ~ 1700. In Zone B, *Staurosira* and *Staurosirella* decreased and there was some growth in the already dominant *Aulacoseira* population. Eutrophic *S. parvus* increased and reached a maximum abundance (< 10%) in ~ 1965 and returned to near pre-European settlement abundances (< 5%) in Zone C. Also in Zone C, *A. granulata*, *A. ambigua*, and *P. brevistriata* increased.

Allouez Bay

Allouez Bay consisted of mostly planktonic diatoms (Fig. 6f). Although the broken stick analysis only found two significant zones, we delineated four zones based on apparent changes in diatom assemblages. The historical assemblage (Zone A, pre-1880) was very diverse, including the phytoplankton *Aulacoseira subarctica* (O. Müller) Haworth, *A. pusilla*, *A. ambigua*, *A. granulata*, *Stephanodiscus* sp. #10, the epiphytic *A. minutissimum* and *Eunotia incisa* Smith ex Gregory, and the benthic *S. construens* var. *venter* (each ~ 5%). In Zone B (1880–1940), *A. ambigua* and *A. granulata* grew to dominate the assemblage, indicating greater planktonic dominance and probable nutrient enrichment. They continued to rise and reached a maximum (together 40% of the assemblage) in Zone C (1940–1960). In Zone D (post-1960), eutrophic indicators *S. binderanus*, *C. meneghiniana*, and *C. dubius* (Stoermer et al. 1987; Hickel and Håkansson 1987) began to increase in abundance, each occupying 5–10% of the assemblage in the upper intervals.

Lake Superior

The species assemblage in Lake Superior was dominated by planktonic, centric diatom species (*Lindavia* (Schutt) De Toni & Forti, *Cyclotella*, *Stephanodiscus*, and *Aulacoseira*) (Fig. 6g). Zone A (pre-1910) was dominated by *S.* sp. #10, *Lindavia ocellata* (Pantocsek) T. Nakov et al., *Lindavia atomus* var. 1, and *Lindavia comensis* (Grunow in Van Heurck) T. Nakov et al., taxa generally reflecting low nutrients. *Stephanodiscus conspicueporus* Stoermer, Håkansson & Theriot, *A. subarctica*, *A. islandica*, and *A. ambigua*, mesotrophic diatoms indicating higher nutrients in oligotrophic Lake Superior (Stoermer 1993) increased in Zone B (1900–1970) but decreased in Zone C (1970–1985). Small centric diatoms, *L. comensis* and *Lindavia* cf. *delicatula* (Hustedt) T. Nakov et al. (Reavie and Kireta 2015), began increasing in Zone C and increased to a combined abundance of ~ 40% in Zone D (post-1985). These low-nutrient taxa may be related to climate-driven physical changes in the lake (Shaw Chraïbi et al. 2014; Reavie et al. 2017a).

Ordination

Based on an initial ordination of diatom samples from all cores, Lake Superior was highly dissimilar to SLRE cores, indicating substantial differences in common taxa between the lake and SLRE (Fig. 7a). Therefore, the analysis was repeated to examine (1) all cores, (2) Lake Superior, and (3) SLRE cores to better visualize historical trajectories in NMDS ordinations.

NMDS of Lake Superior (Fig. 7b) reflected a constant reorganization of the diatom assemblage, from a pre-1900 assemblage dominated by *S.* sp. #10, *L. ocellata*, and *L. comensis*, followed by an increase in higher nutrient taxa (e.g. *A. subarctica* and *A. islandica*) in the upper right quadrant. Migration to the left reflected current conditions dominated by small centrics such as *L. comensis* and *L.* cf. *delicatula*.

With the exception of Billings Park, the oldest intervals of each SLRE core fell within the lower, right quadrant (Fig. 7c), indicating consistent assemblage baselines of *S. construens*, *S. construens* var. *venter*, and *S. pinnata*. Into the twentieth century assemblages migrated to the upper, left quadrant, representing

assemblage shifts associated with higher nutrients (e.g. *C. meneghiniana* and *S. parvus*). The most recent sample scores in Billings Park, Pokegama Bay, and Allouez Bay were especially constrained to the left of the ordination in accordance with higher relative abundances of *C. dubius, S. binderanus*, and *Aulacoseira* spp. In general, fossil assemblages in the SLRE exhibited consistent reorganization, and there was little evidence that recent diatom communities have returned to pre-impact assemblages.

Diatom-inferred modeling

Based on model validation, there was a significant relationship between changes in TP and diatom assemblages in all cores (i.e. DI-TP strongly correlated with the primary gradient of variation in assemblages in each core). Further, analog analyses showed good fit between fossil assemblages and model training sets in all cases (ESM1; Alexson 2016).

DI-TP results (Fig. 8) indicated western Lake Superior had much lower concentrations of TP (3–6 μg L^{-1}) than the SLRE (15–80 μg L^{-1}). DI-TP increased during the mid-twentieth century in open water cores (Lake Superior, Minnesota Point, and North of Clough Island), followed by a decline in western Lake Superior and North of Clough Island cores and stabilization in the Minnesota Point core. Cores taken from SLRE bays (North Bay, Billings Park, Allouez Bay, and Pokegama Bay) generally showed increasing DI-TP since the mid-twentieth century.

DI-TP in western Lake Superior showed increasing TP concentrations in the early 1900s with a maximum concentration of 5.5 μg L^{-1} around 1930 and a secondary peak (5 μg L^{-1}) around 1970. After 1970, TP decreased and stabilized around pre-European settlement concentrations (~ 3 μg L^{-1}), similar to observations in other Lake Superior cores (Shaw Chraïbi et al. 2014).

In the SLRE, the open-water environments (Minnesota Point and North of Clough Island) showed stabilization or a decrease of TP. The DI-TP from North of Clough Island showed an increase from 25 to 65 μg L^{-1}, peaking around 1970. Because there was no diatom record from the North of Clough Island core before 1940, it was not possible to compare pre and post-European settlement conditions. The North of Clough Island reconstruction indicated an increase in the late 1900s from 25 to 35 μg L^{-1} TP. After a peak in 1980, DI-TP stabilized around 30 μg L^{-1}. Again, due to uncertainty in accumulation, we inferred higher nutrients in the 1960s and lower nutrients today, but timing of transitions are ambiguous.

Cores from bay environments showed modern conditions of increasing DI-TP. Cores from Allouez Bay and Pokegama Bay both remained at near-constant concentrations of DI-TP (30 and 45 μg L^{-1}, respectively) until ~ 1950, after which TP concentration increased to as high as ~ 80 μg L^{-1}. DI-TP began to increase around 1920 at North Bay and rose from ~ 50 to ~ 60 μg L^{-1} in modern intervals. In Billings Park DI-TP increased from ~ 1950 to ~ 2000 (from ~ 20 to 38 μg L^{-1}), followed by two modern intervals with lower DI-TP (~ 20 μg L^{-1}).

Compared to historical measured TP from a location in the lower estuary (at Blatnik Bridge; Bellinger et al. 2016), DI-TP concentrations were lower in all cores (Fig. 8); however, the general trend of declining monitored TP in recent decades was similar to DI-TP from North of Clough Island and Lake Superior cores. The monitoring dataset from Bellinger et al. (2016) at Blatnik Bridge spanning 1973–2014 showed a peak of TP in ~ 1980 (~ 180 μg L^{-1}) and afterward a steady decrease in TP concentration to approximately 40 μg L^{-1} (based on the lowess smoothing), closely matching modern DI-TP of ~ 30 μg L^{-1} from Minnesota Point and North of Clough Island cores.

Discussion

These paleolimnological data describe the history of anthropogenic influence on the SLRE and western Lake Superior and reveal where remediation may be occurring. As previously detailed by Reavie and Edlund (2010), paleolimnology in lotic environments can be challenging. We believe we have overcome these limitations through application of multiple fossil indicators and careful selection of core locations.

Evidence of early impacts from logging and subsequent modifications of the drainage basin and the St. Louis River were found in the paleorecord. When logging was at its peak (~ 1850–1900), a transition in SLRE diatom communities from benthic genera (*Staurosira* and *Staurosirella*) to centric,

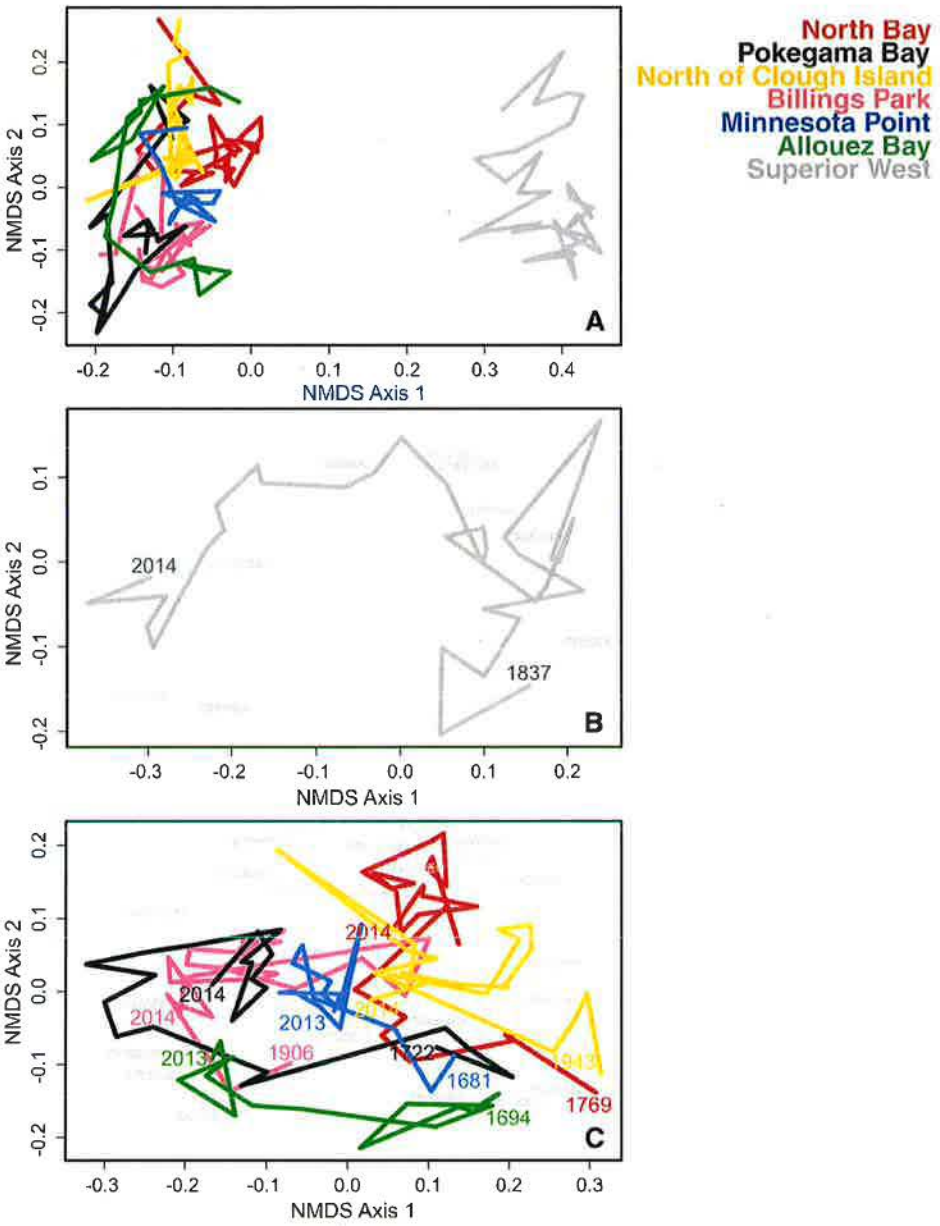

Fig. 7 NMDS analysis of diatom species assemblages (> 5% relative abundance) in **a** all seven cores from the SLRE and Lake Superior (stress of 0.1258), **b** Lake Superior (stress of 0.0889), and **c** the SLRE (stress of 0.1663)

planktonic diatoms (e.g. *Aulacoseira*) in North Bay, Pokegama Bay, Minnesota Point, and Allouez Bay suggested a physical transformation to a more lacustrine (but still fluvial) system because of hydrological manipulation by damming and dredging of the St. Louis River.

By the 1930s, with growing industries and a growing population to support them, the SLRE's ecology changed. Increased sedimentation rates, greater abundance of eutrophic diatom species, and higher DI-TP dominated the paleorecord. This was likely due to the combined effects of untreated wastewater and runoff from a landscape transformed by logging. With the construction of Fond du Lac Dam (upstream of all core locations) in 1924, decreased sedimentation rates were expected due to the retention effect of the new reservoir. However, it is clear other factors (algal production and watershed disruptions leading to increased erosion) contributed to increased sediment loads at some locations. Since 1970, sedimentation decreased in all cores with the exception of Minnesota Point and Pokegama Bay, and nutrient trajectories varied among locations. Cores from SLRE open-water environments suggested a remediation or

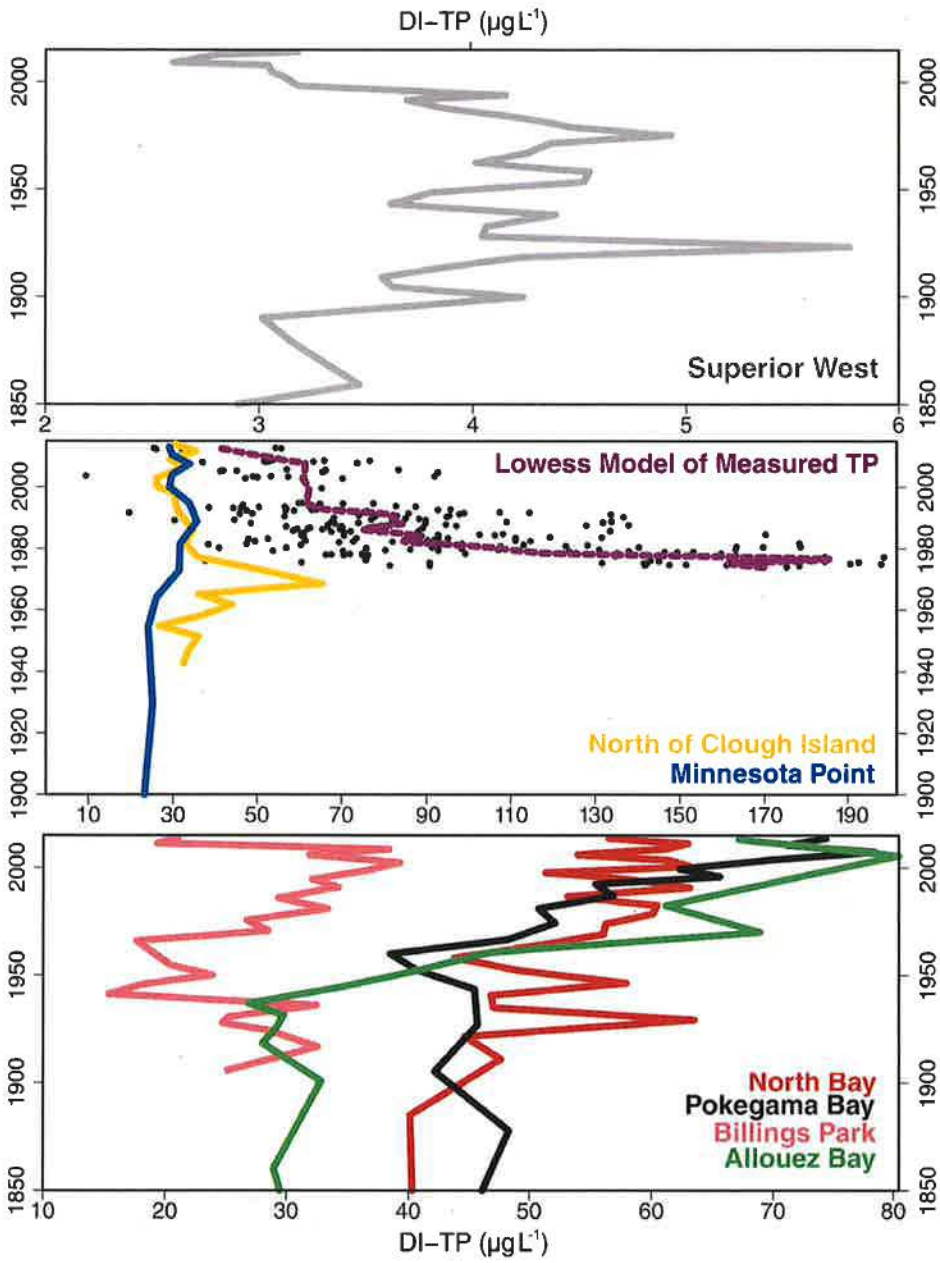

Fig. 8 Diatom-inferred total phosphorus from all cores. The purple line represents a lowess model of total phosphorus measurements (black dots) from the Blatnik Bridge from 1973 to 2012 as reported in Bellinger et al. (2016)

stabilization of phosphorus loading, while excess loading may be continuing in embayments. Fossil pigments corroborate this recent trend with increased concentrations of pigments from total algae and those from cyanobacteria in two bay locations.

Changes in legislation such as the Clean Water Act in 1972 accompanied by restoration efforts are associated with recovery we observed in some cores. The recovery is defined partly by a decrease in nutrient-tolerant diatoms—*Aulacoseira* spp. and *S. conspicueporus* in western Lake Superior, *Aulacoseira* spp. and *C. meneghiniana* in North of Clough Island, and *S. parvus* in Minnesota Point. This was affirmed by a decrease in DI-TP—a reduction in Lake Superior and North of Clough Island and apparent stabilization in Minnesota Point.

Results from these cores mostly agreed with monitoring data from Bellinger et al. (2016). Though the overall measured TP trend matched DI-TP, concentrations found by Bellinger et al. were much higher than those inferred by the model. This discrepancy may be due to the natural variability in the SLRE

as the nearest coring location (Minnesota Point) is ~ 3.5 km away.

Fossil data from four cores taken from bay environments suggest continued high nutrients in these parts of the SLRE. Higher populations of all algae groups (notably cyanobacteria), a growth in abundance of nutrient-tolerant diatoms (*C. dubius*, *C. meneghiniana*, *S. parvus*, and *S. binderanus*), and increased DI-TP all support this conclusion. Recent persistence of high concentrations of nutrients in parts of the SLRE may be due to more localized nutrient sources, potentially from recent residential development and continued presence of industry, or enhanced internal loading of sedimentary nutrient pools. But, contemporary anthropogenic issues facing other water bodies such as those reported in Lake Erie—internal phosphorus loading and higher runoff from high-intensity rain events associated with climate change (Kane et al. 2009; Matisoff et al. 2016)—may also be responsible. Nearby in Lake of the Woods, a shallow, multinational lake bordering Canada and the United States, cyanobacterial blooms and high nutrient levels persist despite a reduction in allochthonous phosphorus. In that case, thermal stratification may be enhancing internal phosphorus loading and altering nutrient stoichiometry to favor nitrogen-fixing cyanobacteria (Reavie et al. 2017b). Similarly in Switzerland's Lake Zurich enhanced stratification due to warmer atmospheric temperatures has aggravated hypoxia and increased sediment phosphorus releases in lakes (North et al. 2014). Such possible drivers need additional study in the SLRE.

There is little doubt efforts to remediate the SLRE reduced the flux and concentration of nutrients in the SLRE (Bellinger et al. 2016). To meet beneficial use impairment removal targets, the portion of Lake Superior in the Area of Concern must have TP concentrations below 10 μg L^{-1}, the upper limit for oligotrophic designation, and the estuary must be below 30 μg L^{-1}, the upper limit for mesotrophic designation according to Minnesota standards (MPCA and WDNR 2013). According to DI-TP, western Lake Superior has always fallen within passing criteria, and is suitable for delisting. Minnesota Point, North of Clough Island, and Billings Park (at least according to the most recent interval) have TP concentrations around or below 30 μg L^{-1}, also meeting delisting criteria; whereas, North Bay, Pokegama Bay, and Allouez Bay exceed desired concentrations and may not be acceptable for delisting. Pre-impact concentrations of DI-TP at North Bay and Pokegama Bay (40–45 μg L^{-1}) surpass delisting criteria, so a criterion of 30 μg L^{-1} may be unrealistic for these areas as they appear to be naturally higher in water column TP. Delisting goals may need reconsideration to accommodate the natural state of and variability within the estuary and address more modern stressors like climate change and internal phosphorus loading not well understood at the time of the Area of Concern listing. Managing agencies may choose to remove the nutrient beneficial use impairment with the intention of addressing these modern issues driving water quality in the estuary.

Presently, only four American and three Canadian Areas of Concern in the Great Lakes have been delisted, leaving 36 still listed. Although there have been paleolimnological studies in Areas of Concern in the past (Reavie et al. 1998; Yang et al. 1993), there have been few studies done intentionally to advise Area of Concern programs. In a similar study to ours, Dixit et al. (1998) examined sedimentary metals, accumulation rates, and diatom taxa to understand the anthropogenic influence in Spanish Harbor of Lake Ontario to inform a management plan. They found similar anthropogenic activities facing the SLRE (paper mills, iron smelting, and untreated wastewater) led to increased metal concentrations and nutrients. In a similar program, the European Union Water Framework Directive has used paleolimnology extensively to aid in management of impacted surface waters (Bennion and Battarbee 2007). As demonstrated here and elsewhere, paleolimnological investigations can be useful in not only developing management plans, but also gauging the success of remediation efforts to ensure progress in degraded surface waters.

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Appendix 3

Saint Louis River Estuary Clay-Influenced Bay Assessment 2018
And
St. Louis River Bays – Douglas County 2017 Fish Community Survey
(Pertains to management action 6.04)

Saint Louis River Estuary
Clay-Influenced Bay Assessment

Pokegama Bay on a fall day. Photo by Craig Roesler, Wisconsin DNR

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Funded by The U.S. Environmental Protection Agency

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About

The Saint Louis River Estuary (SLRE) is a Great Lakes Area of Concern where efforts to improve water quality, including nutrient and sediment loading reductions, have been ongoing. Some bays in the estuary were known to have substantially higher total phosphorus concentrations than the remainder of the estuary. Three bays, Allouez, Pokegama, and Kimballs, were extensively monitored for water quality and biological condition in 2017. Objectives of the monitoring were to:

- Document the current water quality and biotic conditions in these SLRE clay-influenced bays
- Determine if current nutrient and suspended solids concentrations are negatively affecting aquatic life
- Provide data that could be used to determine if site specific water quality goals are warranted

Funding

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Summary

Introduction

The lower St. Louis River (SLR) is part of a Great Lakes Area of Concern (AOC) (Figure 1). The SLR AOC has nine beneficial use impairments (BUI's) listed in the Remedial Action Plan (MPCA and WDNR 2017). One of the BUI's is "Excessive Loading of Sediment and Nutrients."

Beneficial Use Impairment Target

BUI removal target of 30 ug/l for total phosphorus has been established for the St. Louis River portion of the AOC.

This target was established to ensure that anthropogenic sources and activities in the St. Louis River AOC do not result in excessive phytoplankton productivity and nuisance algal conditions within the St. Louis River Estuary (SLRE).

Diatom-inferred (DI) total phosphorus concentrations (TP's) from sediment core analyses (Reavie 2016) indicated that TP's in some SLRE bays (including Allouez and Pokegama Bays) currently exceed the TP goal (30 ug/l) for the SLRE and were at or above this goal prior to watershed development. Therefore, site specific water quality goals for these bays may be appropriate.

Three clay-influenced bays on the Wisconsin side of the SLRE were selected for monitoring: Allouez Bay, Pokegema Bay, and Kimballs Bay (Figure 2). These areas are the largest clay-influenced bays in the estuary. Limited pre-existing water quality data was available for these bays. Direct watersheds for these bays contain clay-rich soils that are highly erodible, and prone to high rates of surface runoff.

The monitoring was intended to:

- Document the current water quality and biotic conditions in these SLRE clay-influenced bays.
- Determine if current nutrient and suspended solids concentrations are negatively affecting aquatic life.
- Provide data that could be used to determine if site specific water quality goals are warranted.

The three bays were monitored during May – October of 2017 for water quality, algae, sediment chemistry, and benthic invertebrates. Tributary streams for the bays were monitored for water quality. Pre-existing water quality and biotic information was reviewed and summarized. A companion project to assess fish communities in the bays was also conducted in 2017 (Nelson 2017). A summary of that assessment is included in this report.

Bay Characteristics and Water Quality

The three bays have some unique characteristics that influence their water quality. Allouez Bay is large (1,011 acres), shallow, and subject to frequent wind-induced mixing. The mouth of the bay is adjacent to the Superior entrance to Lake Superior. Seiche-induced backflows of Lake Superior water influence the bay.

Allouez Bay had minimal thermal and dissolved oxygen stratification. There were indications that seiche-induced inputs of cooler Lake Superior water flowed along the bay bottom at times. Mean total phosphorus concentration was 85 ug/l. Mean total suspended solids concentration was 21 mg/l. Mean chlorophyll a concentration was 7.1 ug/l. Total phosphorus and total suspended solids concentrations were higher in May and October when more watershed runoff was entering the bay. Chlorophyll a concentrations were highest in June and August when watershed runoff was low and water clarity was higher.

Pokegama Bay (441 acres) has the largest direct watershed area and so is heavily influenced by Pokegama River inflow. The bay is also affected by wetlands that fringe its narrow upstream end. Pokegama Bay also had minimal thermal and dissolved oxygen stratification. Lower dissolved oxygen concentrations occurred more frequently at the surface in the upstream end of the bay, probably due largely to decomposition of organic matter in the fringe wetlands.

There may have been occasional releases of sediment phosphorus from the deeper areas in the bay. Phosphorus release from the fringe wetlands may also have occurred. Mean total phosphorus concentration was 121 ug/l. Mean total suspended solids concentration was 32 mg/l. Mean chlorophyll a concentration was 6.2 ug/l. Total phosphorus and total suspended solids concentrations were higher in May and October at the two more downstream monitoring stations when more watershed runoff was entering the bay. Total phosphorus and total suspended solids concentrations were more variable at the most upstream monitoring station which is most strongly influenced by Pokegama River inflow. Chlorophyll a concentrations were highest in July and August when watershed runoff was low and water clarity was higher.

Kimballs Bay (101 acres) is the smallest of the three bays. Steep sloped, wooded banks line the bay's perimeter. The narrowness of the bay and the high wooded banks tend to minimize wind-induced mixing. The greater mean depth (12 ft) also helps minimize mixing. The single water quality monitoring site in the bay was close to the bay mouth and strongly influenced by seiche-induced mixing of SLRE water.

Kimballs Bay had substantial thermal and dissolved oxygen stratification despite the influence of seiche-induced mixing. Sediment phosphorus release was also substantial and prolonged during July and August. Inflow from the small tributary stream appeared to be mostly flowing along the bottom of the bay and producing higher turbidities near the bottom. Mean total phosphorus concentration was 63 ug/l. Mean total suspended solids concentration was 5 mg/l. Mean chlorophyll a concentration was 7.6 ug/l. Total phosphorus concentrations were somewhat higher in May and October, and there was also a pattern of increasing total phosphorus concentrations from mid-June to early September, probably due to sediment phosphorus release. Total suspended solids concentrations were higher in May and October like the other bays. Chlorophyll a concentrations were higher in July through early September when water clarity was higher.

For the three bays, mean total phosphorus concentrations were 2-4 times higher than those found in the rest of the SLRE (Bellinger et al 2015). Mean chlorophyll a concentrations were lower than those found in the rest of the SLRE. Mean total suspended solids concentrations were lower at the Kimballs Bay site and higher at the Allouez and Pokegama Bay sites compared to the rest of the SLRE. The data is summarized below in Table 1.

Table 1. Summary of Mean Total Phosphorus, Total Suspended Solids and Chlorophyll a Concentrations

	Size (acres)	Mean Depth (ft)	Mean TP (ug/L)	Mean TSS (ug/L)	Mean Chl a (ug/L)
Allouez Bay	1,011	6	**85**	**21**	7.1
Pokegama Bay	441	5	**121**	**32**	6.2
Kimball's Bay	101	12	**63**	5	7.6
Estuary Mean	NA	NA	31	11	9.4

(bold #s indicate values higher than the estuary mean)

Bay Chlorophyll a Relationship to Other Trophic State Indices

Chlorophyll a concentrations in the three bays showed non-standard relationships to other trophic state indices (TSI) (trophic state is a water body's level of biological productivity). Chlorophyll a concentrations were only 3 -18% of what is typically found at the total phosphorus concentrations present (Carlson 1977). Water clarities (Secchi depths) are also lower than what is typically found. The poor water clarity due to suspended clay and silt is the probable reason for these altered relationships. Suspended clay and silt is controlling water clarity and the resultant lack of light availability is limiting algal growth. Lack of typical TSI parameter relationships complicate water quality goal setting since it makes it difficult to predict responses to water quality improvements.

Bay Tributary Stream Characteristics and Monitoring Results

Land use in bay tributary stream watersheds is mostly undeveloped, with 77-94% of the watersheds comprised of forest and wetland. Agricultural row crops are absent in four of the watersheds, and only account for 1.2% of the land use in the Bear Creek watershed. Grassland (pasture and hayfield) is the largest agricultural land use and comprises 1.4-20.6% of the watersheds. Streambank and bluff erosion along streams has been identified as the dominant source of fine sediment to clay plain streams. Most runoff and streamflow occurred in May, late September, and October. Total May-October 2017 precipitation was 15% above normal.

Stream dissolved oxygen concentrations were nearly all above 5 mg/l (the WI water quality standard), except for the small tributary to Kimballs Bay that had dissolved oxygen concentrations less than 5 mg/l on two dates. That site was influenced by fringe wetlands and seiche-induced backflows, which likely accounted for the higher dissolved oxygen variability.

Stream total phosphorus concentration means ranged from 106-224 ug/l. Orthophosphate concentration means ranged from 12-33 ug/l. Total suspended solids concentration means ranged from 28-106 mg/l. Watershed non-point sources of phosphorus include pasture and hayfield runoff (including the influence of manure spreading), barnyards, and septic systems. Streambank and bluff erosion along streams is not believed to be a large phosphorus source (Bahnick 1977) but is believed to be the largest source of total suspended solids.

Effluent discharged to the Pokegama River from the Village of Superior wastewater lagoons was the only point source affecting bay tributary streams. During May-October lagoon effluent was estimated to provide about 5.7% of the Pokegama River total phosphorus load and 2.8% of the river's biochemical oxygen demand load. Elevated orthophosphate concentrations in the Pokegama River were seen when stream flow was low and lagoon discharge was occurring.

Elevated concentrations of orthophosphate, ammonia, and nitrate plus nitrite concentrations occurred during low flows in Bear Creek. This may be due to residential septic system discharges. Problems with failing septic systems have been previously documented in the Bear Creek watershed.
Unusually red water was observed in Bluff Creek on one date. Runoff from the rail yard at the taconite storage facility appeared to be the likely source of the color.

Bay Sediment Characteristics

Mean clay content of sediment in all three bays (40 – 46%) was significantly higher than that found in the remainder of the central and lower SLRE, where clay content averages about 14.7% (NOAA DIVER 2018); this is not surprising given the clay rich soils in the watersheds of the bays. Additional findings are summarized below.

- Clay content of sediment (% Clay) was moderately well correlated with phosphorus concentration ($R^2 = 0.75$) and iron concentration ($R^2 = 0.76$). Iron readily attaches to the extensive bonding surfaces of clay particles and phosphorus will attach to the iron.
- Clay content was also moderately inversely correlated with % solids ($R^2 = 0.43$). Clay sediment tends to have a higher water content than coarser grained sediment.
- Allouez Bay sites had the highest mean, median and maximum % sand. There was an inverse correlation between site depth and % sand for the bay ($R^2 = 0.73$). Sediment scouring by wave action is probably removing finer sediments at shallow sites and leaving more sand.
- Soft sediment thickness ranged from 0.9 to 12.9 feet. Water depth and soft sediment thickness showed moderate correlations for individual bays (Allouez Bay (less site ASD), $R^2 = 0.42$; Pokegama Bay, $R^2 = 0.70$). Deeper sites tend to favor long term sediment deposition.

Algae

Total algal cell densities were highest in all bays in July, August, and September. Pokegama Bay had the highest total cell density on July 10th (10,343 cells/ml). All algal phyla occurred in higher densities during those three months. Total suspended solids concentrations and turbidity were lower during these months which increased light availability for algal growth. Water temperatures were higher during these months which can also promote algal growth.

Benthic Invertebrates

The trimetric index (TMI) (Angradi et al 2016) is an index of benthic invertebrate community quality and was developed specifically for the SLRE. Allouez and Pokegama Bays were excluded from the SLRE for the development of the TMI and the accompanying ephemerid density index. However, they are still the most useful benthic invertebrate indices to apply to these bays and provide a basis of comparison to the rest of the SLRE.

The median TMI value for Allouez Bay was poor, for Pokegama Bay was fair, and for Kimballs Bay was poor. The quality of the benthic invertebrate community in all three bays was below average in comparison to the rest of the SLRE. The physical characteristics of sediment with high clay content (and corresponding high-water content) may be restrictive to some benthic invertebrates in all three of the bays, and possibly account for the low TMI values. Periods of anoxia at two sites in Kimballs Bay probably also contributed to the poor median TMI there.

The median ephemerid (mayflies) density index value (Angradi et al. 2016) for Allouez Bay was good, for Pokegama Bay was excellent, and for Kimballs Bay was poor. Median values for Allouez and Pokegama Bays were above average in comparison to the rest of the SLRE. High clay content of sediment does not appear to negatively affect ephemerid species. Periods of anoxia at two sites in Kimballs Bay may have again accounted for the poor median value there.

Aquatic Macrophytes

Results from recent aquatic macrophyte surveys (2004-2015) are available for the three bays (Danz et al. 2017) and are summarized below in Table 2:

Table 2. Aquatic Macrophyte Survey Data for the Bays

	Allouez Bay	Kimballs Bay	Pokegama Bay	All SLRE surveys
Number of species	155	74	148	NC**
Species per plot	8.8	5.0	5.8	NC**
Mean C* value	5.6	3.6	5.4	5.06

*C = coefficient of conservatism, an index of tolerance to disturbance. **NC = not comparable; number of species and species per plot are influenced by size of area surveyed and survey methods, so do not offer a simple means of comparison.

Mean C values for Allouez and Pokegama Bays are better than the mean C value for all SLRE aquatic macrophyte surveys, while the mean C value for Kimballs Bay is poorer. Allouez Bay appears to have the best aquatic plant community, while Kimballs Bay has the poorest.

Wetlands[1]

Recent wetland monitoring data (2011-2017) is available for all three bays from the Great Lakes Coastal Wetland Monitoring Program (Brady 2018).

Wetland nutrient, turbidity, and chlorophyll concentrations were generally similar to those found at open water sampling sites in 2017, although Kimballs Bay total phosphorus concentrations were higher than open water concentrations, suggesting wetland phosphorus release may be occurring at higher rates there.

Daytime dissolved oxygen concentrations in wetlands were below 3 mg/l for a significant percentage of measurements (5-25%), with Kimballs Bay having the highest percentage of such measurements. Less water mixing in Kimballs Bay due to its sheltered nature may account for these differences.
Wetland macroinvertebrate IBI's are available for Allouez and Pokegama Bays for 2011 and 2012. Allouez Bay scores were rated as moderately impacted. Pokegama Bay scores were rated as moderately impacted to most pristine.

Wetland fish IBI ratings for the four years monitored for Allouez Bay ranged from moderately impacted to mildly degraded. The rating for the one year monitored for Kimballs Bay was moderately degraded. The rating for the one year monitored for Pokegama Bay was mildly impacted.

Wetland bird and frog survey results (2012-2013) are available for Allouez and Pokegama Bays (Tozer 2014). Additional wetland bird and frog survey result are available for one or more years during 2014 - 2017 for all three bays (Brady 2018).

For the 2012-2013 bird surveys, Allouez Bay had an index of biotic integrity (IBI) score of 31.8 (fair) which is slightly below the median score of 33.3 found for 14 Lake Superior coastal wetlands (mostly outside of the SLRE). Pokegama Bay had a score of 34.0 (fair) which is slightly above that median score.
For Allouez Bay, three wetland bird surveys (2014, 2016, 2017) had a median index of ecological condition (IEC) rating of high quality. A Kimballs Bay survey (2016) had an IEC rating of degraded. A Pokegama Bay survey (2016) had an IEC of mildly impacted.

For the 2012-2013 frog surveys, Allouez Bay had an IBI score of 60.0 (rated good) which is below the median score of 86.5 found for 13 Lake Superior coastal wetlands (mostly outside of the SLRE).
Pokegama Bay had a score of 70.3 (rated very good) which is also below that median score.
For Allouez Bay, three wetland frog surveys (2014, 2016, 2017) had a median IEC rating of reference condition. A Kimballs Bay survey (2016) had an IEC rating of moderately degraded. A Pokegama Bay survey (2016) had an EIC rating of moderately impacted.

Fishery

Bay fisheries were monitored during 2017 using gill nets and shoreline electrofishing (Nelson 2018). Results are summarized in Table 3 below.

[1] also see Biological Indicators Summary, below

Table 3. Bay Fish Data Summary with Comparison to MN DNR Gill Net Data

Gill Net Data	Allouez Bay	Kimballs Bay	Pokegama Bay	21 MN SLRE gill net sites
Total number of species	12	6	9	19
Median number of species/net lift	9	3	9	8
Mean fish/net lift	39.9	3.6	19.3	27.5
Mean kg fish/net lift	21.9	1.3	8.3	13.0
Gill Net plus Electrofishing Data				
Total number of species	22	15	21	not applicable
Number of native species	18	14	16	not applicable
Number of non-native species	4	1	5	not applicable
Number of intolerant species	4	4	3	not applicable

Allouez and Pokegama Bays gill net data is generally similar to data collected by the Minnesota DNR during 2017 from 21 SLRE gill net sites for median number of species/net lift, mean fish/net lift, and mean kg of fish/net lift. Kimballs Bay gill net data is substantially lower than the Minnesota DNR data for those parameters.

Conclusions of the fishery survey report included, "Despite turbid conditions that may lead to the perception of poor water quality or habitat, locally popular sport fish species like walleye, northern pike, black crappie, and yellow perch were well represented in both Allouez and Pokegama Bays. Other species of interest to anglers and state fisheries management agencies were also found in these bays including lake sturgeon, muskellunge, bluegill, and channel catfish. While Increased turbidity in Allouez and Pokegama Bays may influence the presence or abundance of specific species, it has not diminished the fishery value or eliminated desirable gamefish species from these areas." (Nelson 2018)

Biological Indicators Summary

The available biological indicators for the three bays are summarized below in Table 4.

Table 4. SLRE Bays Biological Indicators

BIOLOGICAL COMMUNITY	INDICATOR	ALLOUEZ BAY	KIMBALLS BAY	POKEGAMA BAY
Benthic invertebrates	Trimetric index[1]	median = poor (poorer than average for SLRE)	median = poor (poorer than average for SLRE)	median = fair (poorer than average for SLRE)
Ephemerid mayflies	Ephemerid density index[1]	median = good (better than average for SLRE)	median = poor (poorer than average for SLRE)	median = excellent (better than average for SLRE)
Aquatic macrophytes	Species richness[2]	155	74	148
Aquatic macrophytes	Species richness per plot[2]	8.8	5.0	5.8
Aquatic macrophytes	Mean C value[2]	5.6; species that tolerate moderate disturbance; better than SLRE mean value of 5.06	3.6; generalist species that are tolerant of disturbance; poorer than SLRE mean value of 5.06	5.4; species that tolerate moderate disturbance; better than SLRE mean value of 5.06
Bay fish	multiple[3-7]; no applicable IBI available	Number fish/gill net lift = 145% of 21 site SLRE mean; kg fish/gill net lift = 168% of 21 site SLRE mean; number species /gill net lift = 112% of 21 site SLRE median; % native species = 92%; number of intolerant species = 4; "...popular sport fish species...are well represented in Allouez ...Bay."	Number fish/gill net lift = 13% of 21 site SLRE mean; kg fish/gill net lift = 10% of 21 site SLRE mean; number species /gill net lift = 38% of 21 site SLRE median; % native species = 99%; number of intolerant species = 4	Number fish/gill net lift = 70% of 21 site SLRE mean; kg fish/gill net lift = 63% of 21 site SLRE mean; number species/gill net lift = 112% of 21 site SLRE median; % native species = 79%; number of intolerant species = 3; "...popular sport fish species ... are well represented in ... Pokegama Bay."

Table 4. SLRE Bays Biological Indicators (Cont.)

BIOLOGICAL COMMUNITY	INDICATOR	ALLOUEZ BAY	KIMBALLS BAY	POKEGAMA BAY
Wetland Macroinvertebrates	Wetland macroinvertebrate IBI[4]	2011, 2012 = moderately impacted; not enough non-clay influenced SLRE surveys to allow comparison.	IBI not available	2011, 2012 median = mildly impacted; not enough non-clay influenced SLRE surveys to allow comparison.
Wetland Vegetation	Wetland vegetation IBI[4]	2011-2017 median = moderately impacted = median for non-clay influenced SLRE surveys	2014, 2016 = moderately degraded, which is poorer than the median for non-clay influenced SLRE surveys (moderately impacted).	2011, 2012, 2016 median = moderately impacted = median for non-clay influenced SLRE surveys
Wetland Fish	Wetland fish IBI[4]	2011-2017 median = moderately impaired to moderately degraded, which is slightly poorer than the median for non-clay influenced SLRE surveys (moderately impaired).	2014 = moderately degraded, which is poorer than the median for non-clay influenced SLRE surveys (moderately impaired).	2012 = mildly impacted, which is better than the median for non-clay influenced SLRE surveys (moderately impaired).
Wetland Birds	Bird IBI[3]	31.8; fair - just below median value of 33.3 found for 14 Lake Superior coastal wetlands, mostly outside of SLRE	no data	34.0; fair - just above median value of 33.3 found for 14 Lake Superior coastal wetlands, mostly outside of SLRE
Wetland Birds	Bird IEC[4]	2014 2016, 2017 median = high quality, which is better than the median for non-clay influenced SLRE surveys (moderately impacted)	2016 = degraded, which is poorer than the median for non-clay influenced SLRE surveys (moderately impacted)	2016 = mildly impacted, which is better than the median for non-clay influenced SLRE surveys (moderately impacted)
Wetland Frogs	Frog IBI[3]	60.0; good - below median value of 86.5 found for 13 Lake Superior coastal wetlands, mostly outside of SLRE	no data	70.3; very good - below median value of 86.5 found for 13 Lake Superior coastal wetlands, mostly outside of SLRE
Wetland Frogs	Frog IEC[4]	2014 2016, 2017 median = reference condition, which is better than the median for non-clay influenced SLRE surveys (mildly impacted)	2016 = moderately degraded, which is poorer than the median for non-clay influenced SLRE surveys (mildly impacted)	2016 = moderately impacted, which is poorer than the median for non-clay influenced SLRE surveys (mildly impacted)

[1]Angradi, TR, Bartsch, WM, Trebitz, AS, Brady, VJ, Launspach, JJ. 2016. A depth-adjusted ambient distribution approach for setting numeric removal targets for a Great Lakes Area of Concern beneficial use impairment: degraded benthos. J Great Lakes Res.

[2]data from Danz, et al. 2017 (get full reference)

[3]Tozer, D. 2014. LSRI nearshore monitoring project: 2012-2013 bird and frog indices of biotic integrity. EPA assistance no. GL00E00500-0.

[4]Uzarski, DG, et al. 2017. Standardized measures of coastal wetland condition: implementation at a Laurentian Great Lakes basin-wide scale. Wetlands (37:15).

[6]Nelson, A. 2018. St. Louis River Bays – Douglas County; 2017 fish community survey. Wisconsin Dept. of Natural Resources, Superior, WI. Unpublished report.

[7]Pinkerton, J. 2018. Personal communication. Minnesota Dept. of Natural Resources fisheries specialist, Duluth, MN.

Allouez and Pokegama Bays both had high turbidities and total phosphorus concentrations. However, biological indicators for these bays tended to have moderate values that were often close to average, and in some cases above average ((ephemerid mayflies, aquatic macrophyte mean C, wetland birds, wetland fish (Pokegama only), wetland frogs (Allouez only)) for the SLRE or other comparable sites. Current water quality conditions do not appear to be having any strong negative effect on biological communities in these two bays. This finding is similar to that of the Red Clay Project conducted during the 1970's (EPA 1980). A conclusion of that project was, "Analysis of areas of Lake Superior and the Nemadji River system which are turbid throughout the year due to erosion of unconsolidated glacial lake deposits indicated that any direct physical effects of this turbidity and resultant low-level sedimentation (on aquatic life) are minimal."

The trimetric index for benthic invertebrates is one biological indicator that is below the SLRE average for Allouez and Pokegama Bays. This may be due to the high clay and water content of the bay sediment, which could be physically restrictive to some benthic organisms.

Kimballs Bay consistently has the poorest values for the biological indicators. Kimballs Bay has the lowest turbidities and total phosphorus concentrations of the three bays. It has a very small direct clay rich soil watershed and no known legacy contaminants. The poorer biological conditions were probably naturally occurring and resulted from the bay's physical characteristics. The bay is narrow and has tall, steep wooded banks that minimize wind-induced mixing. The bay also has steeper bottom contours and greater average depth. The limited mixing results in low summer dissolved oxygen concentrations occurring at depth in the bay. Low summer dissolved oxygen concentrations have also been observed to occur more frequently in the wetlands of this bay than in Allouez and Pokegama Bays, probably also due to the limited mixing.

Low dissolved oxygen concentrations could be impairing the benthic invertebrate, ephemerid mayfly, wetland macroinvertebrate, wetland and bay fish, and possibly frog communities in Kimballs Bay. It is unclear what may be causing the poor biological indicator values for aquatic macrophytes, wetland vegetation, and wetland birds.

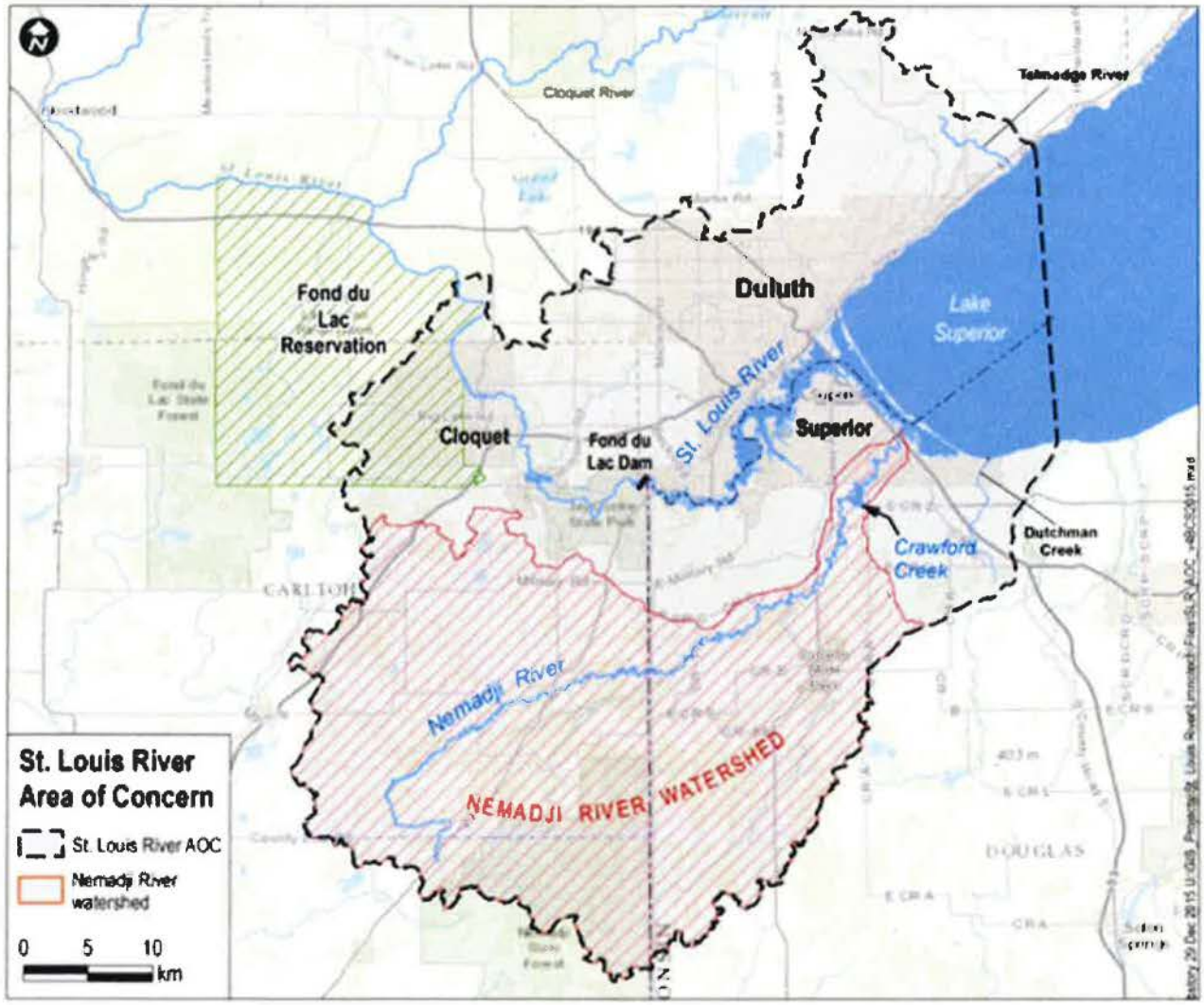
Background

The lower St. Louis River (SLR) is part of a Great Lakes Area of Concern (AOC) (Figure 1). The SLR AOC has nine beneficial use impairments (BUI's) listed in the 2016 Remedial Action Plan (MPCA and WDNR 2017). One of the BUI's is "Excessive Loading of Sediment and Nutrients."

Excessive Loading of Sediment and Nutrients Targets

- A BUI removal target of 30 ug/l for total phosphorus has been established for the St. Louis River portion of the AOC. This target was established to ensure that anthropogenic sources and activities in the St. Louis River AOC do not result in excessive phytoplankton productivity and nuisance algal conditions within the St. Louis River Estuary (SLRE).
- BUI removal targets of 15 mg/l for total suspended solids and 10 ug/l for chlorophyll a ~~are~~ have also been established for the St. Louis River portion of the AOC (MPCA and WDNR 2013).

Figure 1. St. Louis River Area of Concern



Diatom-inferred (DI) total phosphorus concentrations (TP) from sediment core analyses (Reavie 2016) indicate that TP's in three sampled SLRE bays currently exceed the TP goal (30 ug/l) for the SLRE (Table 5). The three bays sampled were North Bay, Pokegama Bay, and Allouez Bay.

Table 5 Diatom-Inferred TP Concentrations from Sediment Cores

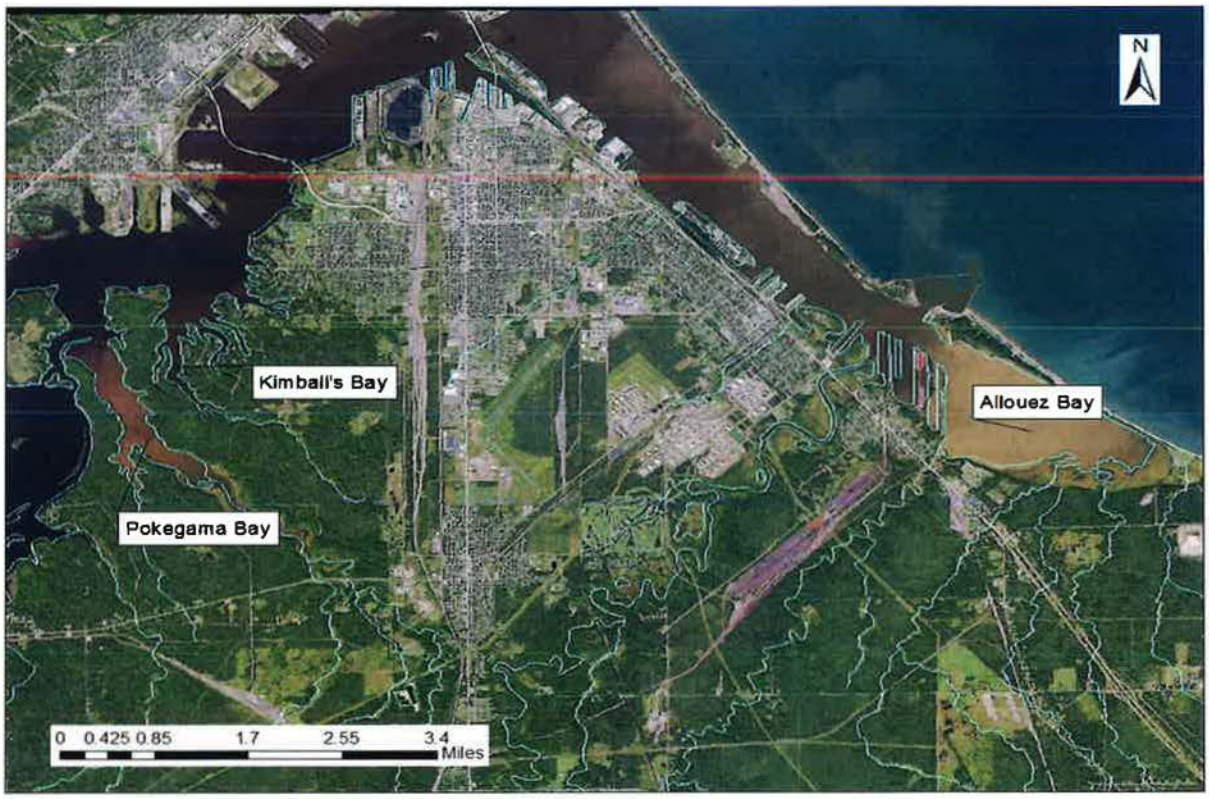
Site	Pre-development DI-TP (ug/l)	Recent DI-TP (ug/l)
North Bay (MN)	40	60
Pokegama Bay (WI)	47	74
Allouez Bay (WI)	29.5	73

Pre-development DI-TP's are nearly at or above the 30 ug/l goal, so site-specific water quality targets for these bays may be appropriate.

Three bays on the Wisconsin side of the SLRE were selected for monitoring (Figure 2. Allouez, Pokegema, and Kimballs Bay). Limited pre-existing water quality data was available for these bays. Direct watersheds for these bays contain clay-rich soils that are highly erodible, and prone to high rates of surface runoff. The sheltered nature of these bays provides some limitations to mixing with SLRE water. The monitoring was intended to:

- Document the current water quality and biotic conditions in these SLRE clay-influenced bays.
- Determine if current nutrient and total suspended solids concentrations are negatively affecting aquatic life.
- Provide data that could be used to determine if site specific water quality goals are warranted.

Figure 2. Monitored Bay Locations within the St. Louis River Estuary



Description of Study Area

High levels of clay turbidity in Pokegama and Allouez Bays and moderate levels in portions of Kimballs Bay can commonly be seen in air photos (Figure 2 above). An aquatic habitat classification system for the SLRE (SLRCAC 2002) classifies Pokegama and Kimballs Bay as clay-influenced river mouths, and classifies Allouez Bay as a clay-influenced bay.

All three bays are influenced by Lake Superior seiches. Periodic seiche flows of about 8 hr. duration and weak semi-diurnal tides cause flow reversals and daily variation in water height in the SLRE of about 13 cm. (Trebitz 2006). Bay and watershed physical characteristics are shown in Table 6.

Table 6. Bay and Watershed Physical Characteristics

Bay	Open Water Area (Acres)	Adjoining Wetland (Acres)	Maximum Depth (ft)	Mean Depth (ft)	Direct Watershed Area (km2)	Watershed to Open Water Ratio	May-Oct Bay Volumes Delivered by Direct Watershed
Allouez	1,011	235	16.6	6	82.4	20.1	2.3
Kimballs	101	6	16.3	12	5.63	13.8	0.8
Pokegama	441	66	10.5	5	89.3	50.0	6.9

Allouez Bay is the largest of the three bays and has the largest area of adjoining wetland. The north shore of the bay is a Lake Superior barrier beach comprised of very sandy soil. The large, open bay can be strongly influenced by wind-induced waves and mixing. The mouth of the bay is adjacent to the Superior entry to Lake Superior. The bay is influenced by seiche-induced backflows of water from Lake Superior and the St. Louis River estuary.

Nearly all of Allouez Bay has water depths ranging from 4 to 8 ft. There is a 35-acre dredged hole near the south side of the bay (at monitoring site ASD) with deeper water, around 16.6 ft. There are also deeper dredged areas at the docking slip and the access channel to the docking slip at the first pier at the mouth of the bay.

Kimballs Bay is the smallest of the three bays and has the least adjoining wetlands. Steep sloped, wooded banks line the bay's perimeter. The narrowness of the bay and the high wooded banks tend to minimize wind-induced mixing. It has a small direct watershed (5.63 km^2) and the lowest watershed to open water ratio (13.8) of the three bays. Bottom contours also have relatively high gradients in this bay. Most of the bay has water depths greater than 9 ft. The single monitoring site in the bay is close to the bay mouth and strongly influenced by seiche-induced mixing of SLRE water.

Pokegama Bay has the largest direct watershed area (89.3 km2), and the highest watershed to open water ratio (50.0) of the three bays. Most of the adjoining wetlands are located near the upstream end where the bay is narrower and more readily influenced by the wetlands. Most of Pokegama Bay has water depths less than 6 ft. Areas greater than 6 ft are found in the downstream third of the bay and in portions of the south arm of the bay. The maximum depth is 10.5 ft.

Methods

Bay Water Quality Monitoring

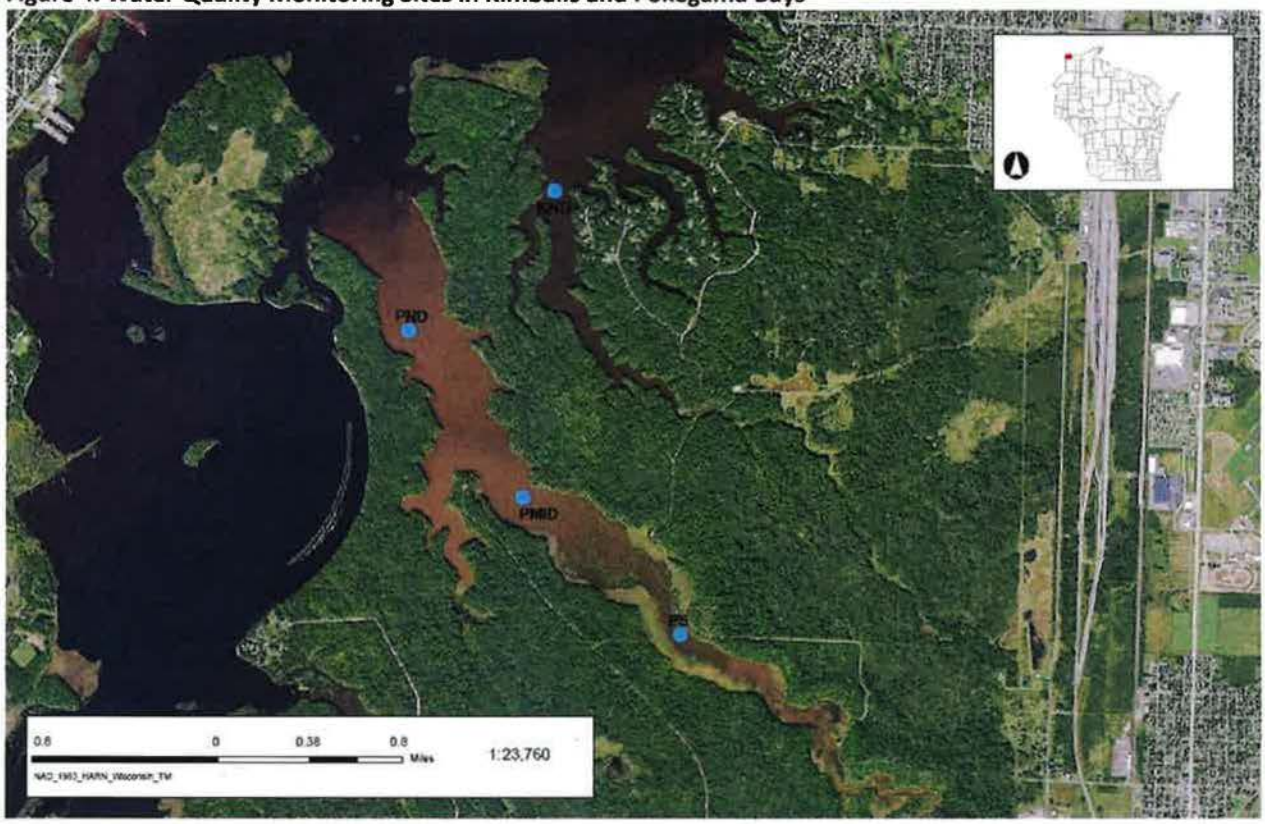
Bay water quality monitoring was conducted at three sites in Allouez Bay, one site in Kimballs Bay, and three sites in Pokegama Bay (Figures 3 and 4). The deepest site in each bay was selected as one monitoring location. Two additional sites were selected in both Allouez and Pokegama Bay, so that each of the three monitoring sites were approximately centered in an equal-sized area of the bay.

Monitoring was conducted twice per month from May through October. Water samples were collected with a 2.2-liter acrylic Kemmerer sampler at 0.5 m (1.6 ft) below the surface, and 0.5 m above the bottom. Samples were acidified, as needed, and kept on ice in the field. Dissolved total Kjeldahl nitrogen samples were field filtered. Algae samples were preserved with 1.5% glutaraldehyde. Duplicates were collected for 10% of samples. Samples were shipped on ice on the day of collection to the Wisconsin State Lab of Hygiene (except algae samples). Lab analyses included:

- Total phosphorus (EPA 365.1)
- Orthophosphate (SM4500-PE)
- Total Kjeldahl nitrogen (EPA 351.2)
- Dissolved total Kjeldahl nitrogen (EPA 351.2)

Figure 3. Water Quality Monitoring Sites in Allouez Bay

Figure 4. Water Quality Monitoring Sites in Kimballs and Pokegama Bays



- Ammonia nitrogen (EPA 350.1)
- Nitrate plus nitrite nitrogen (EPA 353.2)
- Total suspended solids (SM2540D)
- Volatile suspended solids (SM2540E)
- Turbidity (SM2130B)
- Chlorophyll a (EPA 445)

Near bottom samples were only analyzed for total phosphorus, orthophosphate, and ammonia. One shallow site in Pokegama Bay (PS; Figure 4) did not have near bottom samples collected.
Preserved algae samples were shipped on ice on the day of collection to UW-Oshkosh for analysis by Dr. Robert Pillsbury. Algae were identified to species when possible and cell densities were determined (Pilllsbury 2017).

A YSI ProDSS multiparameter meter was used for field measurements of temperature, dissolved oxygen, pH, conductivity (specific conductance), and turbidity. The meter was calibrated daily for dissolved oxygen and pH, and monthly for conductivity and turbidity. Field measurements were made 0.3 m (1 ft) below the surface, 0.3 m above the bottom, and usually at 0.6 m (2ft) intervals in between. A standard black and white Secchi disk was used for water clarity measurements.

Bay Sediment Monitoring

Bay sediment monitoring was conducted at eight sites in Allouez Bay, three sites in Kimballs Bay, and eight sites in Pokegama Bay (Figures 5 and 6). Sites included the water quality monitoring sites and additional sites that were selected to be representative of the common depth ranges in the bays.

Sediment samples were collected in August or September. A stainless-steel petite Ponar grab was used for sample collection. Samples were kept on ice in the field, then refrigerated until being shipped to the lab.

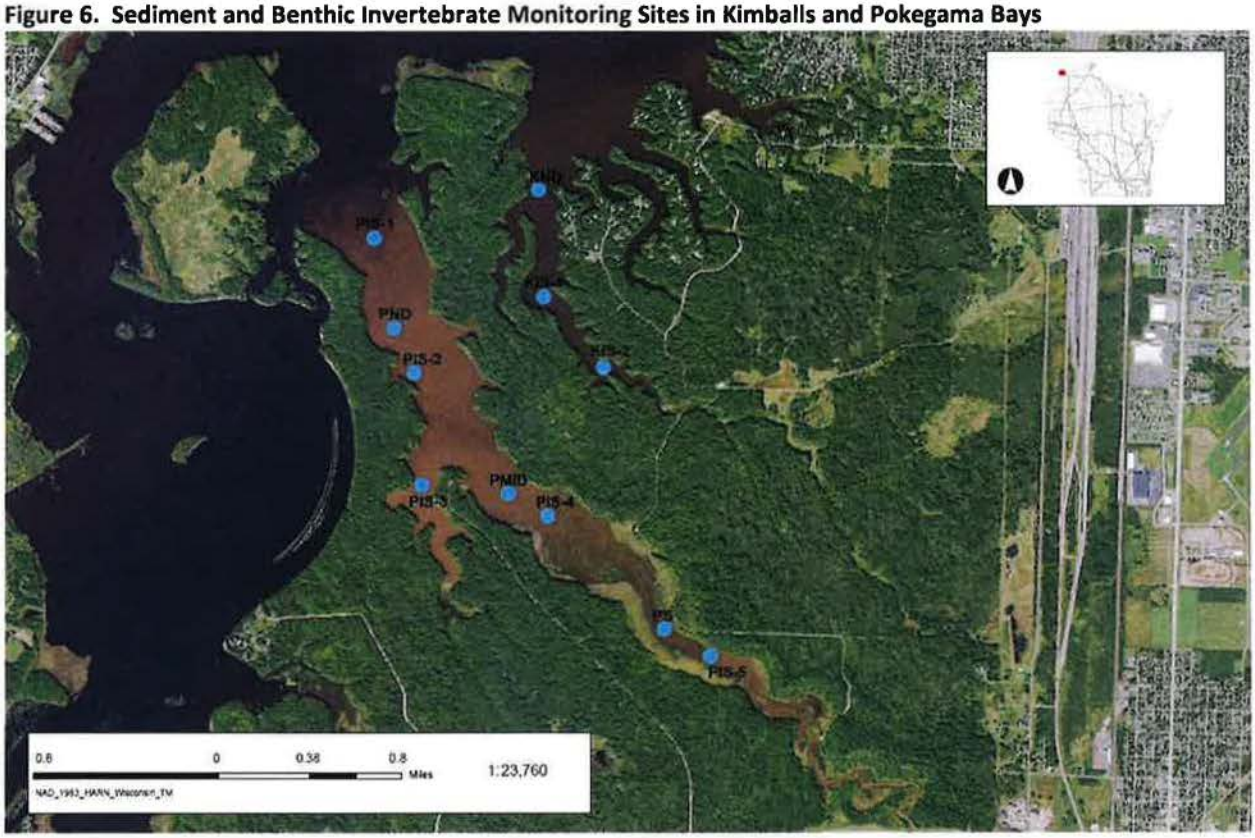
Duplicates were collected for 10% of samples. Samples were shipped on ice to the Wisconsin State Lab of Hygiene. Lab analyses included:

- % Solids (EPA 160.3)
- Total organic carbon (EPA 9060A)
- Phosphorus (SW846 6010B)
- Total Kjeldahl nitrogen (QuickChem 13-107-06-2-D)
- Ammonia nitrogen (QuickChem 12-107-06-1-A)
- Nitrate plus nitrite nitrogen (QuickChem 13-107-06-2-D)
- Iron (SW846 6010B)
- % Sand, silt, and clay (Hydrometer method)

Soft sediment thickness was measured by probing with ¾ inch (19 mm) diameter steel pipe.

Figure 5. Sediment and Benthic Invertebrate Monitoring Sites in Allouez Bay

Figure 6. Sediment and Benthic Invertebrate Monitoring Sites in Kimballs and Pokegama Bays



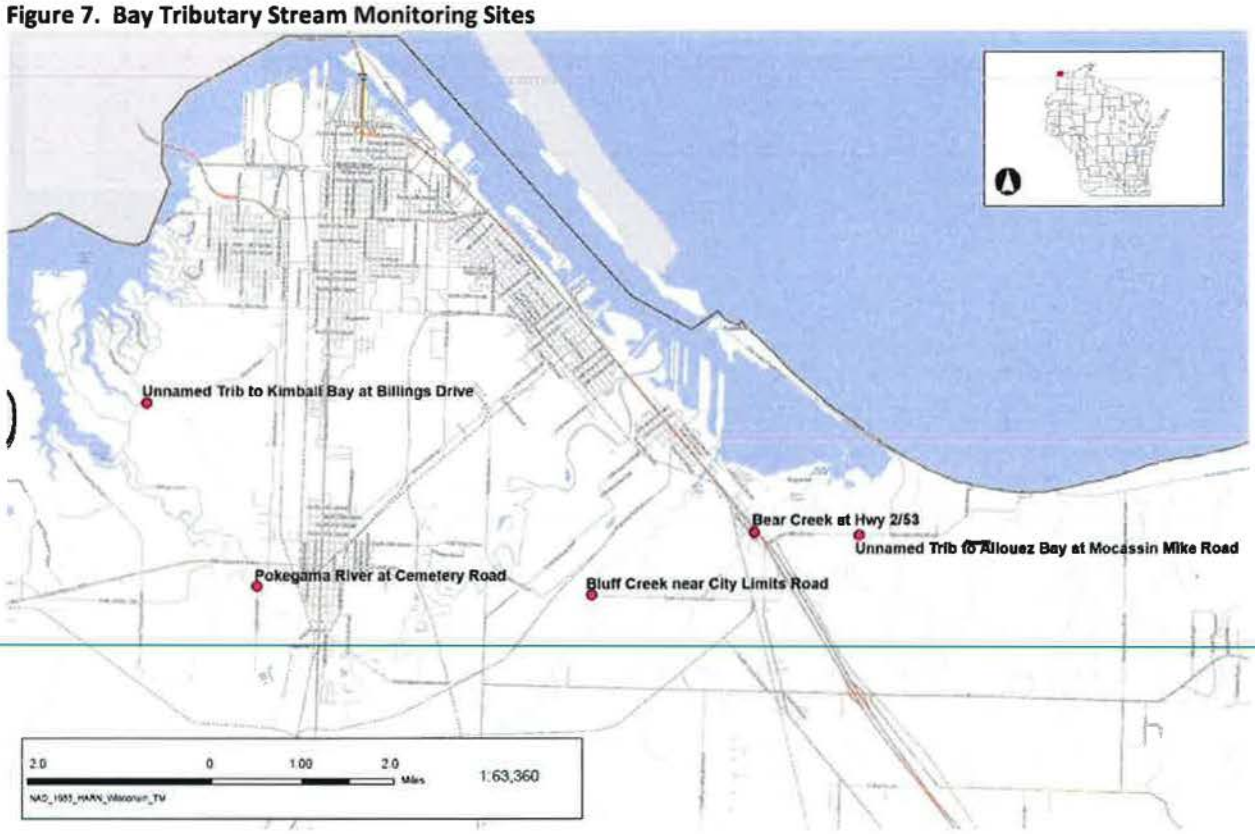
Bay Benthic Invertebrate Monitoring

Benthic invertebrate samples were collected at the same sites as sediment samples (Figures 5 and 6). Samples were collected during August 21st - 22nd. A stainless-steel petite Ponar grab was used for sample collection. Samples were washed of fine sediment using a 250-um screen bottomed bucket, and then preserved with a 10% buffered formalin solution. Duplicates were collected for 10% of samples. Samples were hand delivered to UW-Superior for analysis by Dr. Kurt Schmude using methods that have been commonly used for SLRE benthic invertebrate samples (Schmude 2010).

Bay Tributary Stream Water Quality Monitoring

Tributary stream water samples were collected during May through October at five sites (Figure 7). For the three larger, named tributary streams (Bear Creek, Bluff Creek, Pokegama River), samples were collected four times per month. Sampling was targeted to dates with higher flows, to better represent the total volume of flow. For the two smaller unnamed tributary streams, samples were collected once per month. Grab samples were collected directly in sample bottles at most sites. Due to access limitations, for the Pokegama River and the unnamed tributary to Kimballs Bay, a 2.2-liter acrylic Kemmerer sampler was used to collect samples 0.5 m (1.6 ft) below the surface.

Figure 7. Bay Tributary Stream Monitoring Sites



Samples were acidified, as needed, and kept on ice in the field. Duplicates were collected for 10% of samples. Samples were shipped on ice on the day of collection to the Wisconsin State Lab of Hygiene. Lab analyses included:

- Total phosphorus (EPA 365.1)
- Orthophosphate (SM4500-PE)
- Total Kjeldahl nitrogen (EPA 351.2)
- Ammonia nitrogen (EPA 350.1)
- Nitrate plus nitrite nitrogen (EPA 353.2)
- Total suspended solids (SM2540D)

Monthly samples from the three larger, named tributary streams were also analyzed for:

- Total recoverable iron (EPA 200.7)
- 5-day biochemical oxygen demand (SM5210B)

A YSI ProDSS multiparameter meter was used for field measurements of temperature, dissolved oxygen, pH, conductivity (specific conductance), and turbidity. The meter was calibrated daily for dissolved oxygen and pH, and monthly for conductivity and turbidity.

Stream Flow Monitoring/Estimation

Stream flow monitoring was conducted by the USGS from May 11th through October 24th for the Pokegama River at Cemetery Road (Figure 7). Flows for missing dates in early May and late October were estimated to be watershed area proportional to USGS flows measured for the Nemadji River at County Highway C. Flows for Bear Creek, Bluff Creek, and bay watershed areas were estimated to be watershed area proportional to flows for the Pokegama River.

Load Estimation

Total phosphorus and 5-day biochemical oxygen demand loads for the Pokegama River were estimated by applying flow regression formulas to estimate daily concentrations and daily loads. Total phosphorus and 5-day biochemical oxygen demand loads for effluent discharge from the Village of Superior wastewater lagoons were estimated using daily discharge flows and weekly sample test results provided by the Village. Sample test results from the nearest tested date were applied to dates not sampled. Additional effluent discharge samples were collected by DNR staff and tested for total phosphorus. DNR collected sample results were similar to Village collected sample results (DNR samples, n=3, mean = 1.7mg/l; Village samples, n=14, mean = 1.7 mg/l).

Bay Water Quality Results and Discussion

Bay Stratification / Profile Data

Temperature

Water temperature data is summarized in Table 7. Coolest near surface ("top") temperatures occurred on May 22nd and ranged from 8.3 to 10.6 °C. Warmest top temperatures occurred on July 10th and 24th and ranged from 21.9 to 26.0 °C. Allouez Bay sites were cooler on most dates from May to September 11th. Seiche-induced inputs of Lake Superior water to the bay probably account for this, since Lake Superior temperatures are cooler than SLRE temperatures during that time period (Oost et al. 2010). Temperatures at the shallow sites, PS and PMID tended to decline readily during periods of cool weather (May 22nd, late September, October) making them similar to Allouez Bay at those times.

Site KND showed the most thermal stratification and had the greatest top to near bottom ("bottom") temperature declines on ten of the twelve monitoring dates (Table 7). Site KND is the second deepest site (5 m, 16.3 ft) and is located at the mouth of Kimballs Bay. Kimballs Bay is fairly narrow, relatively deep, and has steep wooded banks. These characteristics provide protection from wind-induced mixing. Site ASD is the deepest site (5.1 m, 16.6 ft), but shows minimal thermal stratification, probably due to the broad expanse of Allouez Bay allowing frequent wind-induced mixing. Site ANW, a shallower site (2.4 m, 7.9 ft) near the mouth of the bay tended to show more stratification than site ASD. This may be due to seiche-induced inputs of cool Lake Superior water back-flowing along the bottom at times. Such underflows have occasionally been observed at the Superior entrance to Lake Superior (Kiesling 2017).

There was very notable stratification in all three bays on August 29th. Most monitoring sites showed their largest top to bottom temperature declines (-2.0 to -5.8° C) on that date (Table 7). The largest top to bottom conductivity declines (-4 to -77 umhos/cm) occurred at all sites on that date (Table 9). The largest top to bottom turbidity increases (24 to 148 ntu) at all sites except PS (Table 10), and the largest top to bottom dissolved oxygen (D.O.) declines (-2.5 to -4.0 mg/l) at all sites except KND and PS (Table 8) also occurred on that date.

There was a substantial runoff event shortly before August 29th which probably accounts for the stratification that was observed. Peak flow for the runoff event occurred on August 27th. Tributary stream data from three sites was collected on August 28th (Table 11). Stream flows were much cooler and had higher turbidities than near-surface water in the bays. This resulted in stream water being denser and flowing along the bottoms of the bays to produce the observed stratification. Stream parameters measured on August 28th can account for most of the top to bottom differences that are observed in the bays on August 29th.

During peak stream flow (August 27th) it is likely that stream conductivities were even lower, and turbidities were even higher than those measured on August 28th. Dissolved oxygen concentrations may have also been somewhat lower. The stream water entering the bays was layered at the bottom of the bays. Dissolved oxygen concentrations in that stream water were likely to have declined over two days due to biochemical oxygen demand present in the water and sediment oxygen demand.

The tributary to Kimballs Bay was not monitored on August 28th. Other measurements of conductivity in that tributary during moderate flows were as low as 113 umhos/cm, so it is certainly possible that conductivity during a high flow event was less than the 93 umhos/cm measured at the bottom of site KND. Stream conductivities during high flows tend to be lower since recent runoff of rainfall, with a very low conductivity, makes up a larger portion of the flow.

Site KND which had the most prior stratification and bottom D.O. depletion, showed a notable increase in near bottom D.O.'s from August 15th (0.8 mg/l) to August 29th (5.9 mg/l), which also indicates stream runoff water was largely replacing the near bottom water at that sampling site.

Despite the relatively shallow depths at the three Pokegama Bay sites, occasional thermal stratification occurred on dates other than August 29th. Site PMID (depth 1.6 m, 5.4 ft) had the most dates (8 of 12) when top to bottom temperature decline more than 1°C. Even site PS with a depth of only 1 m (3.2 ft) had three dates when top to bottom temperatures declined more than 1°C. Surficial heating from absorption of all solar radiation near the surface of the highly turbid water may account for much of this stratification.

Table 7. Bay Sites Top and Bottom Temperature Data

	TOP TEMPERATURE (TOP TO BOTTOM TEMPERATURE DECLINE) (°C)						
	SITES (Depth (ft))						
Dates	ASD (16.6)	ASE (6.2)	ANW (7.9)	KND (16.3)	PND (10.5)	PMID (5.4)	PS (3.2)
5/9/2017	10.6 (0.4)	10.9 (0.7)	10.9 (0.6)	12.4 (1.8)	12.3 (0.2)	13.5 (0.6)	14.5 (0.1)
5/22/2017	8.5 (0.2)	8.8 (0)	8.6 (0)	10.6 (1.5)	9.2 (0.8)	8.4 (0.4)	8.3 (0.1)
6/15/2017	17.2 (1.1)	17.4 (1.3)	17.8 (2.2)	21.6 (3.7)	19.2 (0.4)	20.9 (1.3)	19.7 (0.1)
6/26/2017	17.2 (0.5)	17.3 (0.7)	18.0 (1.3)	19.8 (1.7)	19.2 (0.5)	19.1 (0.6)	18.5 (0)
7/10/2017	21.9 (0.2)	22.6 (0.8)	22.6 (3.2)	24.8 (6.2)	25.7 (2.8)	25.9 (2.5)	23.9 (0.3)
7/24/2017	21.8 (1.1)	21.0 (0.6)	21.3 (1.5)	23.2 (4.0)	23.7 (1.2)	26.0 (3.4)	25.9 (2.7)
8/15/2017	20.5 (0.5)	20.5 (0.7)	21.3 (1.4)	22.1 (3.0)	22.6 (1.2)	22.8 (1.9)	21.7 (0.7)
8/29/2017	19.5 (2.5)	20.3 (3.0)	20.0 (2.0)	21.7 (5.8)	22.1 (5.6)	22.1 (4.8)	20.2 (3.3)
9/11/2017	17.5 (0.2)	17.6 (0.1)	17.7 (0.1)	19.6 (2.6)	19.5 (2.6)	21.8 (3.6)	20.8 (1.1)
9/27/2017	17.0 (0.8)	16.7 (0)	16.5 (0.1)	18.3 (0.8)	18.2 (0.6)	16.8 (1.5)	15.7 (0.2)
10/4/2017	14.8 (0.2)	14.2 (0)	14.7 (0.1)	15.5 (0.4)	14.4 (0.3)	14.4 (0.2)	14.0 (0.1)
10/17/2017	10.6 (0.4)	10.3 (0.1)	10.8 (0.3)	12.5 (1.5)	11.3 (0.5)	11.5 (1.1)	10.2 (0.1)

Top measurements made 0.3 m below water surface; bottom measurements made 0.3 m above bottom.

Table 8. Bay Sites Top and Bottom Dissolved Oxygen Concentration Data

	BOTTOM D.O.'S (D.O. DECLINE FROM TOP TO BOTTOM) MG/L						
	SITE ((DEPTH (FT))						
DATE	ASD (16.6)	ASE (6.2)	ANW (7.9)	KND (16.3)	PND (10.5)	PMID (5.4)	PS (3.2)
5/9/2017	10.2 (0.4)	10.2 (0.7)	10.3 (0.6)	10.6 (0.4)	12.1 (0.2)	12.9 (0.6)	14.4 (0.1)
5/22/2017	10.8 (0.0)	10.6 (0.0)	10.8 (0.1)	9.3 (0.5)	10.0 (0.1)	10.7 (0.0)	10.8 (0.0)
6/15/2017	8.8 (0.6)	8.9 (0.4)	9.4 (0.2)	8.1 (-0.7)	7.3 (0.3)	7.2 (0.2)	4.9 (0.1)
6/26/2017	9.0 (0.0)	9.2 (0.4)	9.1 (0.3)	7.6 (0.2)	7.8 (0.5)	8.5 (0.3)	7.3 (0.0)
7/10/2017	7.5 (0.2)	7.8 (0.9)	8.1 (0.4)	2.2 (5.8)	6.7 (1.8)	6.0 (1.9)	4.0 (0.2)
7/24/2017	6.6 (1.2)	7.2 (0.3)	7.8 (0.4)	0.6 (6.8)	6.1 (0.7)	6.1 (1.5)	3.4 (1.3)
8/15/2017	7.0 (1.6)	8.0 (0.7)	7.7 (1.9)	0.8 (7.8)	5.7 (2.0)	5.6 (1.0)	4.7 (1.5)
8/29/2017	5.5 (4.0)	6.2 (3.8)	6.2 (3.5)	5.9 (2.6)	5.7 (2.5)	6.9 (2.5)	7.2 (-0.2)
9/11/2017	8.2 (0.2)	8.3 (0.1)	8.3 (0.1)	6.3 (1.4)	6.3 (0.7)	5.4 (1.3)	3.7 (0.7)
9/27/2017	7.6 (0.7)	8.3 (0.1)	7.7 (0.2)	7.1 (-0.3)	4.6 (0.7)	6.4 (-0.5)	7.6 (0.0)
10/4/2017	8.2 (0.3)	7.9 (0.1)	8.0 (0.2)	7.3 (-0.5)	7.3 (0.1)	7.7 (0.1)	8.3 (0.0)
10/17/2017	10.0 (0.1)	9.9 (0.0)	9.6 (0.2)	8.7 (-0.6)	8.3 (0.3)	6.2 (0.7)	7.8 (0.0)

D.O. = dissolved oxygen. Top measurements made 0.3 m below water surface; bottom measurements made 0.3 m above bottom. Red values are < 5 mg/l.

Table 9. Bay Sites Top and Bottom Conductivity Data

	TOP CONDUCTIVITY (TOP TO BOTTOM CONDUCTIVITY CHANGE)(umhos/cm)						
	SITES (Depth (ft))						
Dates	ASD (16.6)	ASE (6.2)	ANW (7.9)	KND (16.3)	PND (10.5)	PMID (5.4)	PS (3.2)
5/9/2017	138 (0)	138 (0)	135 (3)	112 (-2)	106 (0)	130 (0)	138 (-1)
5/22/2017	126 (-1)	125 (0)	126 (0)	112 (-4)	114 (2)	112 (0)	107 (0)
6/15/2017	136 (-1)	134 (-2)	132 (-4)	124 (+2)	133 (-1)	139 (+2)	173 (0)
6/26/2017	139 (0)	139 (0)	140 (-1)	143 (+6)	152 (+1)	156 (-1)	177 (0)
7/10/2017	147 (0)	146 (0)	147 (-4)	159 (-2)	161 (0)	168 (+1)	190 (-1)
7/24/2017	155 (-6)	151 (+1)	150 (-4)	155 (+7)	157 (0)	171 (+5)	210 (-3)
8/15/2017	158 (+1)	158 (-1)	156 (0)	176 (-11)	182 (0)	187 (+2)	196 (0)
8/29/2017	162 (-21)	164 (-4)	162 (-7)	170 (-77)	172 (-36)	167 (-34)	134 (-7)
9/11/2017	170 (0)	169 (0)	170 (0)	150 (-4)	146 (-6)	151 (+1)	171 (0)
9/27/2017	166 (-2)	167 (0)	164 (+1)	139 (-4)	158 (-6)	158 (-5)	142 (0)
10/4/2017	150 (-7)	113 (0)	110 (-1)	128 (-1)	95 (-2)	94 (0)	109 (0)
10/17/2017	139 (+1)	141 (+1)	141 (0)	120 (0)	116 (-1)	136 (+22)	165 (0)

Top measurements made 0.3 m below water surface; bottom measurements made 0.3 m above bottom. Conductivity is specific conductance.

Table 10. Bay Sites Top and Bottom Turbidity Data

	TOP TURBIDITY (TOP TO BOTTOM TURBIDITY CHANGE) (NTU) SITES (Depth (ft))						
Dates	**ASD (16.6)**	**ASE (6.2)**	**ANW (7.9)**	**KND (16.3)**	**PND (10.5)**	**PMID (5.4)**	**PS (3.2)**
5/9/2017	91 (10)	83 (12)	85 (10)	13 (1)	25 (2)	135 (-5)	105 (13)
5/22/2017	110 (5)	110 (5)	110 (5)	27 (41)	125 (0)	145 (-5)	117 (0)
6/15/2017	78 (4)	75 (5)	68 (-10)	13 (4)	52 (9)	80 (10)	87 (-2)
6/26/2017	92 (0)	77 (13)	80 (0)	14 (14)	54 (11)	60 (4)	98 (2)
7/10/2017	73 (5)	69 (-2)	77 (-22)	14 (21)	32 (12)	56 (12)	77 (-1)
7/24/2017	54 (24)	55 (2)	55 (6)	13 (20)	36 (6)	40 (7)	45 (2)
8/15/2017	34 (4)	38 (3)	37 (3)	10 (36)	21 (13)	41 (2)	42 (0)
8/29/2017	38 (68)	36 (24)	43 (40)	14 (96)	50 (148)	63 (72)	111 (3)
9/11/2017	48 (0)	47 (0)	49 (1)	15 (10)	52 (0)	59 (7)	56 (0)
9/27/2017	51 (9)	50 (1)	80 (10)	17 (2)	68 (-1)	73 (38)	154 (13)
10/4/2017	80 (30)	195 (-5)	190 (5)	36 (-11)	270 (5)	275 (0)	210 (20)
10/17/2017	76 (4)	70 (10)	73 (1)	29 (-1)	67 (4)	84 (-8)	110 (0)

Top measurements made 0.3 m below water surface; bottom measurements made 0.3 m above bottom.

Table 11. August 27-29th Runoff Event Data for Stream and Bay Sites

Pokegama River Flows

Date	Mean Daily Flow (cfs)
8/26/2017	18
8/27/2017	325
8/28/2017	131
8/29/2017	50
8/30/2017	23

Stream parameters 8/28/2017

Stream	Temperature (°C)	Dissolved Oxygen (mg/l)	Conductivity (umhos/cm)	Turbidity (ntu)
Pokegama	15.7	9.3	120	139
Bear	16	9.2	123	113
Bluff	15.9	8.8	132	135

Bay parameters 8/29/2017

Bay Site	Temperature (°C) (top/bottom)*	Dissolved Oxygen (mg/l) (top/bottom)*	Conductivity (umhos/cm) (top/bottom)*	Turbidity (ntu) (top/bottom)*
ASD	19.5/17	9.5/5.5	162/141	38/106
ASE	20.3/17.3	10/6.2	164/160	36/60
ANW	20/18	9.7/6.2	162/155	43/73
KND	21.7/15.9	8.5/5.9	170/93	14/110
PND	22.1/16.5	8.2/5.7	172/136	50/198
PMID	22.1/17.3	9.4/6.9	167/133	63/135
PS	20.2/16.9	7.0/7.2	134/127	111/114

*top parameters measured 0.3 m below surface; bottom parameters measured 0.3 m above bottom

Dissolved Oxygen

Thermal stratification can allow isolation of near bottom water which can result in dissolved oxygen depletion and sediment phosphorus release. Dissolved oxygen concentration (D.O.) from site profiles are summarized in Table 8. Site KND, which showed the most summer thermal stratification also showed the most near-bottom D.O. depletion, with near-bottom D.O.'s less than 1 mg/l on two dates. Near-bottom D.O.'s at all sites in Allouez Bay were consistently above 5 mg/l. Near-bottom D.O.'s at sites PND and PS were below 5 mg/l at times.

Since D.O. depletion can result in sediment phosphorus release, additional discussion of D.O. results is contained in the "Sediment P Release Indicators" section, below. The large top to bottom declines in D.O. at most sites on August 29th are believed to be due to tributary stream underflow as discussed above.

Conductivity

Conductivity (specific conductance) from site profiles are summarized in Table 9. All three bays showed similar ranges of top conductivities (Allouez, 110-170; Kimballs, 112-176; Pokegama, 94-210). SLRE main channel conductivities are seasonally variable with May-October 2017monthly means ranging from 117 to 201 umhos/cm (NERR 2017). They are not distinctive from bay c conductivities.

Conductivities showed notable declines from top to bottom at all site on August 29th. These declines are believed to be due to tributary stream underflow as discussed above.

Site ANW at the mouth of Allouez Bay showed fairly consistent top to bottom conductivity and temperature declines between June 15th and August 29th, which suggests more frequent bottom inflow of Lake Superior water into that bay. Lake Superior water conductivity is about 103-104 umhos/cm (Oost et al. 2010, Eliot et al. 2014).
Lake Superior has been shown to be a major water source for Allouez Bay. A mixing assessment done during May through mid-July of 2007 using conservative ions estimated that Lake Superior was the source of 46-50% of Allouez Bay water (Hoffman 2018). The St. Louis River was the source of 50-53% of bay water, and direct tributaries (Bluff Creek) were the source of 1% of bay water. The Nemadji River was not providing any significant amount of water to the bay.

Figure 8. June 2017 Air Photo of Kimballs Bay

Turbidity

Turbidities from site profiles are summarized in Table 10. Kimballs Bay (site KND) had significantly lower top turbidities (mean = 18 ntu) than all other sites. Sites PS and PMID had the highest mean top turbidities (101 ntu, 93 ntu), but mean top turbidities for Pokegama and Allouez Bay sites are not significantly different (Figure 52).

The largest top to bottom turbidity increases at all sites occurred on August 29th. These increases are believed to be due to tributary stream underflow as discussed above.

Site KND showed the most pronounced top to bottom turbidity increases through much of the season. The unnamed tributary to Kimballs Bay, with a mean turbidity of 84 ntu's is the likely source of these increases. Water temperature in the tributary was consistently cooler than bay surface temperature. The cooler, high turbidity inflow probably tends to flow along the bottom in this relatively narrow, deep, and wind sheltered bay. The variable top to bottom conductivity changes at site KND also suggest this flow pattern is occurring. Conductivities in the tributary were also variable (113-289 umhos/cm) and could be higher or lower than top conductivities in the bay. A June 2017 air photo (Figure 8) shows clay turbidity was restricted to the upstream end of Kimballs Bay, which may also indicate that turbid inflow is sinking as at moves downstream through the bay.

Bay Water Chemistry

Phosphorus

Range of Total Phosphorus Concentrations and Seasonal Patterns

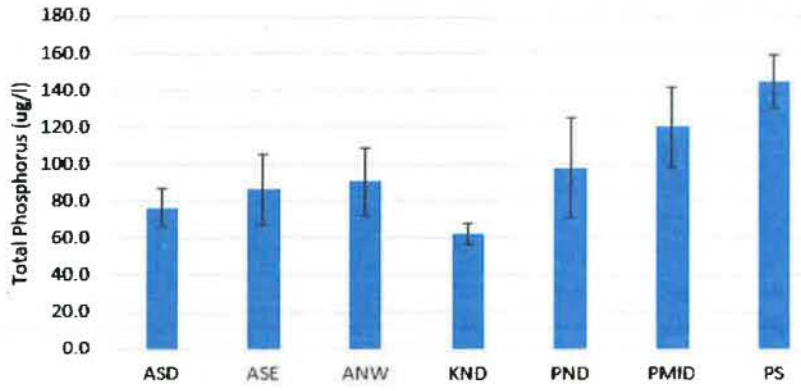
Mean total phosphorus concentrations (TP's) in near surface (top) samples from the bay monitoring sites ranged from 63 ug/l (site KND) to 145 ug/l (site PS) (Figure 9) (Complete bay water quality data is provided in appendix 1). The TP mean for site KND (Kimballs Bay) was significantly lower than the Allouez and Pokegama Bay sites. Sites in Allouez Bay were not significantly different from each other. Site PS in Pokegama Bay was significantly higher than all other sites except site PMD.

The Kimballs Bay site (KND) is close to the mouth of that bay and so is strongly influenced by mixing with estuary water which has lower TP's than the bays. TP inputs from the small unnamed tributary to Kimballs Bay (TP mean = 160 ug/l) are diluted by estuary water, which has lower TP's.

Pokegama Bay mean TP's decreased from the upstream end of the bay (site PS) to the downstream end (site PND). The Pokegama River, which had high TP (mean = 182 ug/l), strongly influenced site PS.

Potential phosphorus release from sediment and fringe wetlands may also have influenced TP at site PS. TP decreases downstream of site PS were probably due to increased mixing with estuary water, which has lower TP's, and sedimentation of particulate phosphorus.

Figure 9. Bay Sites Top Total Phosphorus Means



Error bars are 90% confidence intervals

Some seasonal patterns in near surface (top) TP were evident in Figures 10 - 16, below. All sites, except PS, showed a spring TP peak on May 22nd, and all sites showed a fall TP peak on October 4th. The TP peaks were a result of large runoff events (Figure 17). Sites in Allouez and Pokegama Bay showed lower TP's during mid-summer, generally late July through August, when runoff inputs were low. The Kimballs Bay site showed a different summer pattern, with TP's increasing from mid-June to early September (Figure 13). This was probably due to sediment phosphorus release being more substantial in this bay. Seasonal patterns in near bottom (bottom) TP's are discussed in the "Sediment P Release Indicators" section, below.

Figure 10. Site ASD Top and Bottom Total Phosphorus Concentrations

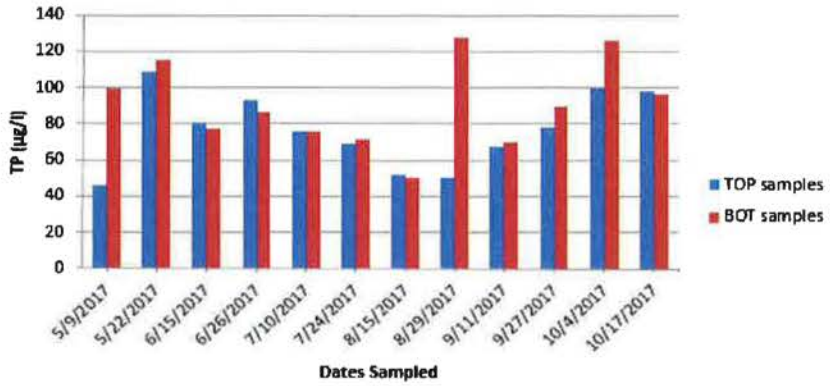


Figure 11. Site ASE Top and Bottom Total Phosphorus Concentrations

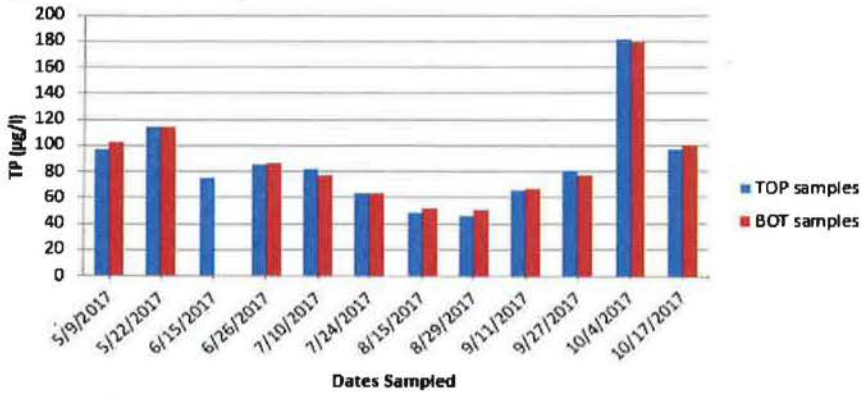


Figure 12. Site ANW Top and Bottom Total Phosphorus Concentrations

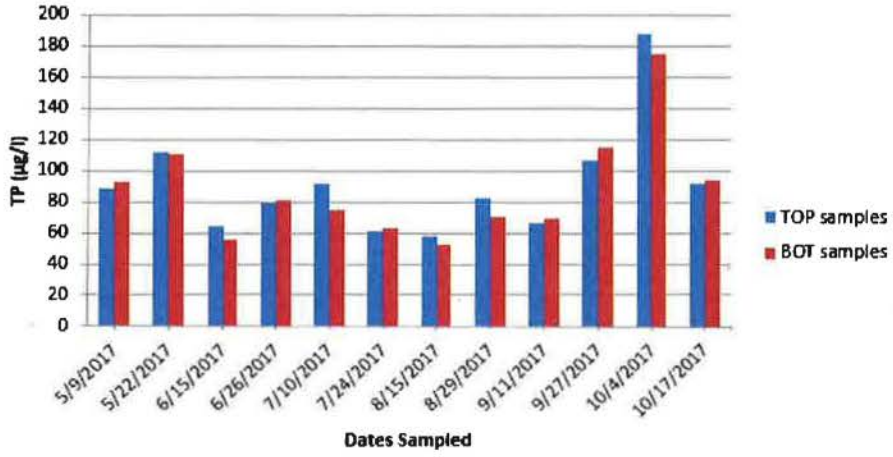


Figure 13. Site KND Top and Bottom Total Phosphorus Concentrations

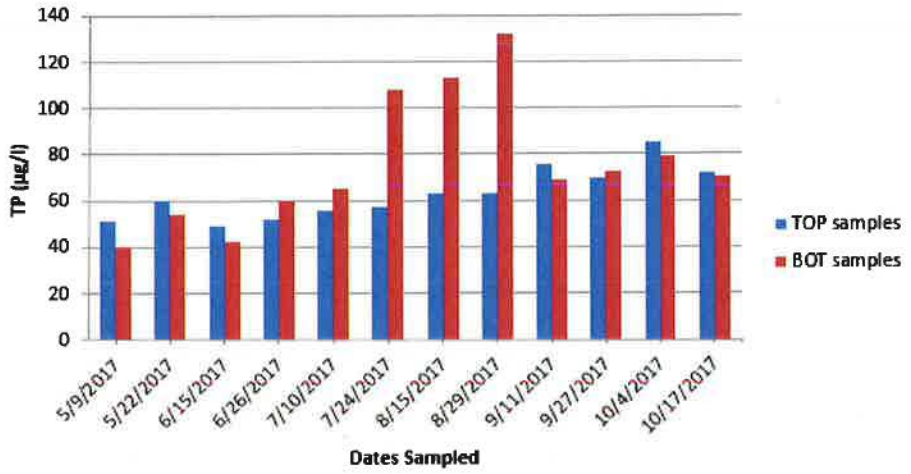


Figure 14. Site PND Top and Bottom Total Phosphorus Concentrations

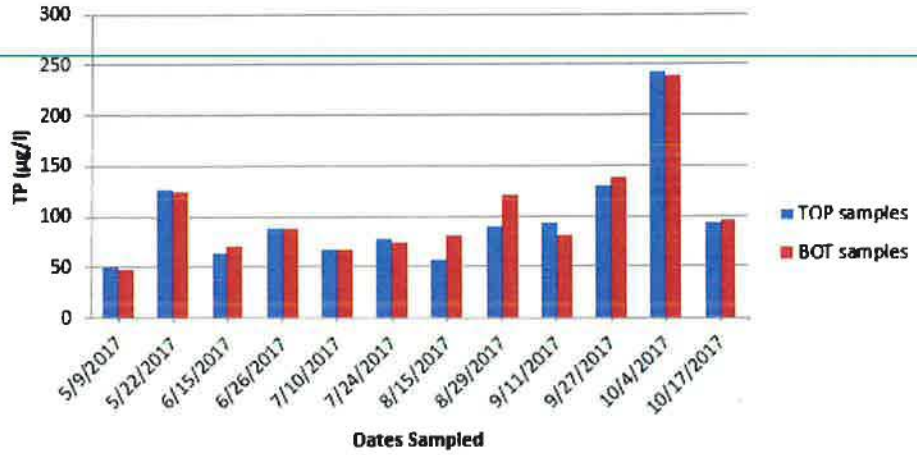


Figure 15. Site PMID Top and Bottom Total Phosphorus Concentrations

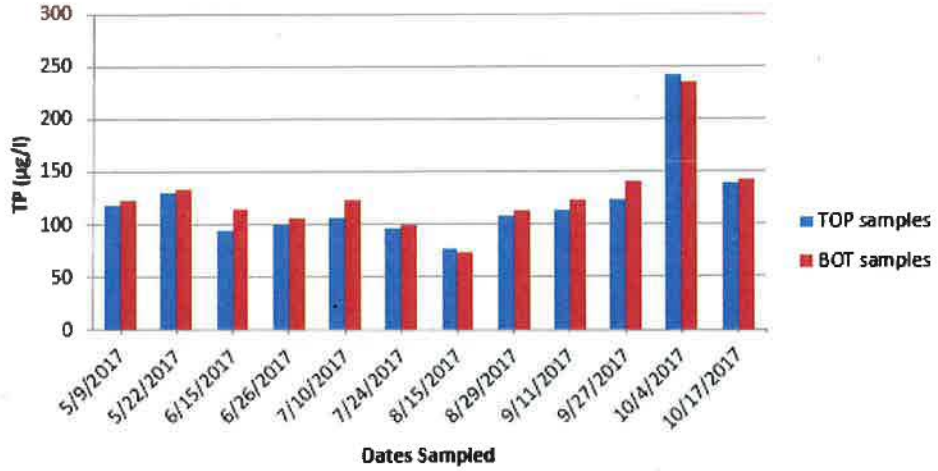


Figure 16. Site PSD Top Total Phosphorus Concentrations

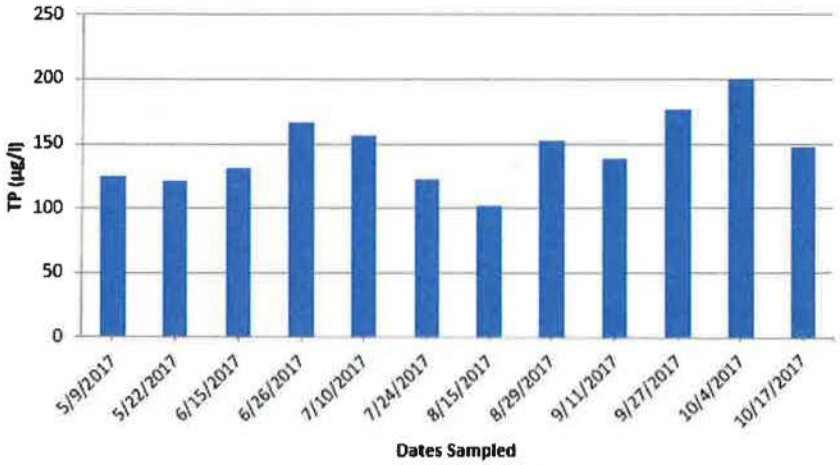
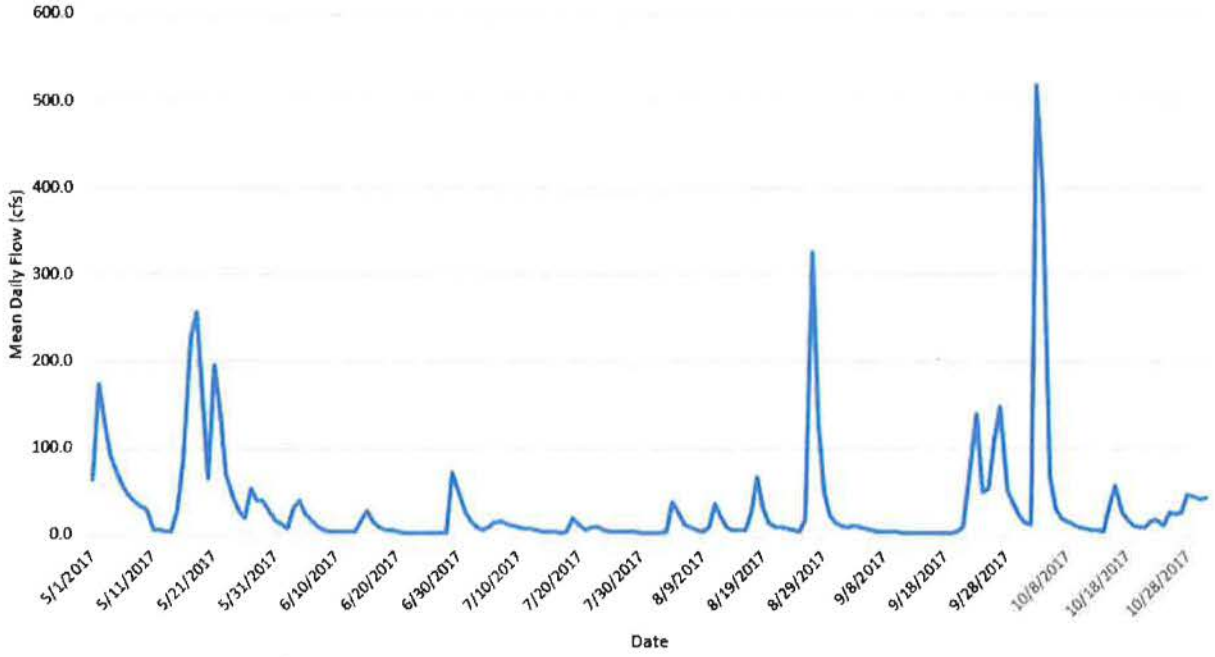


Figure 17. Pokegama River Flows (data from USGS)



Comparison to TP Criteria and Reference TP's

Selecting appropriate TP criteria to apply to these bays is difficult due to their unique hydrologic characteristics. These bays are part of the much larger St. Louis River estuary which is subject to irregular backflows and mixing resulting from Lake Superior seiches.

The WI DNR has a state-wide stream TP impairment threshold of 75 ug/l, and a large river TP impairment threshold of 100 ug/l (applicable to the St. Louis River). However, since these bays are generally not riverine, these thresholds are not suitable for assessing their water quality condition. The St. Louis River Area of Concern 2016 Remedial Action Plan identifies 30 ug/l goal as the TP target for the St. Louis River estuary (SLRE) (MPCA et al. 2017). This target was selected since it was considered to be the upper limit of the mesotrophic range (or eutrophic threshold). This target is intended to apply to the open main channel areas of the SLRE and not sheltered bays and was not based on analyses of local data. Mean bay site TP's (63-145 ug/l) are roughly 2-5 times higher than this target concentration.

Lake-based TP criteria are probably more suitable for the bays than river-based ones. Lake-based TP criteria include:

- 24 ug/l eutrophic threshold for lakes (Carlson 1977)
- 30 ug/l eutrophic threshold for Wisconsin lakes (Lillie and Mason 1983)
- 40 ug/l Wisconsin impairment threshold for shallow lakes (WIDNR 2017)

These lake thresholds are all substantially lower than TP's found at the bay sites. However, lake TP thresholds are usually developed based on a concern for avoiding undesirable levels of algae production. The relationship between TP and algae production (chlorophyll a concentrations) is substantially different in these bays than in most lakes (see chlorophyll a section below), and so lake thresholds are also not well suited to these bays.

TP's measured at 150 sites throughout the SLRE in 2012 and 2013 (Bellinger et al. 2015) provide another basis of comparison. A mean TP of 31 ug/l was found. Mean TP's for the bay sites (63-145 ug/l) are again roughly 2-5 times higher.

Diatom inferred (DI) TP's for Allouez and Pokegama Bay were determined by Reavie et al. (2016) for both recent and pre-development periods (Table 12). Kimballs Bay was not sampled. Pre-development TP's could be used to help guide the selection of TP targets for these bays. The pre-development DI-TP for Allouez Bay (29.5 ug/l) is close to lake eutrophic threshold values. The pre-development DI-TP for Pokegama Bay (47 ug/l) substantially exceeds lake eutrophic threshold values (Table 12).

2017 median sampled TP's exceed recent DI-TP's for both bays. Recent SLRE TP's (Bellinger et al. 2015) at other core sites were also found to exceed DI-TP's (Reavie et al. 2016). Additionally, one year of bay data may not represent average recent conditions. Also, the DI-TP model may not fully account for the influence of light limitation and the limited bio-availability of the clay-bound TP fraction in the bays.

Table 12. Diatom Inferred Total Phosphorus (DI-TP) for Allouez and Pokegama Bays Compared to 2017 Sampled Total Phosphorus

	PRE-DEVELOPMENT*	RECENT**	2017 MEDIAN	SITE OF 2017
BAY	DI-TP	DI-TP	SAMPLED TP	SAMPLING
Allouez	29.5	74	81.3	ASD, ASE, ANW***
Pokegama	47	73	89	PND
	DI-TP values form Reavie, et al. 2016			
	*based on 2 oldest pre-1875 layers			
	*based on 2 most recent post-2000 layers			
	***2017 median is average from 3 sites			

Bay sites were selected that best coincide with the core sampling sites.

Comparison of 2017 TP's and Related Parameters to Data from Other Years

Some TP data along with chlorophyll a (CHL), total suspended solids (TSS), and/or turbidity data is available for other years to compare to 2017 data. Three TP samples collected in 2016 (USGS 2018) during May, July, and August at site PMID in Pokegama Bay ranged from 85 – 293 ug/l. In 2017, TP's at this site were similar and ranged from 77 – 242 ug/l.

Datasets from 2007 (Hoffman 2011), 2011-12 (Bartsch et al 2015), and 2012-13 (Bellinger et al 2015) for Allouez and Pokegama Bays can also be compared to the 2017 data from these bays (Tables 13, 14, 15). In 2007, TP's and CHL's in Allouez and Pokegama Bays were similar to those found in 2017, but TSS's appear to be significantly higher, with 2007 means over double those found in 2017 (Table 13).

Table 13. Comparison of Total Phosphorus, Chlorophyll a, and Total Suspended Solids Concentrations in Allouez and Pokegama Bays During 2007 and 2017

Bay (site)	Parameter	May-July 2007*(n = 5)	May-July 2017 (n = 6)
Allouez (ASD)	TP (ug/l) mean	85.8	79
Allouez (ASD)	TP (ug/l) range	64 - 155	46 - 109
Allouez (ASD)	CHL (ug/l) mean	10.6	6.9
Allouez (ASD)	CHL (ug/l) range	7.3 - 16.4	3.0 - 14.9
Allouez (ASD)	TSS (mg/l) mean	42.3	19.2
Allouez (ASD)	TSS (mg/l) range	19 - 92	7 - 28
Bay (site)	**Parameter**	**May-July 2007* (n = 15)**	**May-July 2017 (n = 6)**
Pokegama (PND)	TP (ug/l) mean	83.5	78.9
Pokegama (PND)	TP (ug/l) range	36 - 263	49 - 127
Pokegama (PND)	CHL (ug/l) mean	8.8	8.0
Pokegama (PND)	CHL (ug/l) range	1.7 - 21.7	0.5 - 18.7
Pokegama (PND)	TSS (mg/l) mean	39.6	15.6
Pokegama (PND)	TSS (mg/l) range	5.2 - 269	7.7 - 37

*Hoffman 2011

TP = total phosphorus; CHL = chlorophyll a; TSS = total suspended solids

Data from 2010-11 (Bartsch et al 2015) (Table 14) was from three site composite samples from sites near tributary mouths, while 2017 data is from mid-bay sites further from tributary mouths. Because of this, the 2010-11 data might be expected to have higher TP's and turbidities, and possibly lower CHL's. However, there were no significant differences in any of the parameter means at the 90% confidence level.

Table 14. Comparison of Total Phosphorus and Chlorophyll a Concentrations, and Turbidities in Allouez and Pokegama Bays During 2010-11 and 2017

Months and Years of Samples	Bay; No. sites; No. samples	TP (ug/l) mean (+/-90% C.I.)	TP (ug/l) range	CHL (ug/l) mean (+/- 90% C.I.)	CHL (ug/l) range	TURB (ntu) mean (+/- 90% C.I.)	TURB (ntu) range
September 2010, May and August 2011*	Allouez; 3 sites; n = 7	94 (+/- 25.1)	68 - 126	6.5 (+/- 5.1)	0.6 - 24	67 (+/-16.2)	36 - 95
May, August, and September 2017	Allouez; 3 sites; n = 18	76 (+/- 9.3)	46 - 115	8.8 (+/- 3.4)	2.1 - 26.6	62 (+/- 13.6)	28 - 123
September 2010, May and August 2011*	Pokegama; 2 sites; n = 6	108 (+/- 20.5)	68 - 142	5.7 (+/- 3.2)	2.6 - 15.1	54.6 (+/-25.7)	19 - 121
May, August, and September 2017	Pokegama; 2 sites; n = 12	101 (+/- 13.4)	49 - 130	6.5 (+/- 3.1)	0.5 - 23.4	70 (+/- 21.8)	17 - 155

*2010 and 2011 data from Bartsch et al 2015. Three site composite samples from sites near tributary mouths were tested.
TP = total phosphorus; CHL = chlorophyll a; TURB = turbidity

Data from 2012-13 (Bellinger et al 2015) (Table 15) showed no significant differences from 2017 data for CHL, TSS, and turbidity in Allouez and Pokegama Bays at the 90% confidence level. However, there was a very significant difference in TP for both bays, with 2012-13 values being much lower than 2017 values.

Table 15. Comparison of Total Phosphorus, Chlorophyll a, and Total Suspended Solids Concentrations, and Turbidities in Allouez and Pokegama Bays During 2012-13 and 2017

	ALLOUEZ BAY		POKEGAMA BAY			ALLOUEZ BAY		POKEGAMA BAY	
	May, Jul, Aug, Sep 2012; May, Jun, Jul, Aug, Sep, Nov 2013	May Oct 2017	Aug, Sep 2012; June, Aug 2013	June, Aug, Sep 2017		May, Jul, Aug, Sep 2012; May, Jun, Jul, Aug, Sep, Nov 2013	May-Oct 2017	Aug, Sep 2012; June, Aug 2013	June, Aug, Sep 2017
Chl a (µg L-1)					TSS(mg L-1)				
mean	10.9	7.1	13.0	7.5	mean	29.8	20.9	23.2	11.6
median	8.5	4.7	11.0	4.7	median	24.7	15.0	19.3	12.2
range	2.6 - 32	1.0 - 26.6	4.6 - 21.1	2.0 - 23.4	range	9.0 - 62.7	5 - 122	7.0 - 36.7	5.4 - 17.5
n	32	18	7	12	n	32	18	7	12
S.D.	6.8	6.2	6.6	6.1	S.D.	16.9	23.3	11.7	4.7
90% CI	2.0	2.4	4.1	2.9	90% CI	4.9	9.0	7.3	2.2
lower 90% CL	8.9	4.7	8.9	4.6	lower 90% CL	24.9	11.9	15.9	9.3
upper 90% CL	12.9	9.5	17.1	10.4	upper 90% CL	34.7	29.9	30.4	13.8
TP (µg L-1)					Turbidity (ntu)				
mean	32.2	84.6	37.1	94.7	mean	81.9	71.4	52.9	50.4
median	27.0	75.4	31.7	93.0	median	63.3	65.1	55.5	49.0
range	20.5 - 59.2	45.5 - 188	16.9 - 64.7	57.6 - 130	range	24.1 - 184	27.5 - 206	13.1 - 105	16.8 - 73.2
n	29	18	6	12	n	32	18	7	12
S.D.	11.5	31.2	16.6	22.1	S.D.	51.6	40.9	31.4	16.6
90% CI	3.5	12.1	11.1	10.5	90% CI	15.0	15.9	19.5	7.9
lower 90% CL	28.7	72.5	25.9	84.2	lower 90% CL	66.9	55.6	33.4	42.6
upper 90% CL	35.7	96.7	48.2	105	upper 90% CL	96.9	87.3	72.4	58.3

For Allouez and Pokegama Bays 2017 TP's were similar to those found in 2007 and 2011-12, but not 2012-13. 2017 TSS's were similar to those found in 2011-12 and 2012-13, but not 2007. The poor correlation between TP and TSS (Figure 49, $R^2 = 0.22$) may help explain the variation in the annual relationships for these two parameters. 2017 CHL's were similar to all three of the other data sets. 2017 turbidities were similar to the two other years with data (2011-12, 2012-13). In general, 2017 water quality conditions were fairly typical of what has been found previously, but the bays have the potential for substantial year to year variability. There was insufficient data for Kimballs Bay in the data sets above to provide meaningful comparisons between years.

Bay wetland site water quality data from July or August 2011-2017 is also compared to 2017 bay water quality data in the "Wetland Water Quality Data" section. Mean concentrations for the wetland water quality parameters were generally within the range of open water values found at nearby sites in the respective bays in July and August of 2017 (Table 36). One exception was Kimballs Bay where the wetland mean TP was about double that found at the open water site. The TP difference suggests wetland phosphorus release is occurring in Kimballs Bay.

Sediment Phosphorus Release

Sediment phosphorus (P) release can be a significant P source under some circumstances. Sediment P release can occur under aerobic conditions during periods of high pH (Anderson 1975). pH values > 8.5-9 are typically required for significant phosphorus release (James 2018). Maximum near surface pH's at the bay sites were 8.1 – 8.3 during July and August (appendix 1). pH's declined to ≤ 8 at a depth of 1 meter, and maximum near bottom pH's were 7.9. pH values are probably too low to make pH-induced sediment P release significant in the open water areas of the three bays.

Sediment P release can also occur if anoxia develops at the sediment surface. Iron bound phosphorus is then released to the water column as orthophosphate (Wetzel 2001). Some of the orthophosphate can be adsorbed by suspended particulates, taken up by bacteria, or re-bound to iron complexes that precipitate as oxygen is re-encountered. An examination of increases in near bottom TP's and orthophosphate concentrations (OP's) along with thermal stratification and decreases in near bottom dissolved oxygen concentrations (D.O.'s) can provide evidence of sediment P release.

Sediment P Release Indicators

Seasonal patterns of top and bottom TP's and OP's provided evidence of sediment P release at some sites. Site KND showed top to bottom TP and DOP increases from July 10th to August 29th (Figures 13 and 22). Those dates had the lowest bottom D.O.'s and the greatest top to bottom temperature decreases for the season. A substantial fraction of bottom TP was OP (25 – 43%) on these four dates. Top to bottom ammonia nitrogen concentrations also increased substantially on these dates (Figure 40), which is also consistent with very low D.O. in bottom water. The two dates with the lowest bottom D.O.'s (July 24th, 0.6 mg/l and August 15th, 0.8 mg/l) had the highest top to bottom OP increases. All these observations indicate sediment P release was substantial and prolonged at this site. On August 29th, Site KND was also influenced by the underflow of stream water entering the bay during a runoff event just prior to that date. Much of the top to bottom differences on that date were due to this (see discussion of August 29th bay profiles in the Bay Stratification / Profile Data section).

Site ASD showed top to bottom TP and OP increases on August 29th (Figures 10 and 19). This probably, again resulted from the underflow of stream water entering the bay just prior to that date (see discussion of August 29th bay profiles in the Bay Stratification / Profile Data section). TP's and OP's measured in Bear and Bluff Creeks on August 28th were higher than those found at the bottom of site ASD on August 29th, and so can account for the observed top to bottom increases observed. Sediment phosphorus release is not indicated.

Site PND showed indications of occasional sediment phosphorus release. Top to bottom DOP increased 5.6 ug/l, and top to bottom TP increased 23.2 ug/l on August 15th (Figures 14 and 23). OP comprised 24% of the top to bottom TP increase. Bottom D.O. on August 15th was moderately low at 5.7 mg/l. Site PS near the upstream end of Pokegama Bay is too shallow (1 m, 3.2 ft) to allow top and bottom TP and OP sampling. However, summer daytime D.O.'s were frequently < 5 mg/l (Table 8). On one date, September 11th, a top D.O. of 2 mg/l was found near the edge of the adjacent wetland, while the top D.O. at site PS was 4.4 mg/l. Pokegama Bay is narrow at this site and bordered by wetlands. There is probably potential for sediment or wetland P release in the vicinity of site PS.

The monitoring design used for this project had limited ability to fully assess the extent of D.O. depletion, sediment phosphorus release, and potential wetland phosphorus release. All site D.O. measurements were made during late morning to early afternoon in open water areas. Diurnal fluctuations in D.O.'s probably result in lower D.O.'s occurring at night. D.O.'s adjacent to, and in wetlands are likely to be lower than at open water sites.

The influence of wetlands adjacent to the bays may be substantial. Decomposition of organic matter in wetlands can result in relatively rapid D.O. depletion and subsequent release of iron bound phosphorus from sediment. Seiche-induced pulses of water into and out of these wetlands may enhance the phosphorus cycling process.

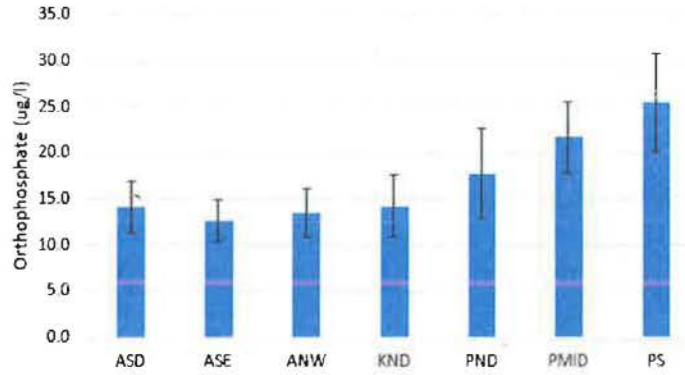
Orthophosphate (Top)

Orthophosphate is the phosphorus form immediately available for algal uptake. Orthophosphate is also the phosphorus form released from anoxic sediment. The relationship between top to bottom TP increases and top to bottom orthophosphate concentration (OP) increases at bay sites is discussed in the preceding section.

Mean OP's in top samples ranged from 12.7 ug/l (site ASE) to 25.5 ug/l (site PS) (Figure 18). The three sites in Pokegama Bay had the highest mean OP's. Sites PS and PMID had significantly higher mean OP's than the Allouez and Kimballs Bay sites. This may be partially due to release of OP from the wetlands that fringe the upstream end of Pokegama Bay and the downstream end of the Pokegama River. The timing of discharges of wastewater lagoon effluent from the Village of Superior to the Pokegama River (primarily May and October) did not appear to be correlated with higher OP's in Pokegama Bay (Figures 23-25), and so are unlikely to be a major source of the elevated OP's in the Bay. However, lagoon effluent phosphorus delivered to the bay will contribute to the pool of phosphorus available for release at other times of the year. Lower OP's in Allouez Bay were probably influenced by inputs of Lake Superior water with low OP's.

Suspended red clay in the SLRE area has been shown to adsorb orthophosphate when high OP's occur (Bahnick 1980). Orthophosphate adsorption by clay occurs when an equilibrium concentration of 20 - 42 ug/l is exceeded. Mean bay site OP's (12.7 – 25.5 ug/l) are within or below this concentration range, suggesting suspended clay may be contributing to the maintenance of lower OP's.

Figure 18. Bay Sites Top Orthophosphate Concentration Means



Error bars are 90% confidence intervals

The percent of mean top TP as OP ranged from 15% (ASE) to 23 % (KND) (Table 16). Allouez Bay tended to have lower percentages partly due to low OP's. Kimballs Bay (KND) had the highest percentage partly due to low TP.

Table 16. Bay Sites % of Total Phosphorus as Orthophosphate

Bay Site	ASD	ASE	ANW	KND	PND	PMID	PS
Mean top TP (ug/l)	76.7	86.4	90.8	62.8	98.2	120.5	144.8
Mean top OP (ug/l)	14.1	12.7	13.5	14.3	17.7	21.7	25.5
% TP as OP	18.4	14.7	14.9	22.7	18.0	18.1	17.6

Top OP's were lowest at most sites on August 15th and August 29th (Figures 19 - 25). High chlorophyll a concentrations occurred at that time (Figure 27) and algal uptake converts OP to TP. Tributary stream OP's were also relatively low at that time (Figure 68). Top Bay OP's tended to be highest at most sites during late September and October. The Pokegama Bay sites were more variable. Release of orthophosphate from decomposition of algae and aquatic macrophytes and reduced orthophosphate uptake by declining algae populations (Figure 27) may account for the higher fall OP's at most sites.

Figure 19. Site ASD Top and Bottom Orthophosphate Concentrations

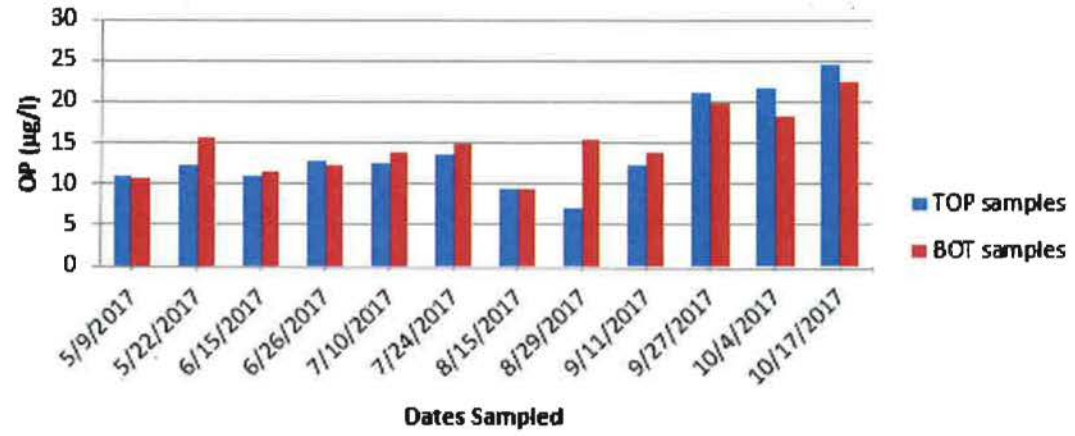


Figure 20. Site ASE Top and Bottom Orthophosphate Concentrations

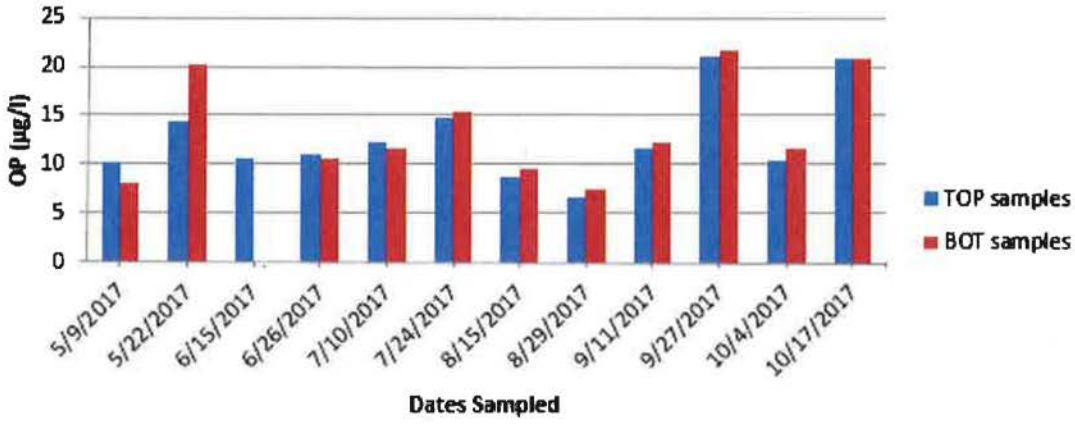


Figure 21. Site ANW Top and Bottom Orthophosphate Concentrations

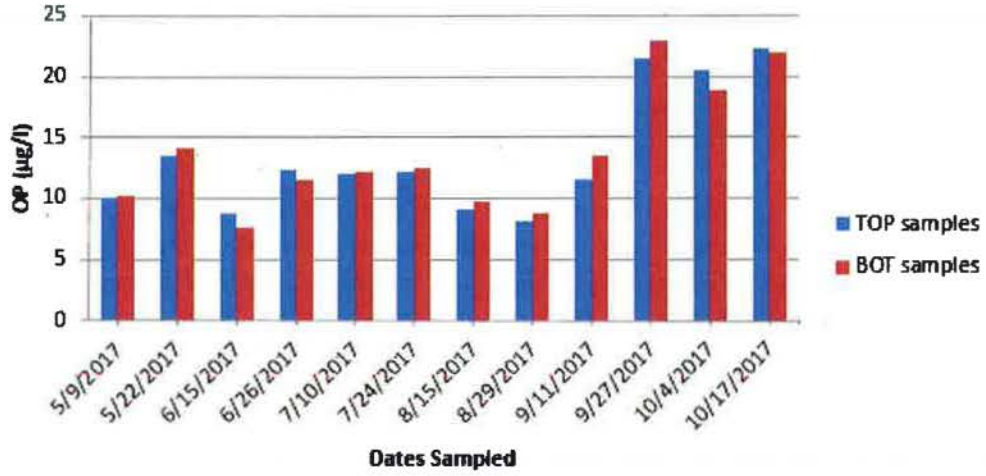


Figure 22. Site KND Top and Bottom Orthophosphate Concentrations

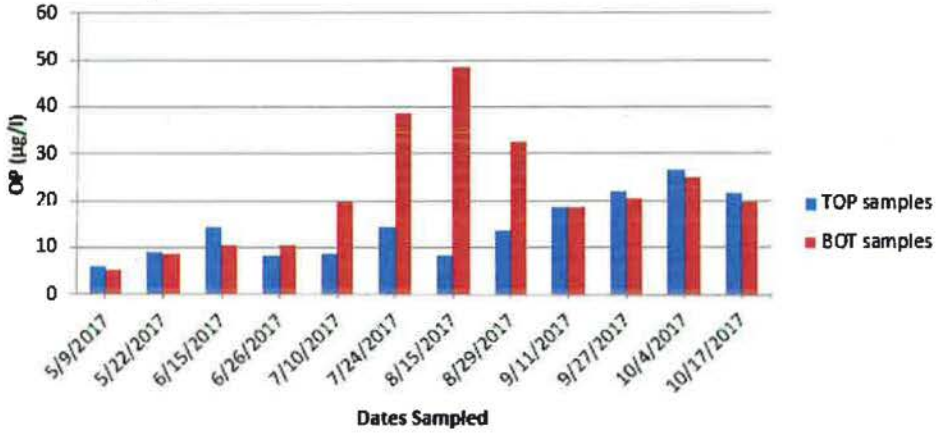


Figure 23. Site PND Top and Bottom Orthophosphate Concentrations

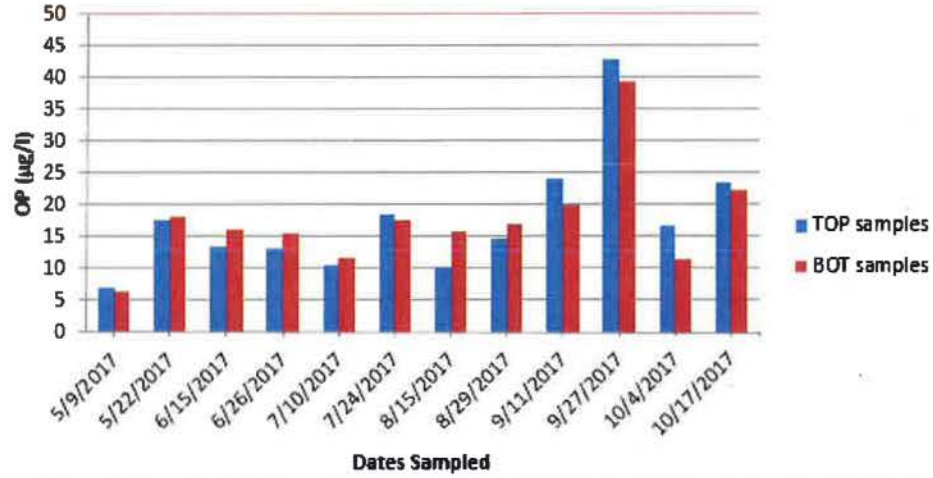


Figure 24. Site PMID Top and Bottom Orthophosphate Concentrations

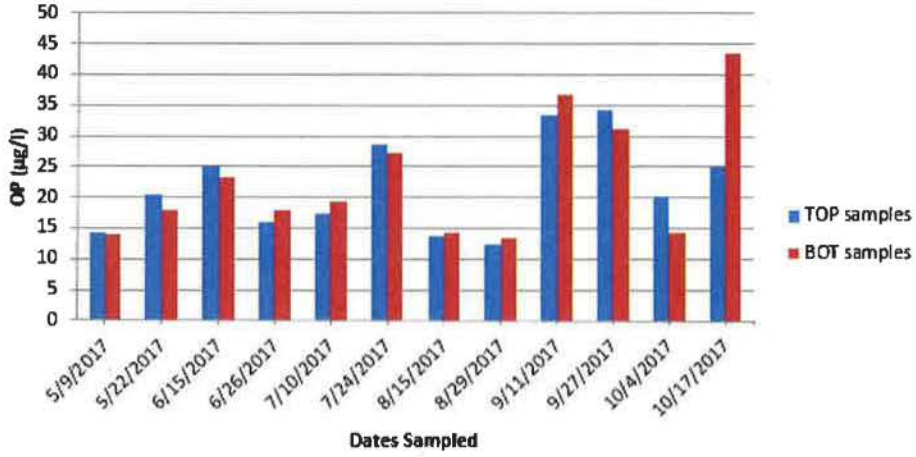
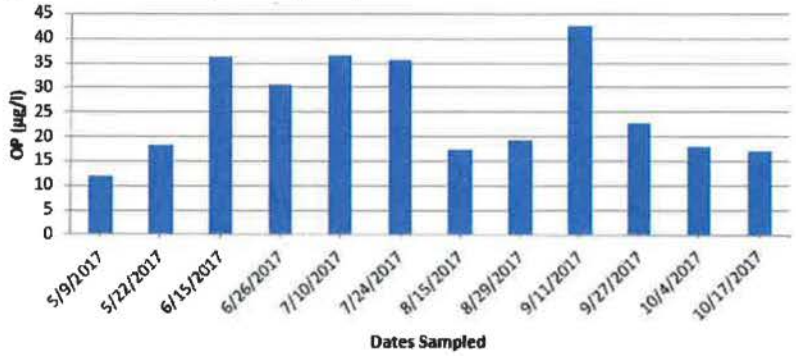


Figure 25. Site PS Top Orthophosphate Concentrations

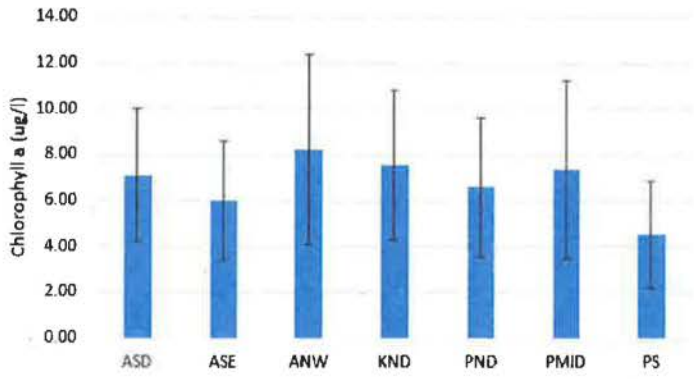


Chlorophyll a

Range and Seasonal Patterns of Chlorophyll a Concentrations

Mean chlorophyll a concentrations (CHL's) ranged from 4.5 ug/l (site PS) to 8.2 ug/l (site ANW) (Figure 26). Site PS at the upstream end of Pokegama Bay had the lowest mean CHL, but differences between sites were not significant.

Figure 26. Bay Sites Chlorophyll a Concentration Means



Error bars are 90% confidence intervals

Highest CHL's tended to occur in July and August (Figure 27). Three July and August samples exceeded 20 ug/l, with the highest CHL at 26.6 ug/l in Allouez Bay at site ANW. Turbidity and total suspended solids concentrations were low during these months (Figures 47 and 53), so potential light limitation of algae growth was reduced. Warmer water temperatures in those months may have contributed to algal growth, especially blue-green algae growth. Total algal cell densities were also highest in July and August (Figure 81).

Allouez Bay also had moderately high CHL's on May 9th (7-11 ug/l) and June 15th (11-15 ug/l) (Figures 27 and 28). Allouez Bay also had higher algal cell densities than the other two bays in May and June (Figure 81).

Figure 27. Allouez, Kimballs, and Pokegama Bays Chlorophyll a Concentrations

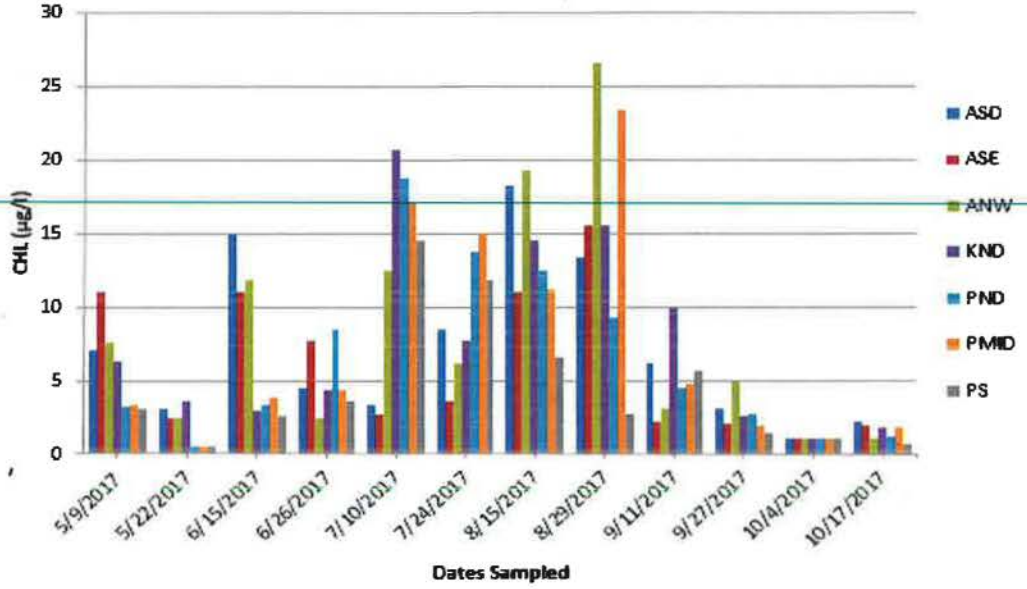


Figure 28. Allouez Bay Chlorophyll a Concentrations

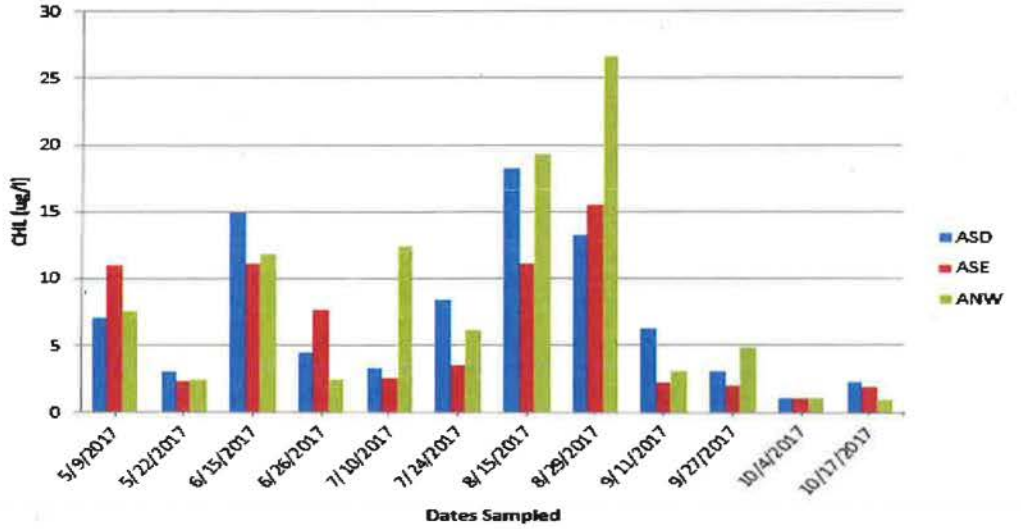
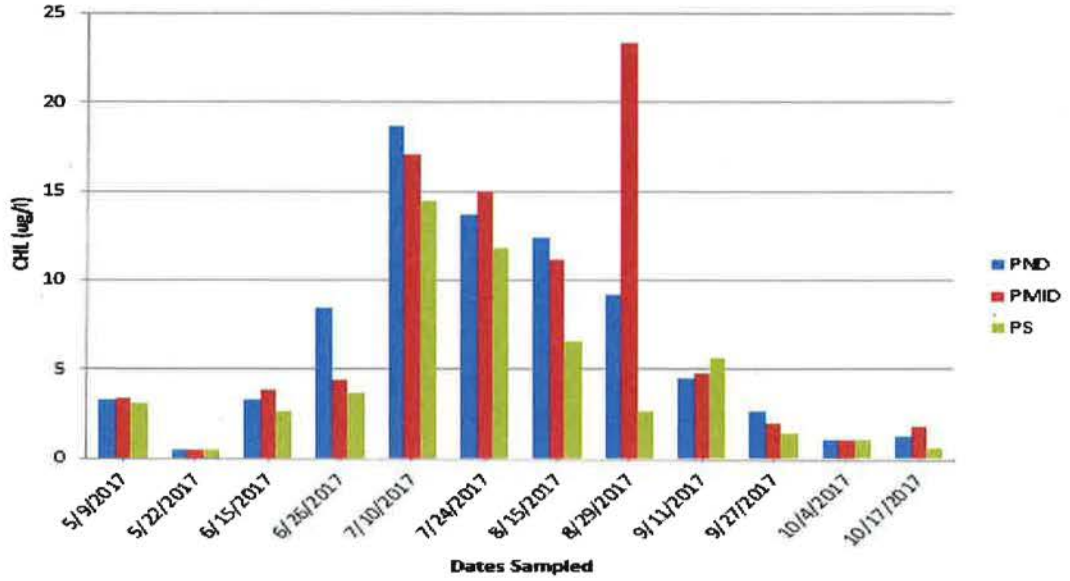


Figure 29. Pokegama Bay Chlorophyll a Concentrations



Comparison to CHL Criteria and Reference CHL's

The St. Louis River Area of Concern 2016 Remedial Action Plan identified 10 ug/l goal as the CHL target for the St. Louis River estuary (SLRE) (MPCA et al. 2017). This target was intended to apply to the open main channel areas of the SLRE but not sheltered bays. Mean CHL's at the bay sites were below this goal.

CHL's were measured at 150 sites throughout the SLRE in 2012 and 2013 (Bellinger et al. 2015). Median CHL's of 6.0 ug/l and 6.3 ug/l were found for 2012 and 2013 respectively. These medians are higher than those found at sites in the three bays (2.9 – 5.5 ug/l), despite the much higher TP's in the bays. This also suggests limitation of algal growth by factors other than TP in the bays.

A CHL of 10.7 ug/l was identified as a threshold for eutrophic lakes in Wisconsin by Lillie and Mason (1983). A CHL of 20 ug/l is considered to indicate a nuisance algal bloom (WI DNR 2017). The Wisconsin Dept. of Natural Resources (WI DNR) (2017) has a recreational impairment threshold for CHL in shallow lakes based on >30% of days having CHL's exceeding 20 ug/l during July 15th through September 15th. The upper 90% confidence limit of the mean number of days must be $\leq$ 30% for a lake to "clearly meet" this criterion. The combined sites from Pokegama Bay clearly met this criterion (mean = 9%; upper 90% C.I. = 25%). The combined sites from Allouez Bay "may meet" this criterion (mean = 15%; upper 90% C.I. = 33%). The site in Kimballs Bay also may meet this criterion (mean = 7%; upper 90% C.I. = 40%). Additional monitoring might lower these 90% C.I.'s below 30%. All bay mean values are well below 30%. The WI DNR also has a fish and aquatic life impairment threshold for CHL in shallow lakes of 27 ug/l (based on the upper bound of the 90% confidence interval of the mean CHL). CHL's from all bay sites fell below this threshold.

Bay CHL's in 2017 were similar to CHL's measured in 2007, 2010-11, and 2012-13 (Tables 13, 14, 15).

CHL relationship to other Trophic State Indices

Trophic state indices (TSI's) are often used to categorize lake nutrient and productivity levels. TP's, CHL's, and Secchi depth (a measure of water clarity) have been used to develop TSI's (Carlson 1977), since these parameters typically provide inter-related measures of lake nutrient and productivity levels. TSI's for bay site median TP's, CHL's and Secchi depths (SD's) are shown in Table 17. If these parameters have a typical relationship, their TSI values will be similar. These relationships were largely derived from water bodies where algal biomass (i.e. chlorophyll) was limited by TP and where water clarity (i.e. Secchi depth) was regulated by algal particulate material.

Bay site TSI values were very different for the three parameters (Table 17) indicating an atypical relationship. For the TP's present, much higher CHL's would normally be expected. CHL's were only a small fraction, 2.9 – 18.5%, of expected concentrations. SD's were also lower than would normally be expected. SD TSI's were much higher than TP TSI's.

Table 17. Bay Sites Trophic State Index (TSI) Values

SLRE BAYS TSI* VALUES

SITE	MEDIAN TP (UG/L)	TP TSI	MEDIAN CHL (UG/L)	CHL TSI	TP TSI "NORMAL" CHL (UG/L)	MEDIAN CHL AS % OF "NORMAL" CHL	MEDIAN SECCHI (M)	SECCHI TSI
ASD	77.3	67	5.4	47	40.2	13.4	0.23	81
ASE	81.3	68	3	41	43.3	6.9	0.24	81
ANW	85.4	68	5.5	47	46.6	11.8	0.21	82
KND	61.4	64	5.3	47	28.7	18.5	0.73	65
PND	89	69	3.9	44	49.5	7.9	0.30	77
PMID	111	72	4.2	45	68.5	6.1	0.23	81
PS	143	76	2.9	41	99.4	2.9	0.21	82

**TSI relationships from Carlson (1977).*

The poor water clarity due to suspended clay and silt, rather than algal cells, is the probable reason for these altered relationships. High turbidities due to suspended inorganic particles have been shown to cause light limitation of algal growth in other water bodies (Wetzel 2001). Also, a portion of the TP in the three bays is bound to suspended clay particles and is less bio-available, as has been observed in the SLRE area (Bahnick 1980) and elsewhere (DePinto et al. 1981). Short residence times might also limit full algal response to available phosphorus at some sites, especially site PS. The poor water clarity (low SD's) is controlled more by suspended inorganic particles than suspended algae. This greatly limits the use of TSI's for these bays.

Lack of typical lake-derived TSI parameter relationships complicates water quality goal setting. It makes it difficult to predict responses to water quality improvements.

Nitrogen

Mean nitrogen parameter values for the bay sites are shown in Figures 30 – 32, 35, 36, and 44. Median nitrogen parameter values for the bay sites are shown in Table 18.

Table 18. Bay Sites Median Values for Nitrogen Parameters

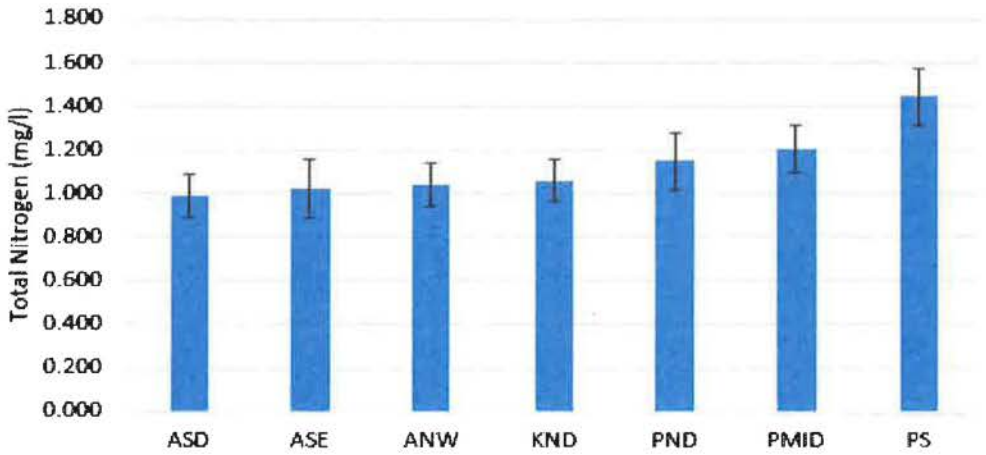
	SITES						
	ASD	ASE	ANW	KND	PND	PMID	PS
NH_3-N top (ug/l)	39.5	41.3	34.8	43.8	35.9	39.9	49
NH_3-N bottom (ug/l)	42.6	39.5	42.7	57.8	53.5	44.2	no data
$NO_3 + NO_2$-N (ug/l)	125	116	126	34.8	51.8	34.5	9.5
TKN (ug/l)	924	880	913	977	1030	1090	1420
Organic N (ug/l)	885	839	878	933	989	1050	1370
% TKN as organic N	96	95	96	96	96	96	96
Dissolved TKN (ug/l)	667	608	637	764	895	820	929
% TKN dissolved	72	69	70	78	87	75	66
Dissolved organic N (ug/l)	627	567	602	720	859	780	880
Particulate organic N (ug/l)	258	272	276	213	130	268	486
Total dissolved N (ug/l)	792	724	763	798	947	855	939
Total Nitrogen (ug/l)	1050	1000	1040	1010	1080	1120	1420
Diss. org. N / Part. org. N	2.4	2.1	2.2	3.4	6.6	2.9	1.8

NH_3-N = ammonia; NO_3+NO_2-N = nitrate plus nitrite; TKN = total Kjeldahl nitrogen; organic N, dissolved organic N, particulate organic N, total dissolved N, and total N concentrations were determined by calculations using lab-measured parameters; Top samples collected 0.5 m below water surface; Bottom samples collected 0.5 m above sediment surface.

Total Nitrogen, Total Kjeldahl Nitrogen and Organic Nitrogen

Mean total nitrogen concentrations (TN's) ranged from 0.989 mg/l (site ASD) to 1.44 mg/l (site PS) (Figure 30). The mean TN at site PS was significantly higher than at all other sites. This was probably due to the strong influence of the Pokegama River which had a mean TN of 1.57 mg/l. The mean TP at site PMID was significantly higher than at site ASD, the site with the lowest mean TP. This, again, was mostly due to Pokegama River influence on site PMID. Samples collected in 2012 and 2013 throughout the SLRE had slightly lower median TN's (2012 – 0.963 mg/l, 2013 – 0.905 mg/l) (Bellinger 2015) than the bay sites (1.00 – 1.42 mg/l).

Figure 30. Bay Sites Total Nitrogen Concentration Means



Error bars are 90% confidence intervals

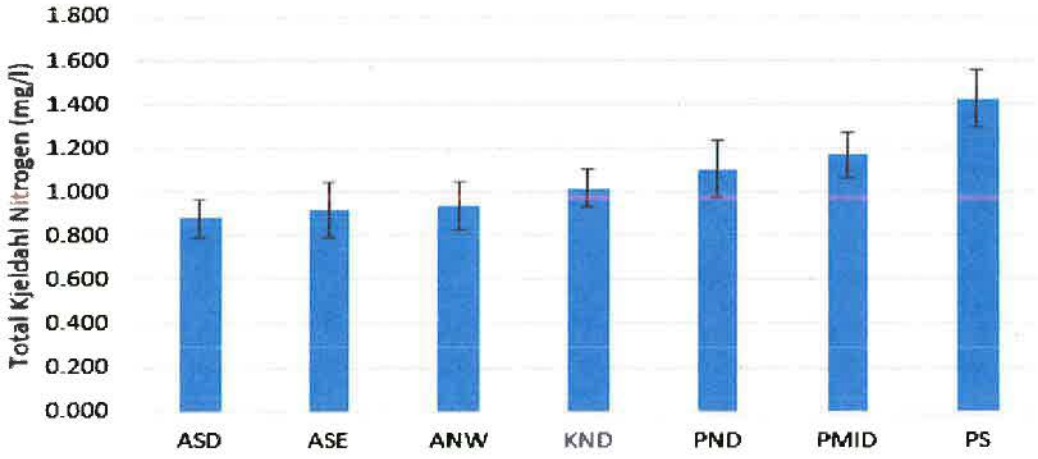
Mean total Kjeldahl nitrogen concentrations (TKN's) ranged from 0.881 mg/l (site ASD) to 1.43 mg/l (site PS) (Figure 31). The mean TKN for site PS was significantly higher than all other sites. The mean TKN for site PMID was significantly higher than the three Allouez Bay sites. Pokegama River influence probably accounted for the higher TKN values at the two Pokegama Bay sites. The Pokegama River had a mean TKN of 1.51 mg/l.

Mean dissolved total Kjeldahl nitrogen concentrations (DTKN's) ranged from 0.649 mg/l (site ANW) to 0.974 mg/l (site PS) (Figure 32). All Pokegama Bay sites had significantly higher mean DTKN's than Allouez Bay sites. The mean DTKN for site PS was significantly higher than for site KND. Pokegama Bay sites were probably, again, influenced by Pokegama River inputs. Dilution with Lake Superior water may have accounted for the lower mean DTKN's in Allouez Bay.

Seasonal TKN patterns were minimal (Figure 33). In Allouez Bay, lowest TKN's occurred in mid-summer, and highest TKN's occurred in October. In Kimballs and Pokegama Bay there were no notable mid-summer TKN declines. Higher TKN's for most sites occurred in late September and October. Release of dissolved organic nitrogen from decomposing vegetation may contribute to this. Stream TKN's also tended to be somewhat higher at that time. Seasonal DTKN patterns generally appear similar to TKN patterns (Figure 34).

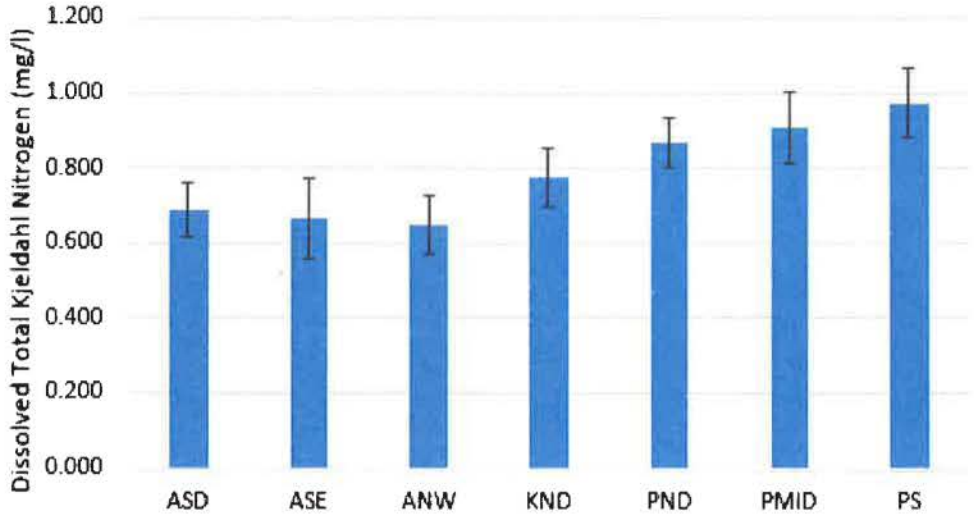
Greater than 95% of TKN was present as organic nitrogen at all sites (Table 18). Most TKN was in a dissolved form (65.7 – 87.3 %). Dissolved TKN is comprised of dissolved organic nitrogen and ammonia. Much of dissolved organic nitrogen is in forms resistant to bacterial degradation, while some forms can be readily utilized by bacteria (Chan and Campbell 1978).

Figure 31. Bay Sites Total Kjeldahl Nitrogen Concentration Means



Error bars are 90% confidence intervals

Figure 32. Bay Sites Dissolved Total Kjeldahl Nitrogen Concentration Means



Error bars are 90% confidence intervals

Figure 33. Bay Sites Total Kjeldahl Nitrogen Concentrations

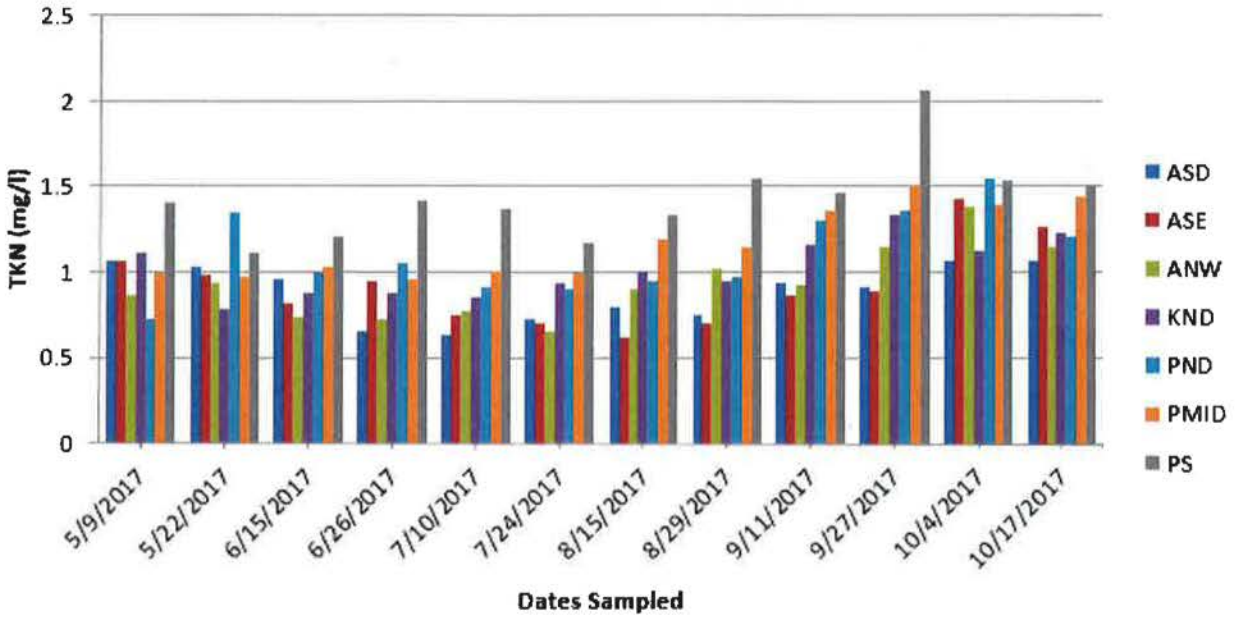
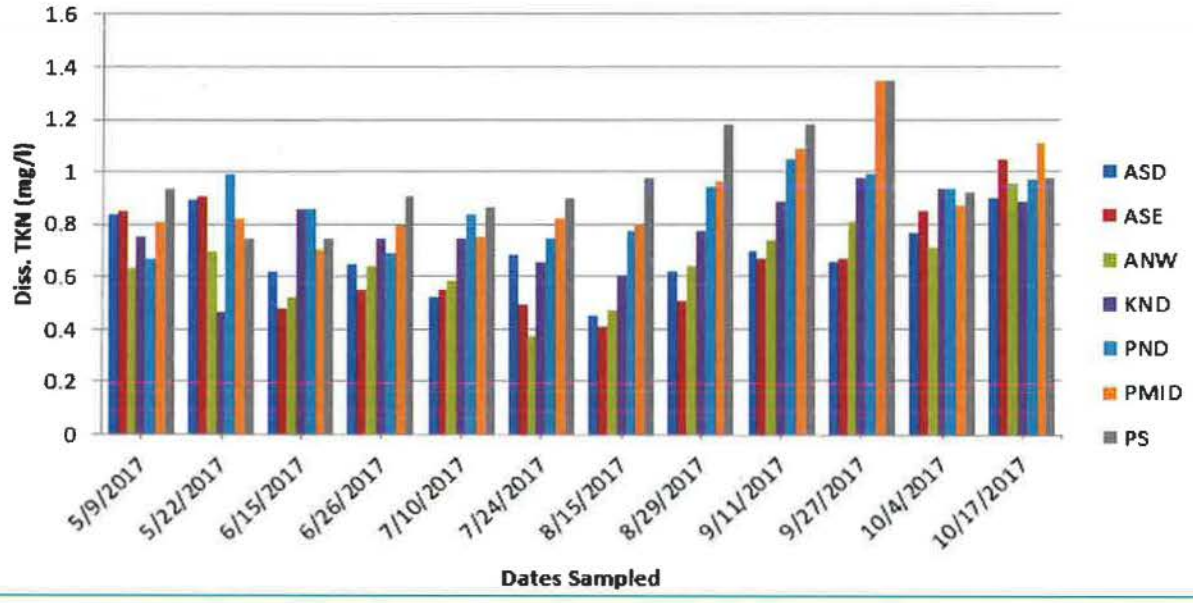


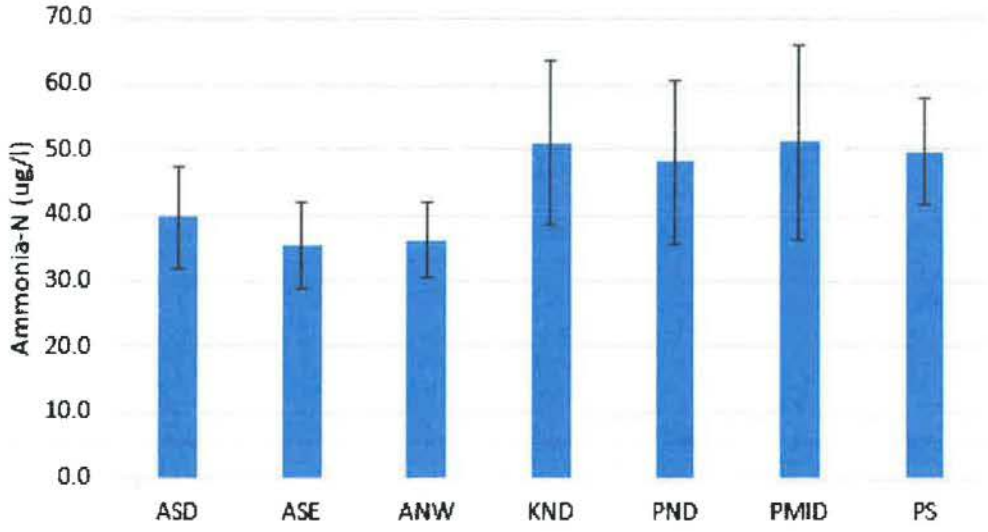
Figure 34. Bay Sites Dissolved Total Kjeldahl Nitrogen Concentrations



Ammonia Nitrogen

Mean top ammonia nitrogen concentrations (NH_3's) were not significantly different across sites and range from 35.4 ug/l (site ASE) to 51.2 ug/l (site PMID) (Figure 35). The pooled top NH_3's from Allouez Bay sites were significantly lower than the pooled top NH_3's from the Pokegama Bay sites. This was probably due to dilution with Lake Superior water in Allouez Bay and the influence of Pokegama River water on Pokegama Bay. Samples collected in 2012 and 2013 throughout the SLRE had median NH_3's (2012 – 29.5 ug/l, 2013 – 26.6 ug/l) (Bellinger 2015) that were lower than bay site medians (34.8 – 49.0 ug/l) (Table 18). Higher NH_3's in the bays were probably due to greater influence of organic matter decomposition in wetlands and in shallow water sediments. Additionally, bay tributary streams had higher median NH_3's (41-46 ug/l) than the SLRE.

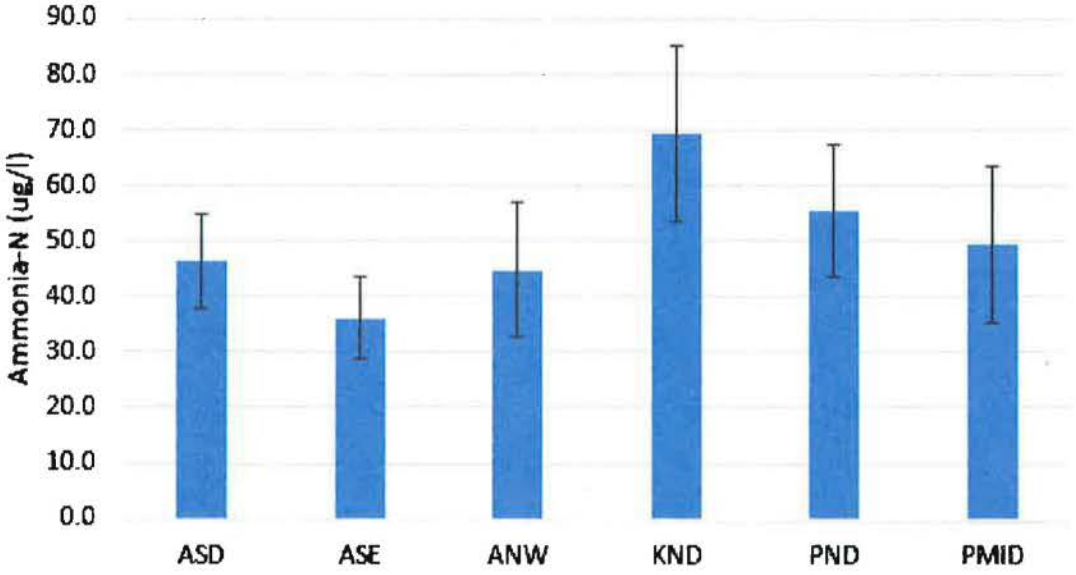
Figure 35. Bay Site Top Ammonia-N Concentration Means



Error bars are 90% confidence intervals

Mean bottom NH_3's ranged from 36.2 ug/l (site ASE) to 69.3 ug/l (site KND). The mean bottom NH_3 was significantly higher at site KND than at site ASE. Site KND had the most extensive bottom oxygen depletion, which can contribute to increased NH_3's by reducing bacterial nitrification of ammonia to nitrate. Increased mineralization of ammonia from sediments rich in organic nitrogen will also lead to higher levels of NH3 in bottom waters. The three Allouez Bay sites had mean bottom NH_3's that were lower than the means for Kimballs and Pokegama Bay. This may again be due to dilution with Lake Superior water in Allouez Bay

Figure 36. Bay Site Bottom Ammonia-N Concentration Means



Error bars are 90% confidence intervals

In Allouez Bay, NH_3 tended to be lower in early May and July through August (Figures 37, 38, 39). Uptake of NH_3 by algae probably accounted for this. CHL's in Allouez Bay were high during those periods (Figure 28). A spike in NH_3 in the bottom sample at site ASD on August 29th was probably influenced by the underflow of stream water entering the bay just prior to that date (see discussion of August 29th bay profiles in the Bay Stratification / Profile Data section).

At the Kimballs Bay site (KND), NH_3 in top samples tended to be lower in May and late June through August (Figure 40). This was again, likely due to algal uptake of NH_3, since CHL's also tended to be highest during those periods (Figure 27). Bottom sample NH_3's were highest during July and August, probably due to a combination of oxygen depletion and high rates of organic matter decomposition. In Pokegama Bay, NH_3 patterns were more variable, with somewhat of a tendency to be lower in May and summer (Figures 41, 42, 43). Summer CHL's were also higher in the bay (Figure 29). NH_3's were higher at sites PND and PMID on August 15th, possibly due to a period of dissolved oxygen depletion, also suggested by elevated dissolved orthophosphate in the bottom sample at site PND on that date (Figure 23).

Figure 37. Site ASD Top and Bottom Ammonia-N Concentrations

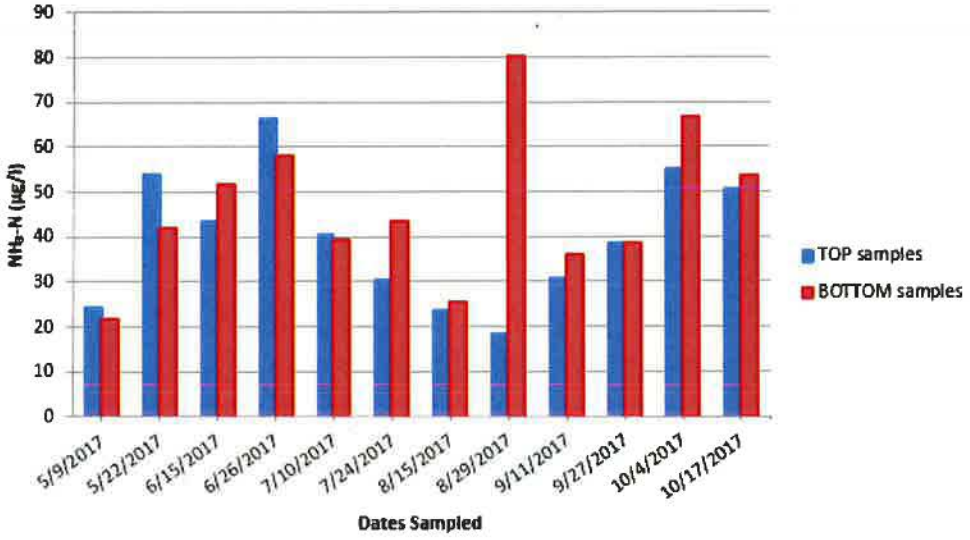


Figure 38. Site ASE Top and Bottom Ammonia-N Concentrations

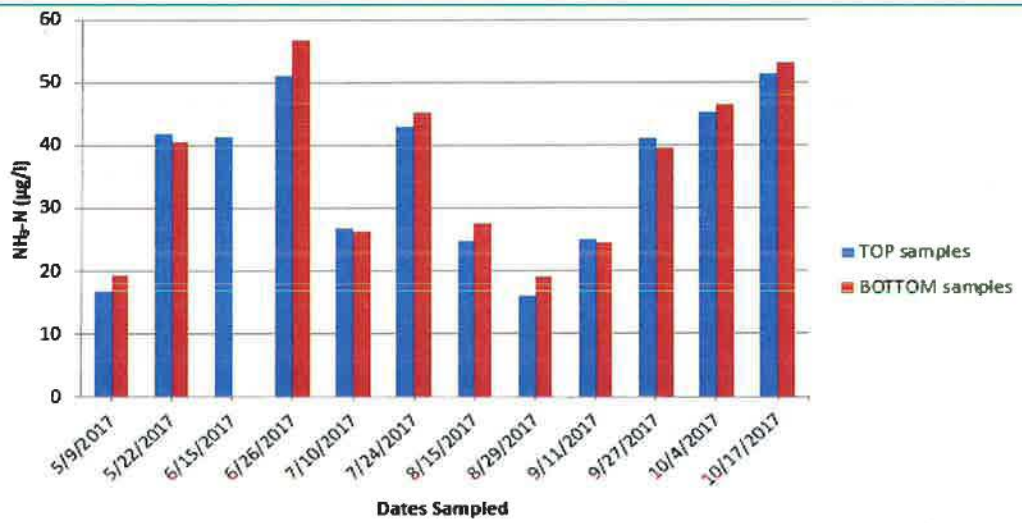


Figure 39. Site ANW Top and Bottom Ammonia-N Concentrations

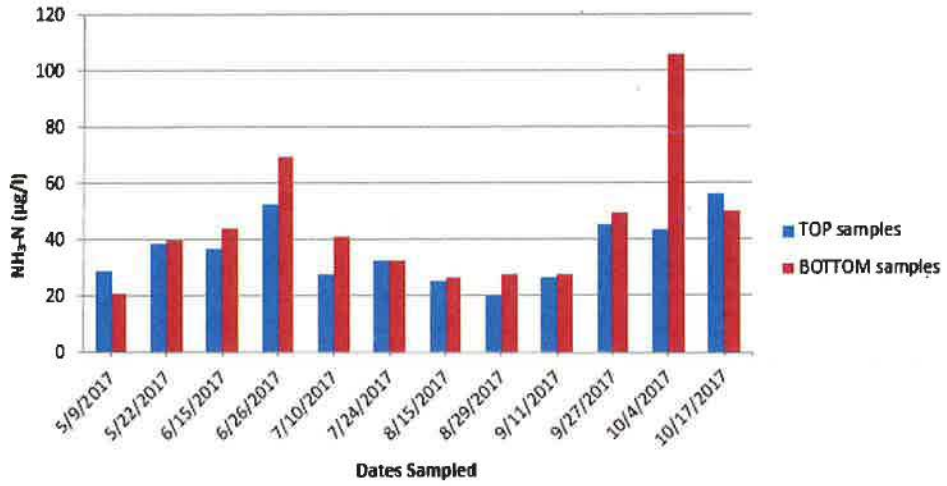


Figure 40. Site KND Top and Bottom Ammonia-N Concentrations

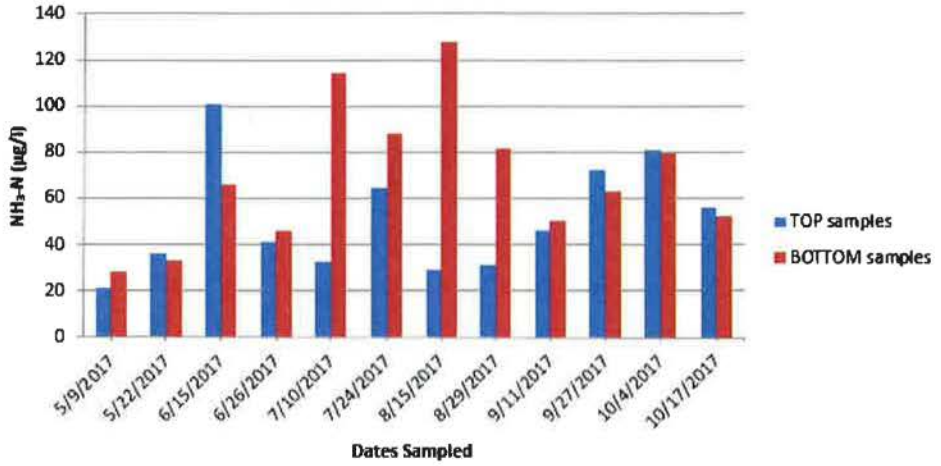


Figure 41. Site PND Top and Bottom Ammonia-N Concentrations

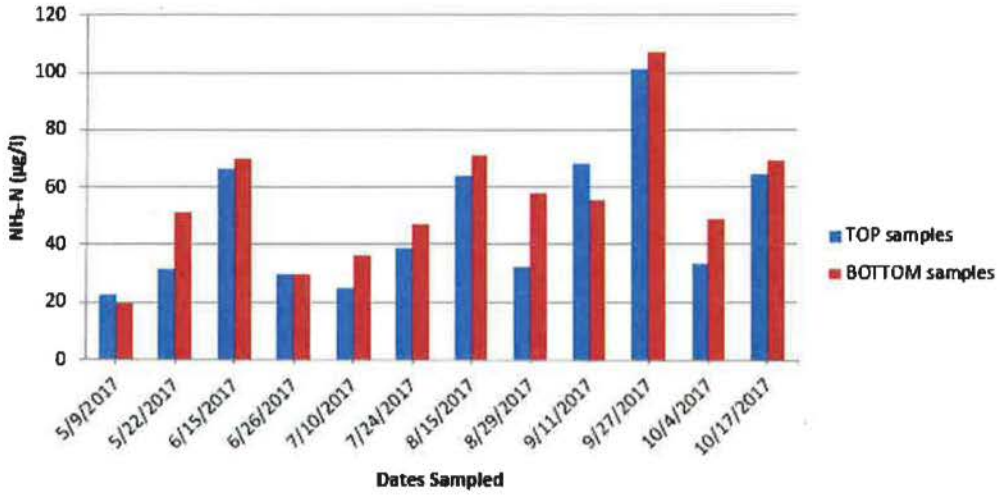


Figure 42. Site PMID Top and Bottom Ammonia-N Concentrations

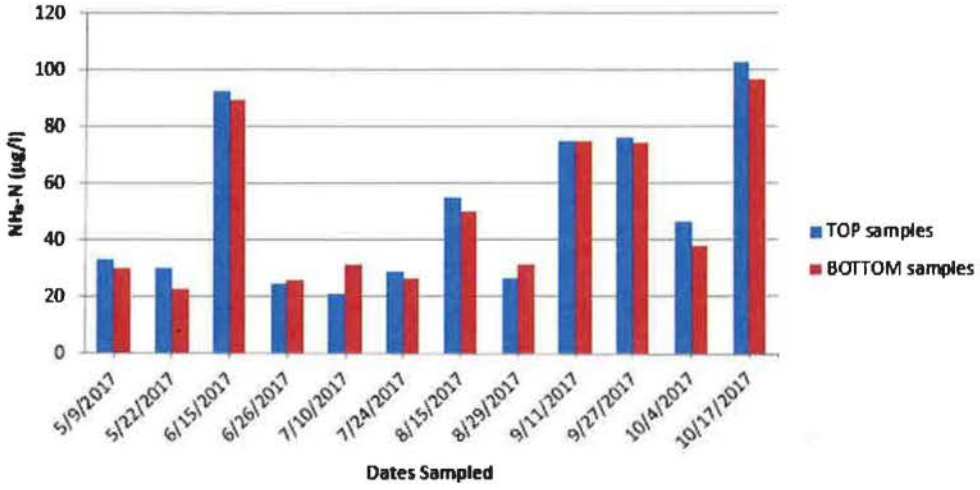
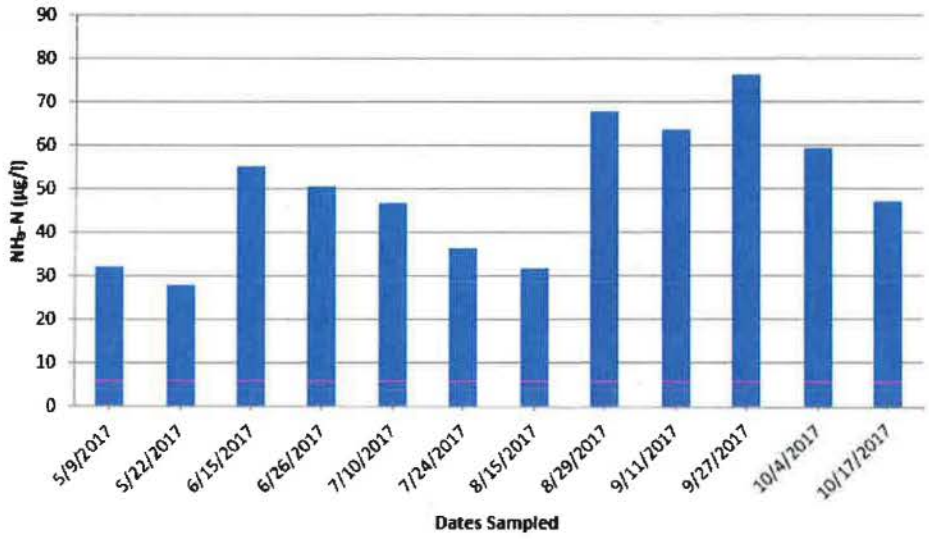


Figure 43. Site PS Top Ammonia-N Concentrations

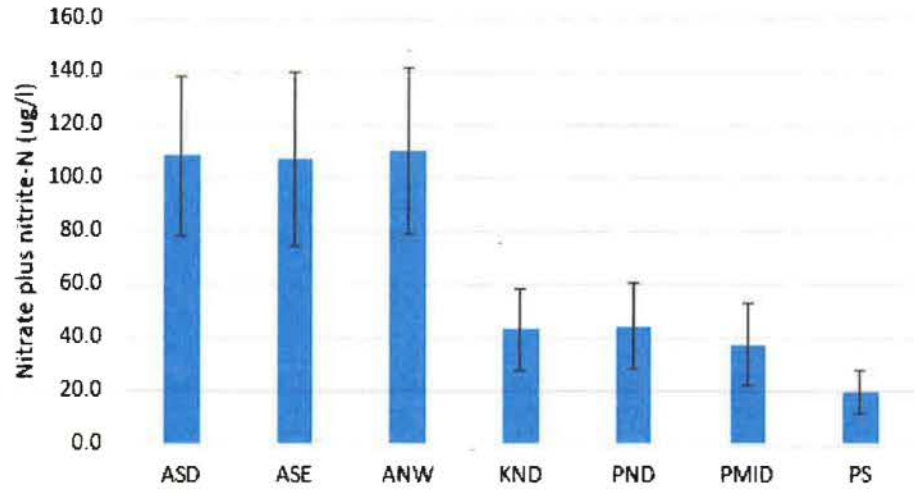


Nitrate plus Nitrite Nitrogen

Mean nitrate plus nitrite nitrogen concentrations (NOx's) ranged from 19.4 ug/l (site PS) to 110 ug/l (site ANW) (Figure 44). The mean NOx for site PS was significantly lower than site PND and the three Allouez Bay sites. Mean NOx's for the three Allouez Bay sites were significantly higher than all other sites. Site PS had the lowest mean NOx, probably due to greater rates of denitrification in this wetland influenced area, with periodic dissolved oxygen depletion. The Allouez Bay sites had the highest mean NOx's (106.9 – 110.4 ug/l), probably due to inputs of water from Lake Superior. The mean May – October 2013 NOx in the near-shore waters of Lake Superior was 321.5 ug/l (Bellinger 2015).

Samples collected in 2012 and 2013 throughout the SLRE had higher median NOx's (2012 – 135.4 ug/l, 2013 – 153.4 ug/l) (Bellinger 2015) than the bay sites (9.5 -126 ug/l). Inputs of Lake Superior water also raise median NOx values in the SLRE, while denitrification due to wetland influence and dissolved oxygen depletion lower median NOx values in the bays.

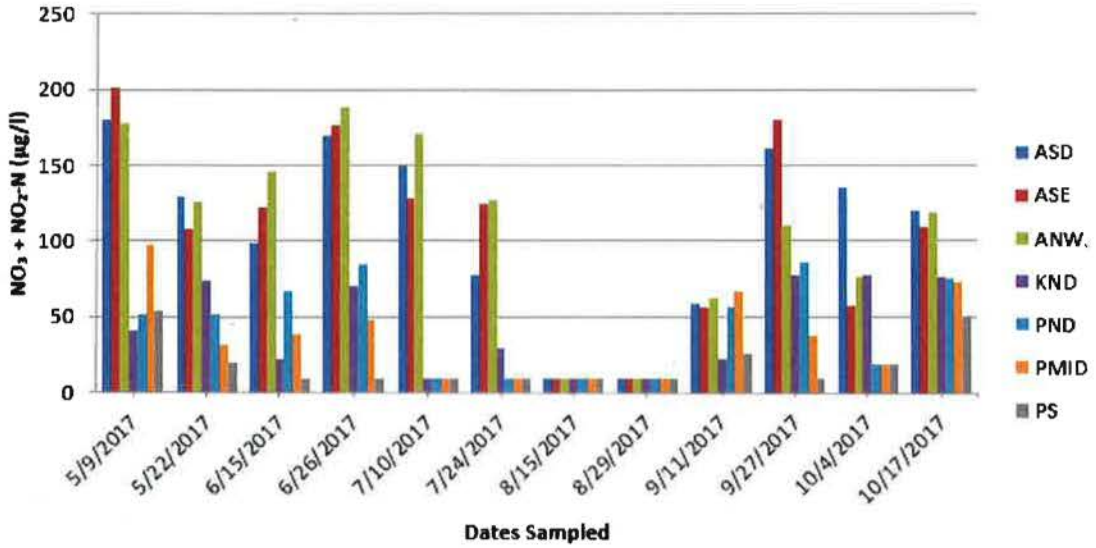
Figure 44. Bay Sites Nitrate plus Nitrite-N Concentration Means



Error bars are 90% confidence intervals

NOx's were generally lowest in the summer (Figure 45). NOx's in Kimballs and Pokegama Bays were almost entirely below detection levels in July and August. NOx's in Allouez Bay were also below detection levels in August. Uptake of nitrate by algae probably accounts for this pattern. Chlorophyll a concentrations peaked during July and August (Figure 27).

Figure 45. Bay Sites Nitrate plus Nitrite-N Concentrations



Nitrogen to Phosphorus Ratios

Nitrogen to phosphorus (N:P) ratios can, in some cases, provide an indication of whether nitrogen or phosphorus is likely to be the limiting nutrient for algal growth. A guide for evaluating Wisconsin lake N:P ratios indicates N:P ratios >15 indicate phosphorus limitation, N:P ratios < 10 indicate nitrogen limitation, and N:P ratios of 10 -15 are transitional (Shaw, et al. 1993).

Summarized total nitrogen to total phosphorus (N:P) ratios for the seven bay sites are shown in Table 19. Most median N:P ratios at the bay sites were in the transitional range (10-15). Site KND's (Kimbals Bay) median N:P ratio slightly exceeded 15, suggesting phosphorus limitation, while site PS's (Pokegama Bay, south) median N:P ratio was slightly less than 10, suggesting nitrogen limitation. Site KND probably receives the greatest contributions from estuary water. Site PS is the most riverine site and is strongly influenced by Pokegama River water inputs. N:P ratios for the primary tributary streams to Allouez and Pokegama Bays are shown in Table 20. Median N:P ratios for the stream sites (8.3 – 10.4) were lower than most bay site ratios, except for site PS

Nutrient limitations suggested by N:P ratios for the bays may be of limited value. TP is only partially bioavailable due to bonding to suspended clay particles (Bahnick 1980, DePinto et al. 1981). The potential for light limitation of algal growth also complicates the use of N:P ratios.

Table 19. N:P Ratios for Bay Sites

	SITE						
	ASD	ASE	ANW	KND	PND	PMID	PS
MEAN:	13.7	12.5	12.2	17.1	13.0	10.6	10.1
MEDIAN:	12.6	13.0	12.2	16.6	13.2	10.6	9.9
MIN:	8.9	8.2	7.7	14.0	6.5	5.8	7.7
MAX:	27.0	15.7	15.7	22.6	16.9	15.6	13.1

Table 20. N:P Ratios for Bay Tributaries

	STREAM		
	Bear Ck.	Bluff Ck.	Pokegama R.
MEAN:	9.6	8.1	9.8
MEDIAN:	10.4	8.3	10.3
MIN:	5.2	2.5	4.4
MAX:	13.8	11.9	16.2

Suspended Solids and Turbidity

Median suspended solids and turbidity values are shown in Table 21. Mean suspended solids and turbidity values are shown in Figures 46, 50, and 52. Mean total suspended solids concentrations (TSS's) ranged from 5.2 mg/l (site KND) to 42 mg/l (site PS). The mean total suspended solids concentration for Site KND was significantly lower than all other sites except PND and ASE. Site KND was strongly influenced by mixing with SLRE water.

Table 21. Bay Sites Suspended Solids and Turbidity Parameter Medians

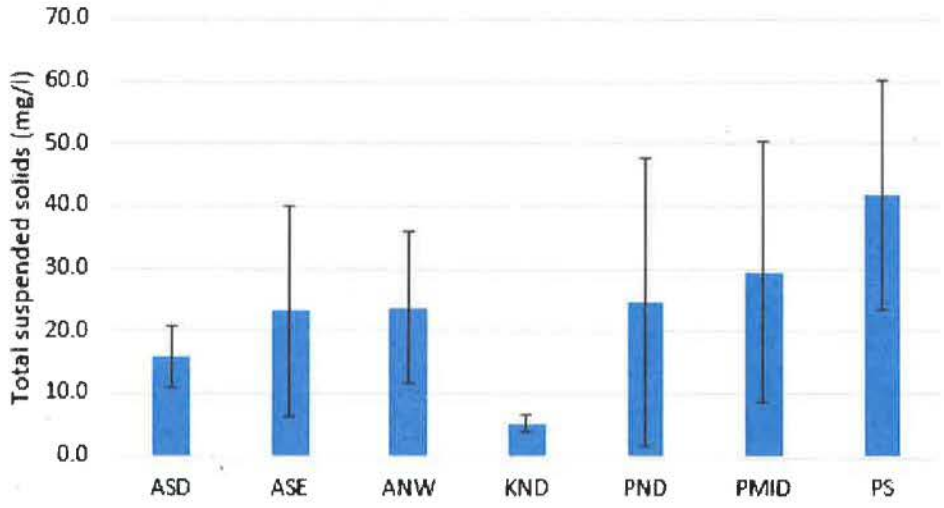
	SITES						
	ASD	ASE	ANW	KND	PND	PMID	PS
TSS (mg/l)	11.6	15	17.0	4.3	10	15	33.3
Volatile TSS (mg/l)	2.5	2.5	1.9	1.6	2	2.7	4.7
% TSS volatile	21.5	16.3	11.4	36.0	20.0	18.0	14.0
Turbidity (ntu)	68.5	61.1	67.4	13.4	44.5	67.8	97.9

Median TSS's found throughout the SLRE in 2012 and 2013 (2012 – 10.1 mg/l, 2013 – 8.0 mg/l) (Bellinger et al. 2015) were generally lower than those found in Allouez and Pokegema Bays (10 – 33.3 mg/l) (Table 21). The bays are influenced by drainage from clay rich watersheds. Stream TSS data from the primary tributaries to Allouez and Pokegama Bay is shown in Table 22. Median stream TSS's ranged from 32 to 39 mg/l and were substantially higher than those for the bay sites, except for site PS which had a median TSS very similar to the Pokegama River. Partial sedimentation of tributary inputs of suspended solids would be expected in the bays. Frequent resuspension of partially sedimented inputs of clay and silt probably maintains the very noticeable turbidity in Allouez and Pokegama Bays throughout the season.

Resuspension results from wind generated waves, seiche-induced flow reversals, and bioperturbation, especially by bottom feeding fish.

TSS's for all bay site samples were moderately correlated with TP's ($R^2 = 0.71$) (Figure 48). However, for samples with TP's < 100 ug/l the correlation was much weaker ($R^2 = 0.22$) (Figure 49).

Figure 46. Bay Sites Total Suspended Solids Concentration Means



Error bars are 90% confidence intervals

Table 22. Total Suspended Solids Concentrations (mg/l) for Bay Tributaries

	Tributary Stream		
	Pokegama R.	Bear Ck.	Bluff Ck.
Mean:	75.0	66.6	106.3
Median:	32.1	39.2	38.5
Max.:	464	257	936
Min.:	10.8	15	12

Figure 47. Bay Sites Total Suspended Solids Concentrations

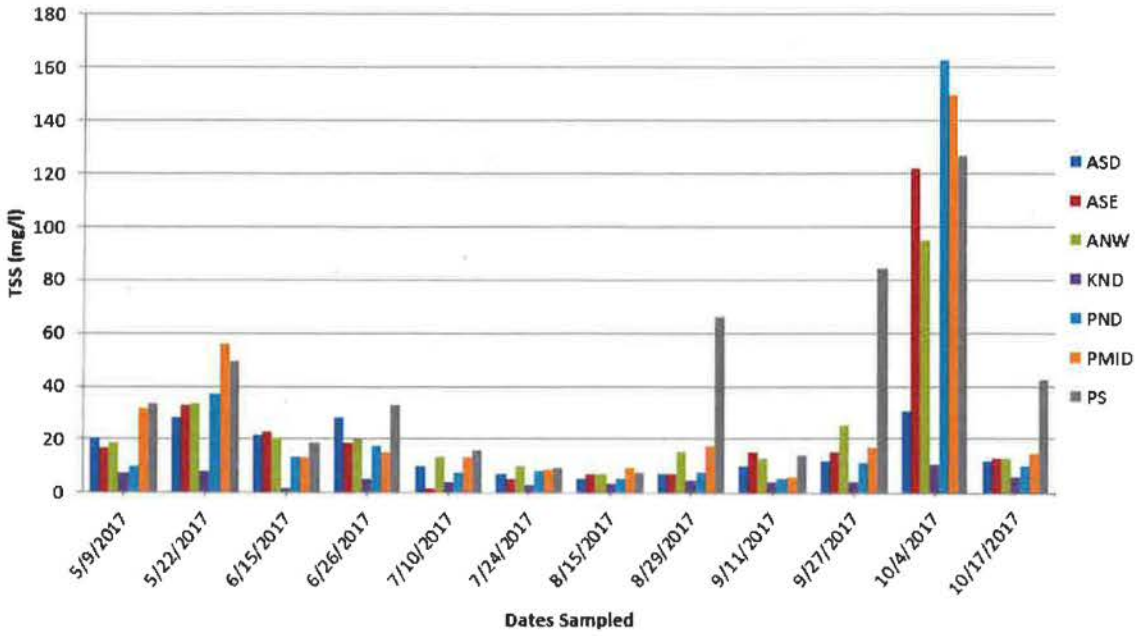


Figure 48. Bay Sites Total Phosphorus vs. Total Suspended Solids Relationship (for all data)

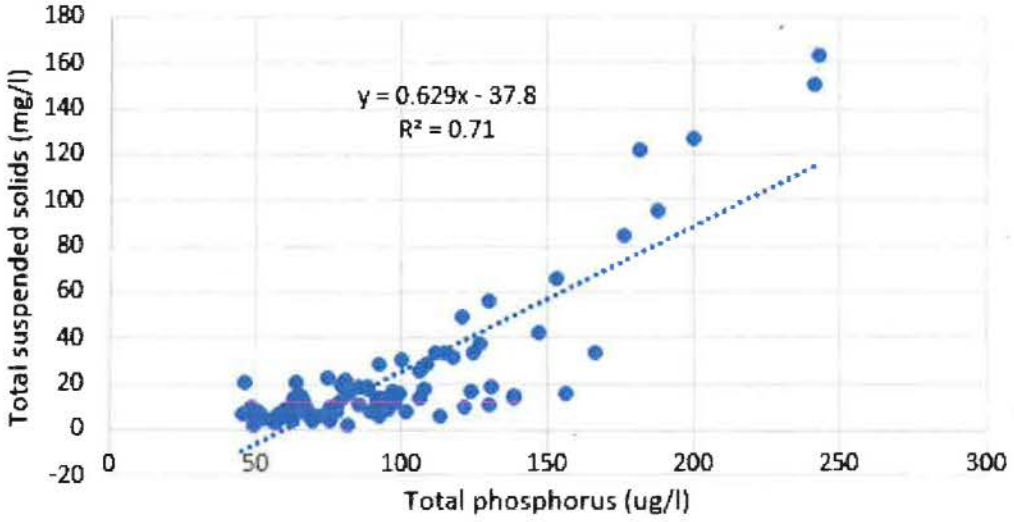
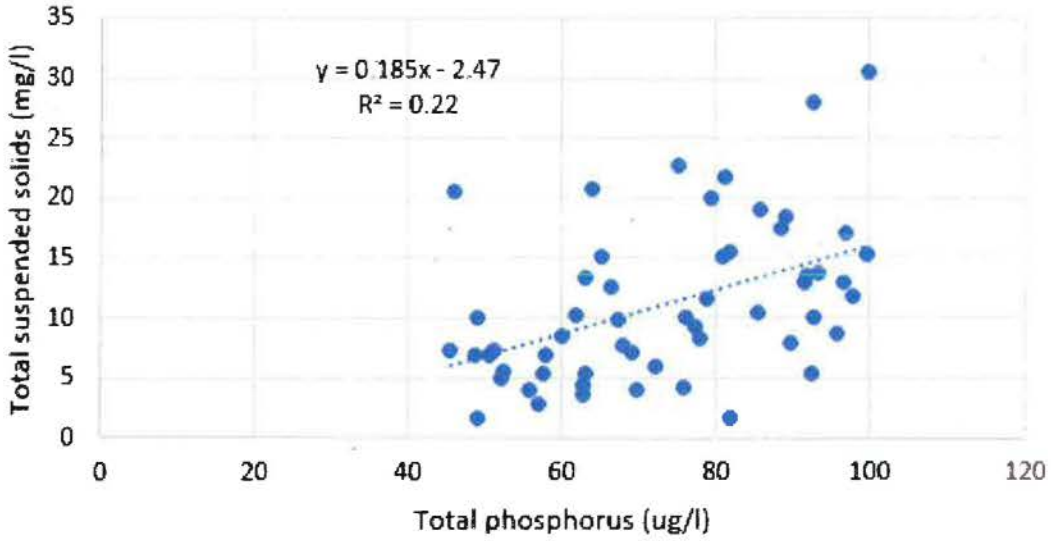


Figure 49. Bay Sites Total Phosphorus vs. Total Suspended Solids Relationship (for TP's < 100 ug/l)



Despite the relatively high TSS's in the bays, median values (Table 21) were at, or below the SLRE BUI TSS goal of 15 mg/l at five of the seven sites. Only sites ANW and PS had median values exceeding the goal. TSS's were highest in May and early October (Figure 47) when high rates of watershed runoff occurred (Figure 17). Site PS showed occasional TSS spikes in other months due to the strong influence of the Pokegama River. A Pokegama River flow spike that peaked on August 27th at 325 cfs accounted for the high TSS at site PS on August 29th when river flow was still 50.5 cfs.

For Allouez and Pokegama Bays, 2017 TSS's were similar to those found in 2011-12 and 2012-13, but not 2007 (see "Comparison of 2017 TP's and Related Parameters to Data from Other Years").

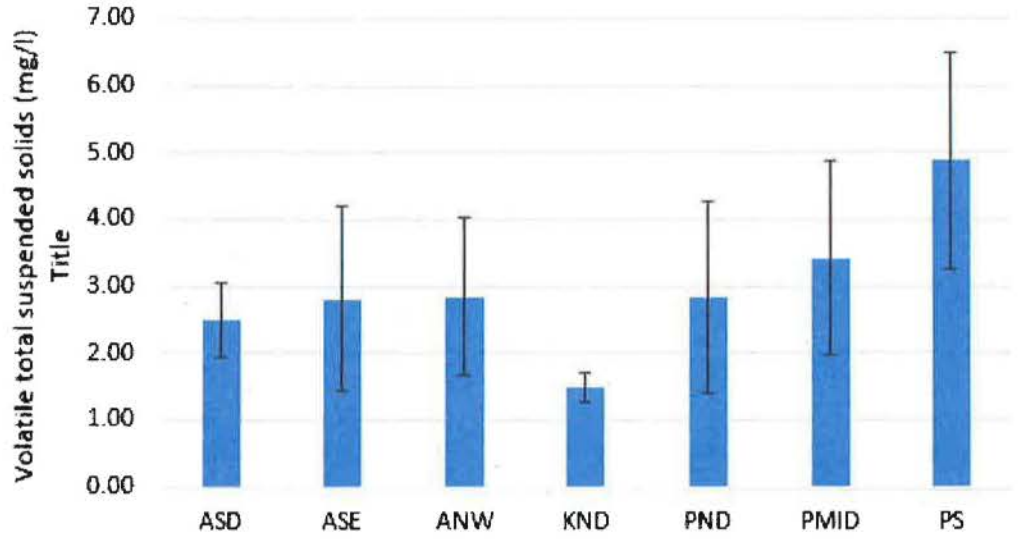
Mean volatile total suspended solids concentrations (VTSS's) ranged from 1.49 mg/l (site KND) to 4.90 mg/l (site PS) (Figure 50). The mean VTSS for site KND was significantly lower than means for sites ASD, PMID, and PS. The mean VTSS for site PS was significantly higher than means for sites KND and ASD. The VTSS for

site KND was probably lower due to the strong influence of SLRE water on that site. The mean VTSS for site PS was higher due to the strong influence of Pokegama River water on that site.

Median VTSS's (Table 21) were low, and a small percentage (11.4–36.0 %) of TSS's. This indicates the majority of TSS is inorganic material (such as clay and silt) rather than organic material (such as algae and detritus). VTSS measurements were below detection limits (2.0–6.3 mg/l) for 56% of samples. Values of one-half the detection limit were used for these samples, so statistics generated were somewhat inaccurate.

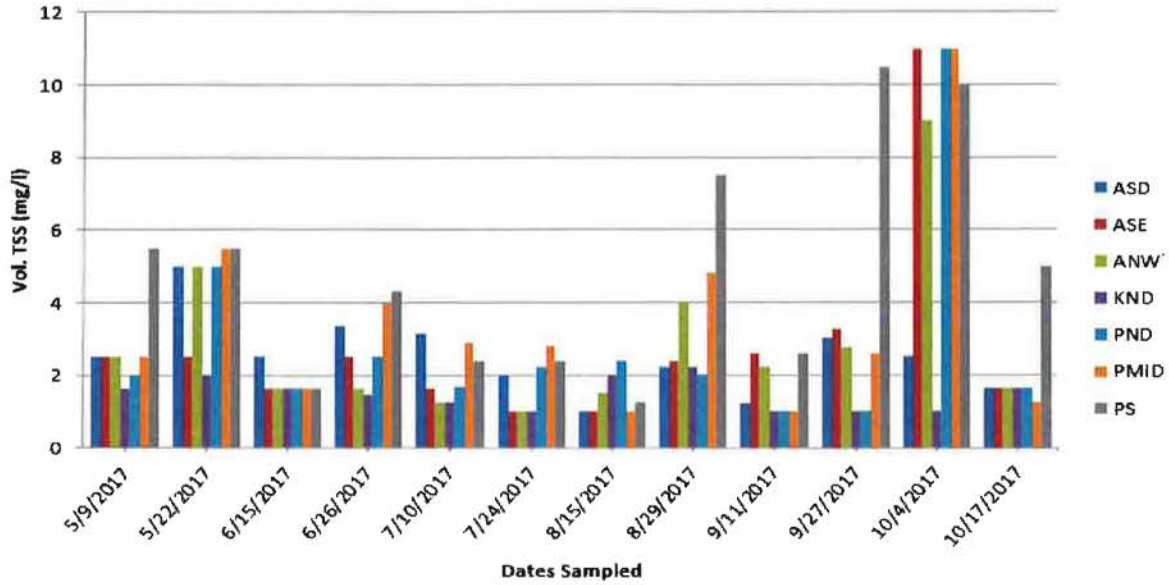
VTSS's appear to be roughly correlated with TSS (Figures 47 and 51). This suggests organic detritus delivered by runoff was a large component of VTSS. VTSS's appear to be very poorly correlated with CHL's (Figures 51 and 27), which suggests algae were a minor component of VTSS.

Figure 50. Bay Sites Volatile Total Suspended Solids Concentration Means



Error bars are 90% confidence intervals

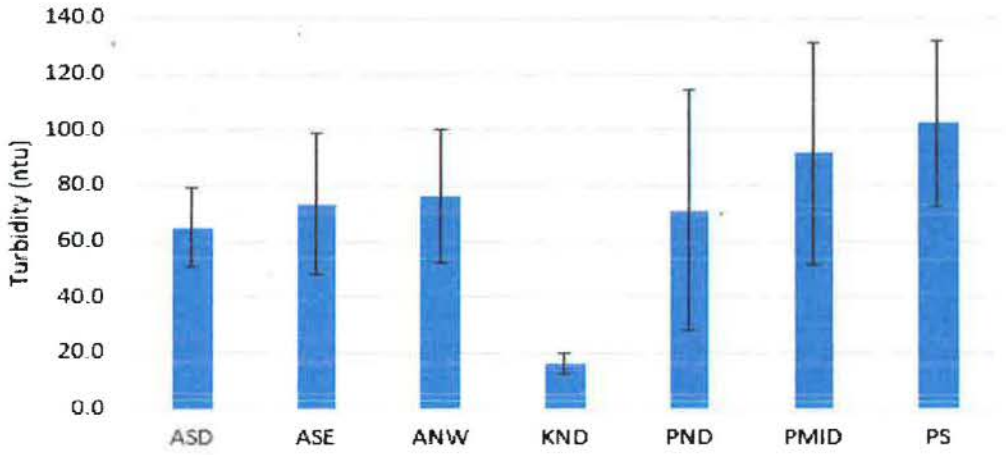
Figure 51. Bay Sites Volatile Total Suspended Solids Concentrations



Mean turbidities ranged from 16.4 ntu (site KND) to 102 ntu (site PS) (Figure 52). Site KND, which is strongly influenced by mixing with SLRE water had the lowest turbidity. Site PS, which is strongly influenced by Pokegama River water had the highest turbidity. Like TSS's, turbidities were highest in May and early October when high rates of watershed runoff occurred (Figure 53). Site PS showed occasional turbidity spikes in other months due to the strong influence of the Pokegama River.

For Allouez and Pokegama Bays, 2017 turbidities were similar to those found in 2011-12, and 2012-13 (see "Comparison of 2017 TP's and Related Parameters to Data from Other Years"). There is a strong correlation between turbidities and TSS's for all bay site samples ($R^2 = 0.90$) (figure 54). The correlation is weaker, but still moderate for samples with turbidities < 100 ntu's ($R^2 = 0.63$) (figure 55). Turbidity is a measure of light scattering by suspended particulates, while total suspended solids is a measure of the mass of suspended particulates, so a correlation would be expected. Clay particles produce more light scattering per unit mass than silt particles.

Figure 52. Bay Sites Turbidity Means



Error bars are 90% confidence intervals

Figure 53. Bay Sites Turbidities

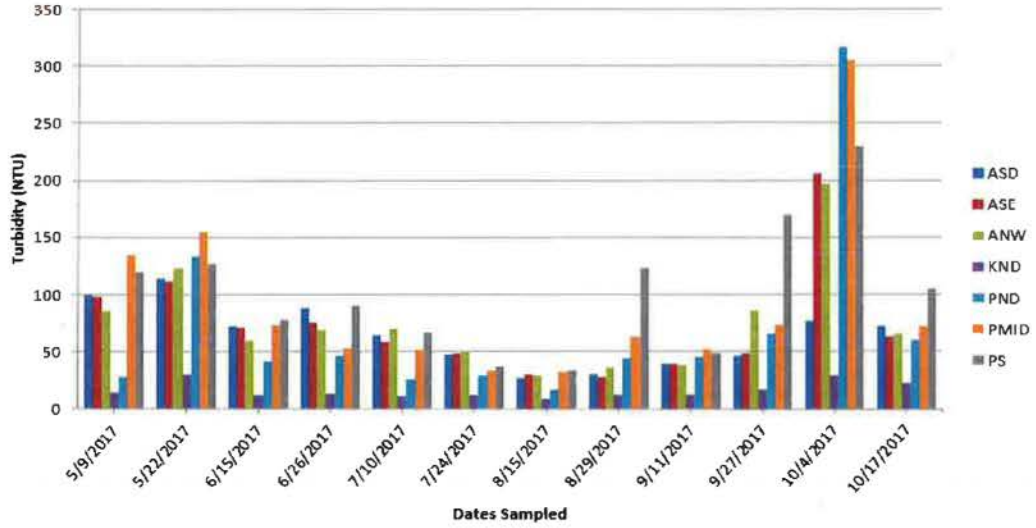


Figure 54. Bay Sites Turbidity vs Total Suspended Solids Relationship (for all data)

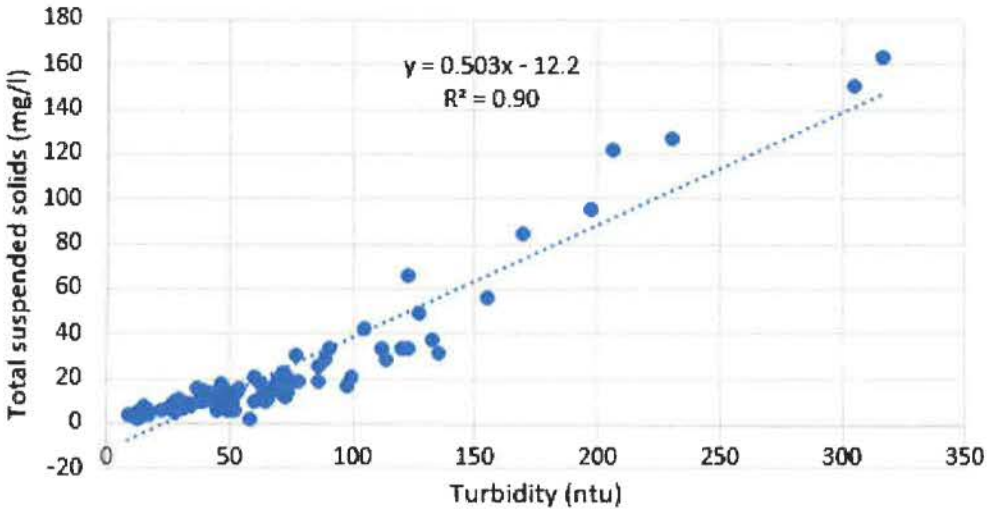
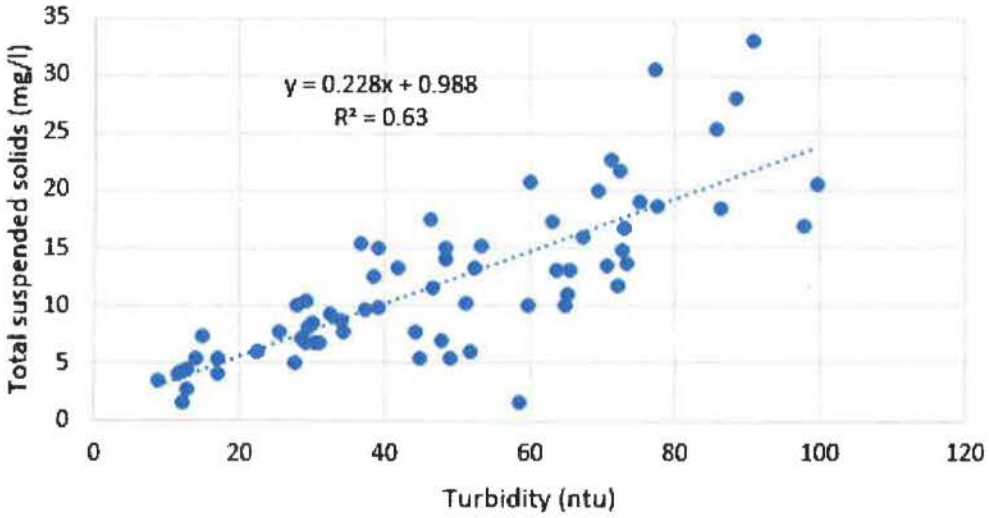


Figure 55. Bay Sites Turbidity vs Total Suspended Solids Relationship (for turbidities < 100 ntu)



Tributary Stream Results and Discussion

Stream Watershed Characteristics

Watershed Areas, Land Use, Sediment and Nutrient Sources

Watershed area and land use for the five monitored tributaries are shown in Table 23. The Pokegama River has the largest watershed (73.2 km^2), while the unnamed tributary at Billings Drive has the smallest watershed (4.1 km^2). Agricultural row crops are absent in four of the watersheds, and only account for 1.2% of land use in the Bear Creek watershed. Grassland (pasture and hayfields) is the largest agricultural land use and comprises 1.4 – 20.6% of the watersheds. Wetland is the most common land use in all watersheds, ranging from 39.9 – 73.4%.

All stream watersheds are located in the Lake Superior Clay Plain. Soils in the area are clay rich and highly erodible. Erosion to the Nemadji River, which is also strongly influenced by clay plain soils, has been extensively assessed, and those findings are probably applicable to other clay plain streams. For the Nemadji River, eroding bluffs along streams were estimated to be the source of 98% of the fine sediment reaching the stream mouth. Roadside erosion, and sheet and rill erosion, were each estimated to be the source of an additional 1% of fine sediment (NRCS 1998). A second erosion estimate for the Nemadji River estimated that 89% of fine sediment originated from streambank and bluff erosion along streams (Carlton County 2002). A third erosion assessment for the Nemadji River estimated that channel erosion provides 75% of the total sediment load at the stream mouth, and upland erosion provides 25% of the load (Butcher 2016). Natural background sources account for 55% of the upland erosion (Butcher 2016). These conditions make sediment control for clay plain streams difficult.

Streambank and bluff erosion along streams is believed to be the largest source of total suspended solids to bay tributary streams. However, it is not believed to be a large phosphorus source (Bahnick 1977).

Four-site composite samples of eroding stream bank soil were collected from both Bear and Bluff Creeks (Roesler 2018). The samples had a mean TP concentration of 472 mg /kg (range = 454-491 ug/l). Bear and Bluff Creeks had a mean TP concentration of 198 ug/l and a mean TSS concentration of 86 mg/l. Adjusting the TSS concentration for volatile TSS (-14%; from riverine bay site PS data) gives a non-volatile TSS concentration of 74 mg/l. This data suggests that, at most, stream bank erosion could be supplying 18% of the mean stream TP concentration for Bear and Bluff Creeks (if all non-volatile TSS is supplied by stream bank erosion).

Watershed non-point sources of phosphorus include pasture and hayfield runoff (including the influence of manure spreading), barnyards, and septic systems. The low infiltration rates and high runoff rates of clay plain soils are likely to limit the retention of phosphorus by soils.

The Village of Superior wastewater lagoon outfall is a point source of phosphorus for the Pokegama River. That discharge is about 5.7 % of the Pokegama River's May-October TP load (see "Total Phosphorus" section below).

Table 23. Tributary Stream Watershed Areas and Land Use

	Tributary Streams and Receiving Bays				
	Allouez Bay			Kimballs Bay	Pokegama Bay
	Bear Creek	Bluff Creek	Unnamed @ Moccasin Mike Rd	Unnamed @ Billings Drive	Pokegama River
Watershed Area (km2)	24	50.6	6.6	4.1	73.2
Land Use %					
Urban or developed	8.2	2.7	9.1	3.8	3.9
Agriculture (Row Crops)	1.2	0	0	0	0
Grassland (Pasture, Hay)	13	20.6	4.8	1.4	9.2
Forest	16.3	36.8	15.8	20.8	30.2
Open Water	0.1	0.1	0	0.6	0.2
Wetland	61.3	39.9	70.4	73.4	56.5
Barren	0	0	0	0	0
Shrubland	0	0	0	0	0

Figure 56. Eroding Clay Banks along a Well Vegetated Section of the Pokegama River

Point Sources

The Village of Superior wastewater lagoon outfall discharges to the Pokegama River. There are no point source discharges to Bear or Bluff Creeks or the two unnamed streams.

Streamflow in 2017

The clay-rich watershed soils have low infiltration rates and high runoff rates. Streamflow is flashy with most flow produced by runoff events. Measured flows for the Pokegama River during May – October of 2017 are shown in figure 57 (Complete daily mean stream flows are contained in appendix 3). Monthly rainfall was below normal in June, July, and September, and above normal in May, August, and October. Total rainfall during May-October of 2017 was 15% above normal (Table 24).

Figure 57. Pokegama River Flows (data from USGS)

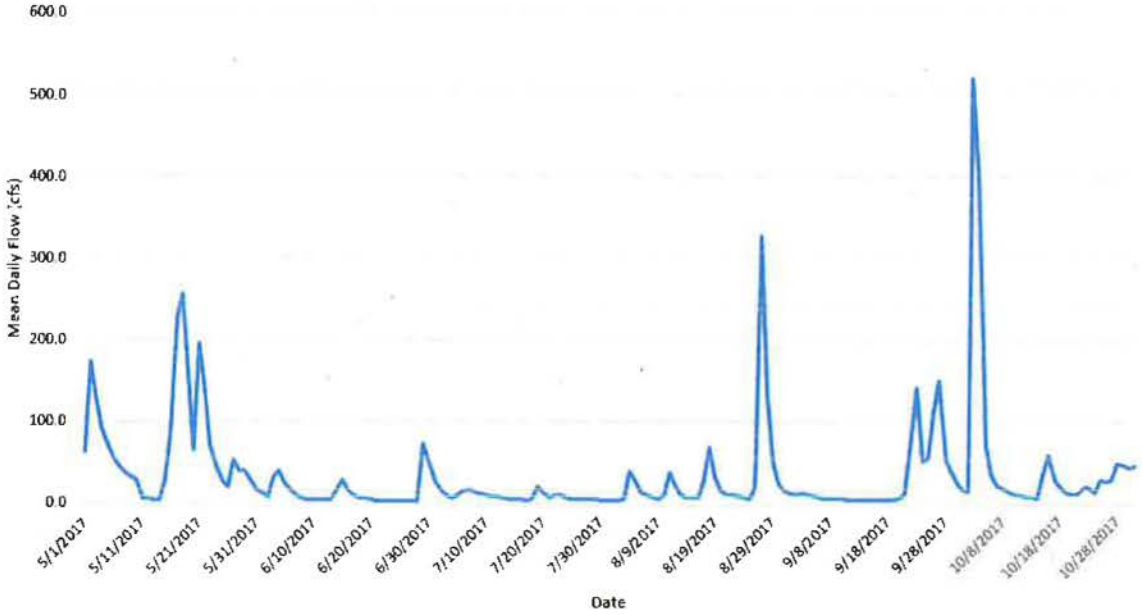


Table 24. Monthly Precipitation for May-October 2017

	2017	
Months	Total Precip. (in)	Avg. Precip. (in)
May	5.03	3.13
June	2.66	4.1
July	3.01	3.95
August	7.4	3.76
September	2.95	4.11
October	4.34	3.01
May-Oct	25.39	22.06
May-Oct 2017 difference from average	15%	

Note: - Avg Precip data obtained from Superior WI station USC00478349
Note: - Precip, cumulative precip, and total precip data obtained from NOAA National Estuarine Research Reserve System, Pokegama Bay weather station LKSPOMET

Table 25 shows the number of bay volumes of water that are delivered to the bays by drainage from their direct watersheds during May through October. This provides an indication of how strongly water quality in the bays is influenced by direct watershed drainage versus seiche-induced backflows of St. Louis River and/or Lake Superior water. Kimballs Bay had the lowest relative delivery of direct watershed water, with only 0.8 bay volumes of water delivered over the six-month period. Kimballs Bay was more strongly influenced by seiche-induced backflows. Pokegama Bay had the highest relative delivery of direct watershed water, with 6.9 bay volumes of water delivered. Pokegama Bay was more strongly influenced by direct watershed runoff than the other two bays.

Table 25. Bay Volumes of Water Delivered by Direct Watersheds May-October 2017

Bay	Area (acres)	Estimated Mean Depth (ft)	Volume (acre-ft)	Direct Watershed Area (km2)	May-Oct Bay Volumes Delivered by Direct Watershed*
Allouez	1011	6	6066	82.4	2.3
Pokegama	441	5	2205	89.3	6.9
Kimballs	101	12	1212	5.6	0.8

**Watershed flows assumed to be area-proportional to measured Pokegama River flows.*

Field Parameter Results

Table 26 shows the summarized field monitoring results for the three named tributary stream sites. Table 27 shows the summarized field monitoring results for the two unnamed tributary stream sites. Figures 58 through 62 display means and 90% confidence intervals for field parameters from all five stream sites. The primary (named) streams were monitored 4x/month, while the unnamed streams were monitored 1x/month. The mean flow for the dates the unnamed streams were monitored was about half the mean flow for all dates the named streams were sampled. This needs to be considered when comparing named and unnamed streams for water quality field parameters that are significantly correlated with flow (turbidity and conductivity).

Table 26. Field Parameter Data for Named Streams

PRIMARY STREAM FIELD MONITORING PARAMETERS							
Stream	Statistic	Temp. (°C)	D.O. (mg/l)	pH (s.u.)	Conductivity (umhos/cm)	Turbidity (ntu)	Transparency Tube (cm)
BEAR CREEK	Mean:	13.4	9.7	7.6	183	142	9
	Median:	14	9.4	7.7	170	123	10
	Max.:	19.4	12.3	8	328	315	16
	Min.:	6.5	8.1	6.9	81	83	3
BLUFF CREEK	Mean:	13.5	8.7	7.6	178	218	8
	Median:	14.4	8.2	7.6	168	145	9
	Max.:	19.6	11.7	7.9	330	879	13
	Min.:	6.9	6.2	7.1	91	105	1.5
POKEGAMA RIVER	Mean:	14.2	9.2	7.7	168	138	11
	Median:	14.9	9.1	7.7	173	106	10
	Max.:	21.6	12.1	8	258	475	24
	Min.:	7.1	4.9	7.3	86	49	2

Named streams were monitored 4x per month, May through October. Conductivity is specific conductance.

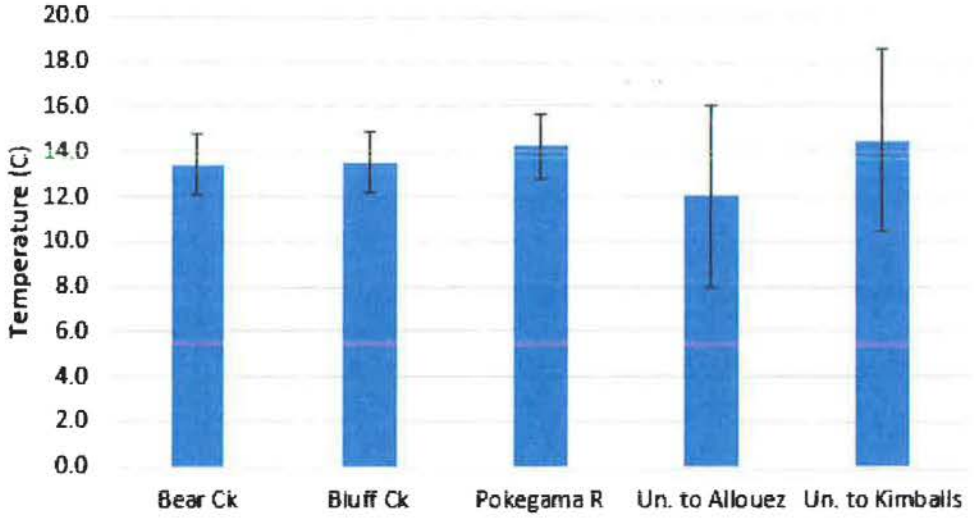
Table 27. Field Parameter Data for Unnamed Streams

UNNAMED STREAM FIELD MONITORING PARAMETERS							
Stream	Statistic	Temp. (°C)	D.O. (mg/l)	pH (s.u.)	Conductivity (umhos/cm)	Turbidity (ntu)	Transparency Tube (cm)
Unnamed tributary to Allouez Bay at Moccasin Mike Rd.	Mean:	12	9.6	7.4	357	98	13
	Median:	13.8	9.1	7.6	378	95	12
	Max.:	16.4	12.2	7.8	491	123	20
	Min.:	5.9	7.4	6.6	204	63	9
Unnamed tributary to Kimballs Bay at Billings Drive	Mean:	14.5	6.7	7.5	211	84	15
	Median:	16.1	6.2	7.4	233	74	17
	Max.:	19.5	11.5	7.7	289	145	22
	Min.:	7.4	3.8	7.3	113	48	8

Unnamed streams were monitored 1x per month, May through October. Conductivity is specific conductance.

Mean stream temperatures ranged from 12°C (unnamed tributary to Allouez Bay) to 14.5 °C (unnamed tributary to Kimballs Bay). Mean stream temperatures for all sites were not significantly different (Figure 58). While the seasonal mean temperature for the unnamed tributary to Allouez Bay was not significantly different, the site had the coolest temperature on five of the six dates of monitoring. This suggests that this tributary has greater groundwater inputs than the other streams.

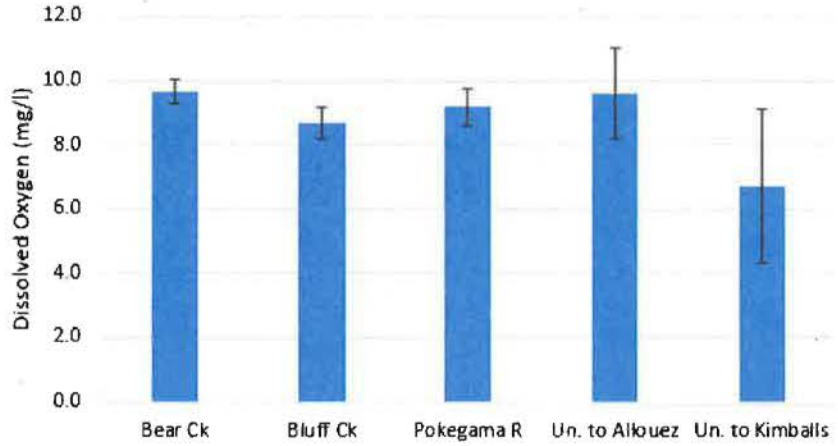
Figure 58. Stream Temperature Means



Error bars are 90% confidence intervals

Mean dissolved oxygen concentrations (D.O.'s) ranged from 6.7 mg/l (unnamed tributary to Kimballs Bay) to 9.7 mg/l (Bear Creek) (Tables 26 and 27). Mean D.O.'s for Bear Creek were significantly higher than for Bluff Creek and the unnamed tributary to Kimballs Bay (Figure 59). One measurement for the Pokegama River (4.9 mg/l) was just below the 5 mg/l water quality standard. The unnamed tributary to Kimballs Bay had two of six dates with D.O.'s less than 5 mg/l (July 19th – 3.8 mg/l; Sept 20th – 4.1 mg/l). That stream site is fringed with wetlands and subject to seiche-induced backflows.

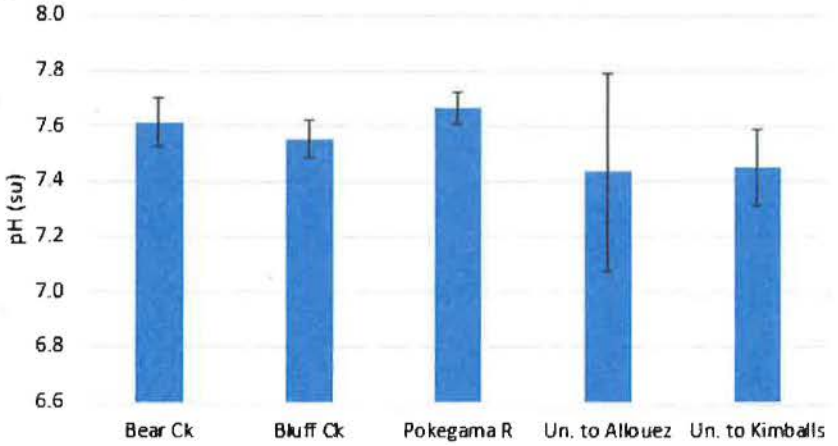
Figure 59. Stream Dissolved Oxygen Concentration Means



Error bars are 90% confidence intervals

Mean pH's for all streams were similar and ranged from 7.4 (unnamed tributary to Allouez Bay) to 7.7 (Pokegama River) (Tables 26 and 27), with no significant differences between streams (Figure 60).

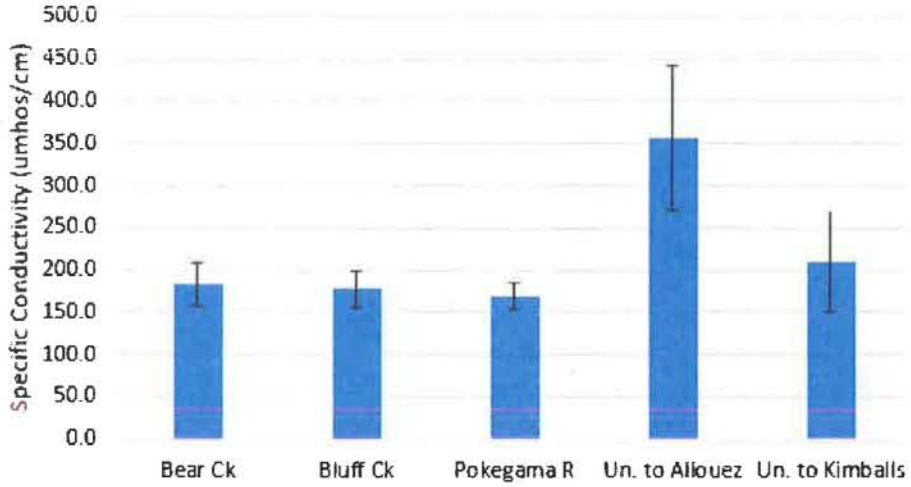
Figure 60. Stream pH Means



Error bars are 90% confidence intervals

Mean conductivities (specific conductance) ranged from 168 umhos/cm (Pokegama River) to 357 umhos/cm (unnamed tributary to Allouez Bay) (Tables 26 and 27). The mean conductivity for the unnamed tributary to Allouez Bay was significantly higher than all other sites (Figure 61). This, along with cooler temperatures, suggests greater groundwater inputs to this stream. However, the high conductivities could also be influenced by the high density of highway road surfaces in the watershed. There are 1.6 km of 2-lane highway per km2 of watershed. State highways 2/53 and 13, and County Highways Z and UU are present. Chloride from road salt could be a contributor to stream conductivities if there is a lag time (winter to summer) in chloride transport through the watershed. Conductivity was moderately inversely correlated with stream flow (Pokegama R $R^2 = 0.66$, Bear Ck $R^2 = 0.60$, Bluff Ck $R^2 = 0.67$). The higher mean conductivity at this site was probably also influenced by the lower flows on the dates sampled, when groundwater inputs (with high conductivity) supply a greater portion of the flow.

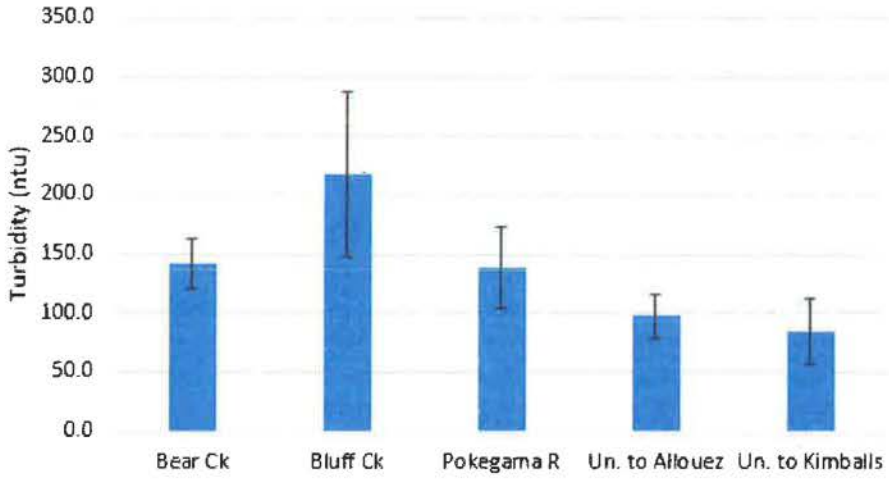
Figure 61. Stream Conductivity Means



Error bars are 90% confidence intervals

Mean turbidities ranged from 84 ntu (unnamed tributary to Kimballs Bay) to 218 ntu (Bluff Creek) (Tables 26 and 27). Mean turbidities for the unnamed tributary to Allouez Bay and the unnamed tributary to Kimballs Bay were significantly lower than means for Bear and Bluff Creek, but not for the Pokegema River (Figure 62). The watersheds for the two unnamed streams have the highest percentages of wetlands (70.4%, 73.4%) and the lowest percentages of grassland (pasture, hay) (1.4%, 4.8%) (Table 23). This may have contributed to the lower turbidities. Turbidity was poorly to highly correlated with stream flow (Pokegama R $R^2 = 0.87$, Bear Ck $R^2 = 0.34$, Bluff Ck $R^2 = 0.03$). The lower mean turbidities at the unnamed sites may also have been influenced by the lower flows on the dates sampled, since less runoff (with high turbidity) was contributing to stream flow.

Figure 62. Stream Turbidity Means



Error bars are 90% confidence intervals

Lab Parameter Results

Table 28 shows the summarized lab testing results for the three named tributary stream sites. Table 29 shows the summarized lab testing results for the two unnamed tributary stream sites. Complete data for tributary streams is contained in appendix 2. Stream water quality parameter means are shown in figures 63, 65, 67, 69, 71, 73, 75, and 79. Trends for stream water quality parameters at the three named tributary stream sites are shown in figures 64,66, 68, 70, 72, 74, 76, and 80. The lower mean flow for the dates the unnamed streams were monitored needs to be considered when comparing unnamed and named streams for water quality lab parameters that are significantly correlated with flow (total phosphorus, total suspended solids, total Kjeldahl nitrogen).

Table 28. Lab Parameter Data for Named Streams

PRIMARY STREAM LAB RESULTS									
Stream	Statistic	TP (µg/l)	OP (µg/l)	TSS (mg/l)	NH_3-N (µg/l)	$NO_3 + NO_2$-N (µg/l)	TKN (mg/l)	Iron (mg/l)	BOD (mg/l)
BEAR CREEK	Mean:	173	24	67	50	85	1.38	4.1	1.8
	Median:	145	21	39	46	74	1.45	4.1	1.8
	Max.:	364	64	257	145	216	1.89	5.7	3.9
	Min.:	96	12	15	15.5	9.5	0.11	3.2	1
BLUFF CREEK	Mean:	224	24	106	42	79	1.46	4.9	2.3
	Median:	170	23	39	44	57	1.50	4.2	1.6
	Max.:	987	45	936	68	259	2.41	9.9	5.5
	Min.:	107	12	12	22	18	0.85	3.0	1
POKEGAMA RIVER	Mean:	182	31	75	42	49	1.52	3.9	1.8
	Median:	161	20	32	41	42	1.51	2.8	1.5
	Max.:	514	154	464	86	152	2.19	8.3	3.4
	Min.:	103	10	11	16	9.5	0.83	2.5	1

TP = total phosphorus; OP = orthophosphate phosphorus; TSS = total suspended solids; NH_3 - N = ammonia nitrogen; NO_3+NO_2- N= nitrate plus nitrite nitrogen; TKN = total Kjeldahl nitrogen; BOD = 5-day biochemical oxygen demand.
Named streams were monitored 4x per month, May through October.

Table 29. Lab Parameter Data for Unnamed Streams

UNNAMED STREAM LAB RESULTS							
Stream	Statistic	TP (µg/l)	OP (µg/l)	TSS (mg/l)	NH_3-N (µg/l)	$NO_3 + NO_2$-N (µg/l)	TKN (mg/l)
Unnamed tributary to Allouez Bay at Moccasin Mike Rd.	Mean:	106	11.6	28.3	43.4	82.2	1.57
	Median:	110	11.9	24.7	42.6	55.9	1.61
	Max.:	129	13.8	53.7	57.4	178	1.94
	Min.:	66.9	7.9	9.2	30.8	9.5	1.16
Unnamed tributary to Kimballs Bay at Billings Drive	Mean:	160	32.7	30.4	37.1	39.1	1.30
	Median:	169	34.4	16.7	41.5	13.75	1.14
	Max.:	204	47.3	82.7	57.7	95.8	2.06
	Min.:	77.9	11.2	7.2	7.5	9.5	1.08

Unnamed streams were monitored 1x per month, May through October.

Total Phosphorus

Mean total phosphorus concentrations (TP's) ranged from 106 ug/l (unnamed to Allouez Bay) to 224 ug/l (Bluff Creek) (Tables 28 and 29). TP's exceeded the 75 ug/l WI DNR stream standard at all sites on all dates, except for one sample from the unnamed tributary to Allouez Bay (May 3rd; 66.9 ug/l). The mean TP for the unnamed tributary to Allouez Bay was significantly lower than for the named streams. At least part of this

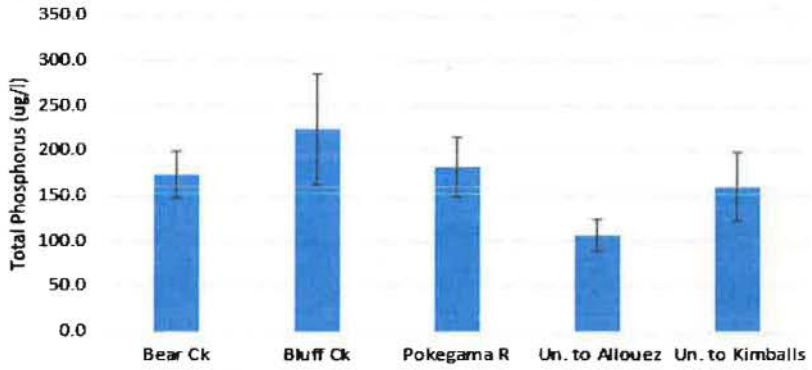
difference may have been due to the lower mean flows on the dates the unnamed streams were sampled, since TP was poorly to moderately correlated with stream flow (Pokegama R $R^2 = 0.53$, Bear Ck $R^2 = 0.13$, Bluff Ck $R^2 = 0.45$).

A large TP spike was evident for Bluff creek on June 29th (Figure 64). A relatively minor flow spike occurred on the Pokegama River on that date (Figure 57) and precipitation of 1.3 inches was reported. Localized precipitation may have differed. There is a ~~A~~ cattle raising operation with a large area of ~~heavily~~ pastured slopes and stream channels in the upper Bluff Creek watershed. There is also a dairy farm located on a Bluff Creek tributary (Birch Creek). These are ~~is one~~ possible sources of this spike.

A large TP spike was evident for the Pokegama River on October 3rd (figure 64). The highest flow of the season occurred on the Pokegama River on that date (Figure 57) and the river was flowing out of its banks. Streambank erosion during this peak flow may have been a source. Additionally, construction of an oil pipeline across the river over the previous months had left some areas of soil not fully stabilized, which may also have contributed to the TP spike.

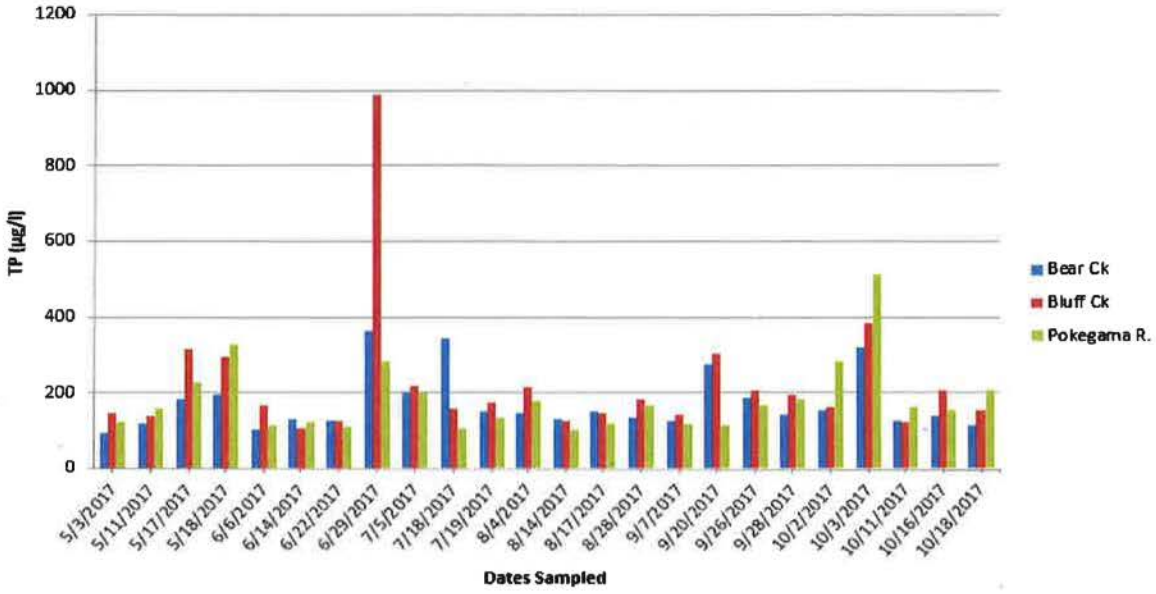
The 2017 May-October TP load for the Pokegama River was 3,302 kg. The Village of Superior wastewater lagoons discharged 190 kg of TP to the river during that period. The discharge was about 5.7 % of the river's May-October TP load.

Figure 63. Stream Total Phosphorus Concentration Means



Error bars are 90% confidence intervals

Figure 64. Stream Total Phosphorus Concentrations

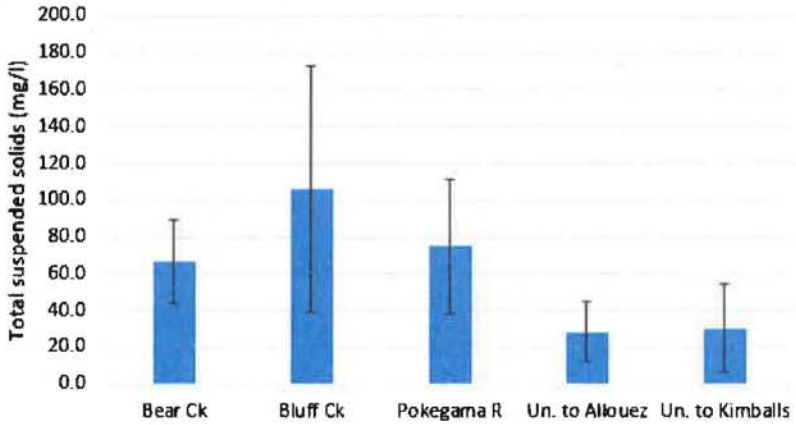


Total Suspended Solids

Mean total suspended solids concentrations (TSS's) ranged from 28.3 mg/l (unnamed tributary to Allouez Bay) to 106 mg/l (Bluff Creek) (Tables 28 and 29). There were no significant differences between site means (Figure 65). TSS was poorly to highly correlated with stream flow (Pokegama R $R^2 = 0.93$, Bear Ck $R^2 = 0.17$, Bluff Ck $R^2 = 0.11$).

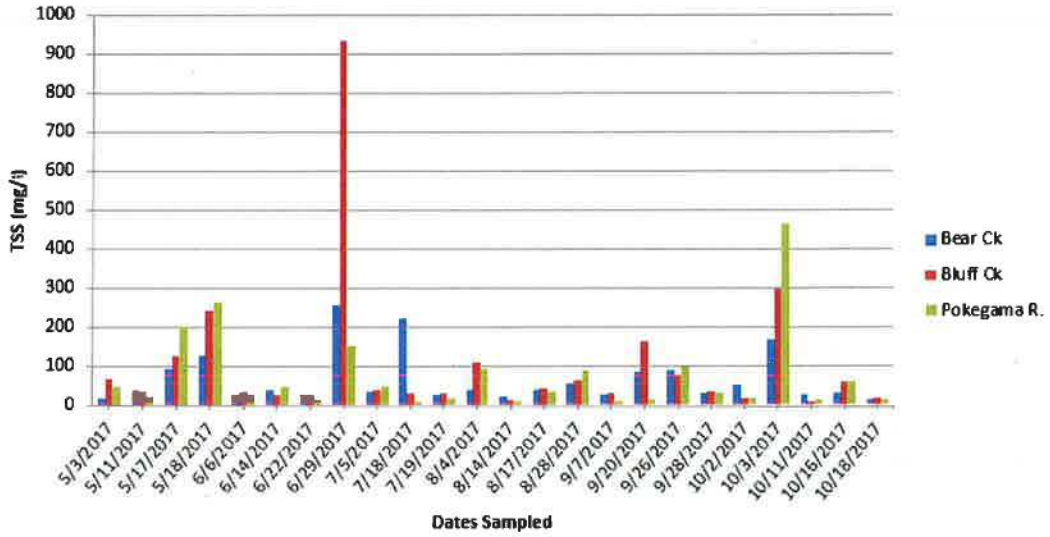
Similar to TP, a large TSS spike was evident for Bluff creek on June 29th (Figure 66). A relatively minor flow spike occurred on the Pokegama River on that date (Figure 57) and precipitation of 1.3 inches was reported. Localized precipitation may have differed. A cattle raising operation with a large area of pastured slopes and stream channels in the upper Bluff Creek watershed is one possible source of this spike.
Also similar to TP, a large TSS spike was evident for the Pokegama River on October 3rd (Figure 66). The highest flow of the season occurred on the Pokegama River on that date (Figure 57) and the river was flowing out of its banks. Streambank erosion during this peak flow event may have been a source. Additionally, construction of an oil pipeline across the river over the previous months had left some areas of soil not fully stabilized, which may also have contributed to this spike.

Figure 65. Stream Total Suspended Solids Concentration Means



Error bars are 90% confidence intervals

Figure 66. Stream Total Suspended Solids Concentrations



Orthophosphate

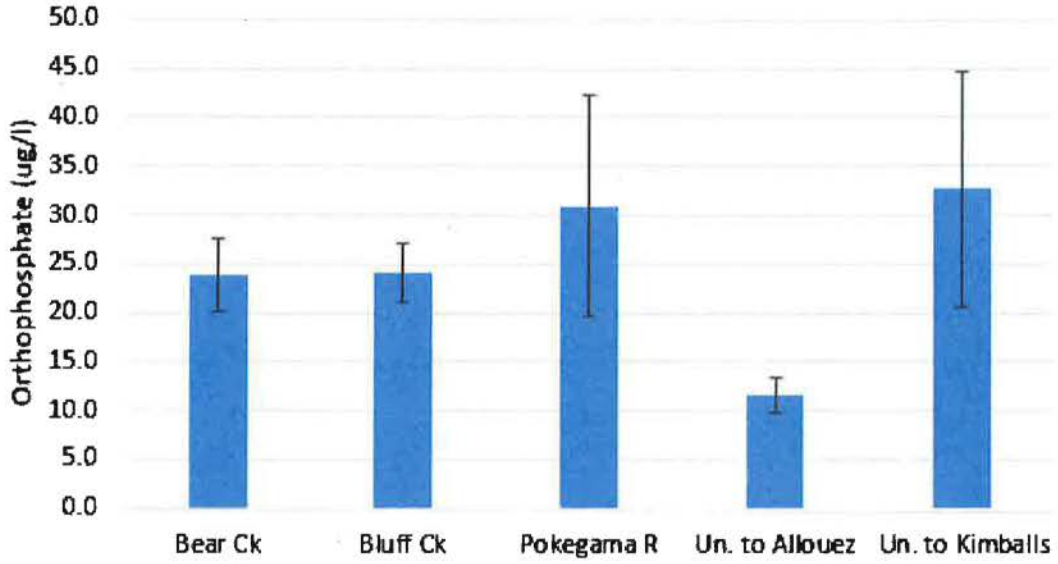
Mean orthophosphate concentrations (OP's) ranged from 11.6 ug/l (unnamed tributary to Allouez Bay) to 32.7 ug/l (unnamed tributary to Kimballs Bay) (Tables 28 and 29). The OP mean for the unnamed tributary to Allouez Bay was significantly lower than all other sites.

Suspended red clay in the SLRE area has been shown to adsorb OP when high concentrations occur (Bahnick 1980). OP is adsorbed by suspended clay when an equilibrium concentration of 20 - 42 ug/l is exceeded. Mean OP's (12.6 – 32.7 ug/l) were within or below this concentration range, suggesting suspended clay may be responsible for maintaining lower OP's.

The Pokegama River showed notable OP spikes on May 11th, and October 2nd, 11th, and 18th, with concentrations as high as 154 ug/l (Figure 68). The Village of Superior wastewater lagoons were discharging on all these dates and were the likely source of the OP spikes. Pokegama River flows were relatively low (4.9 – 17.7 cfs) on all dates with OP spikes, so there was limited dilution of lagoon discharge.

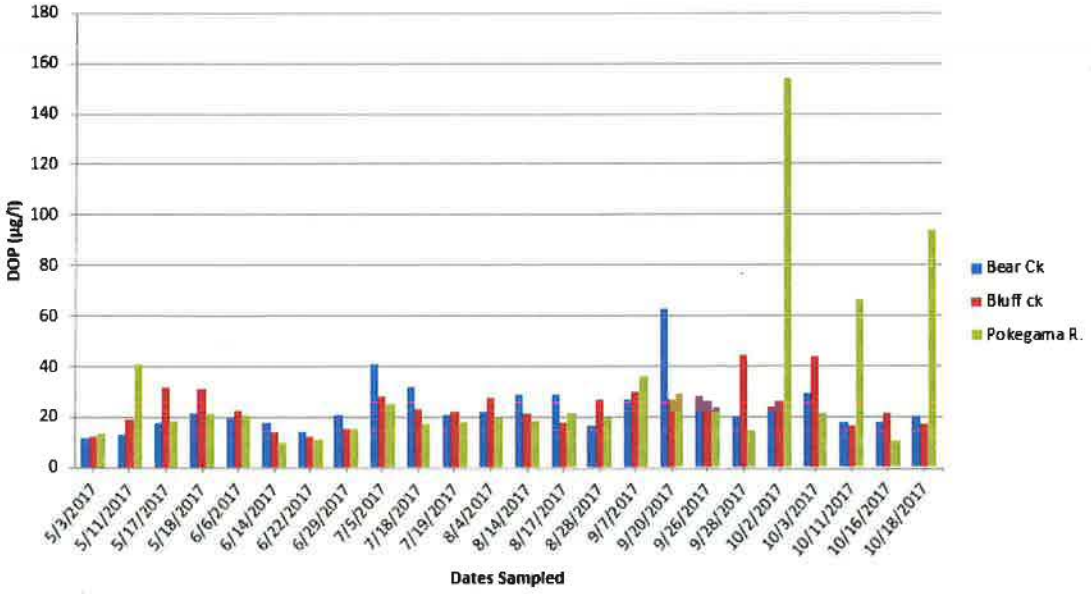
Bear Creek showed notable OP spikes on July 5th and September 20th. Stream flows were low on both these dates (2.9 and 1.6 cfs, respectively). Septic system discharge may have been the source of these spikes. Septic system influence tends to be more noticeable during low flow conditions when less dilution water is available. Failing septic systems have previously been found in the Bear Creek watershed. The Town of Parkland Sanitary District was formed to deal with failing septic systems in a portion of the watershed. The District installed sewers to collect household wastewater, which is partially treated and pumped to the City of Superior wastewater treatment plant for final treatment.

Figure 67. Stream Orthophosphate Concentration Means



Error bars are 90% confidence intervals

Figure 68. Stream Orthophosphate Concentrations

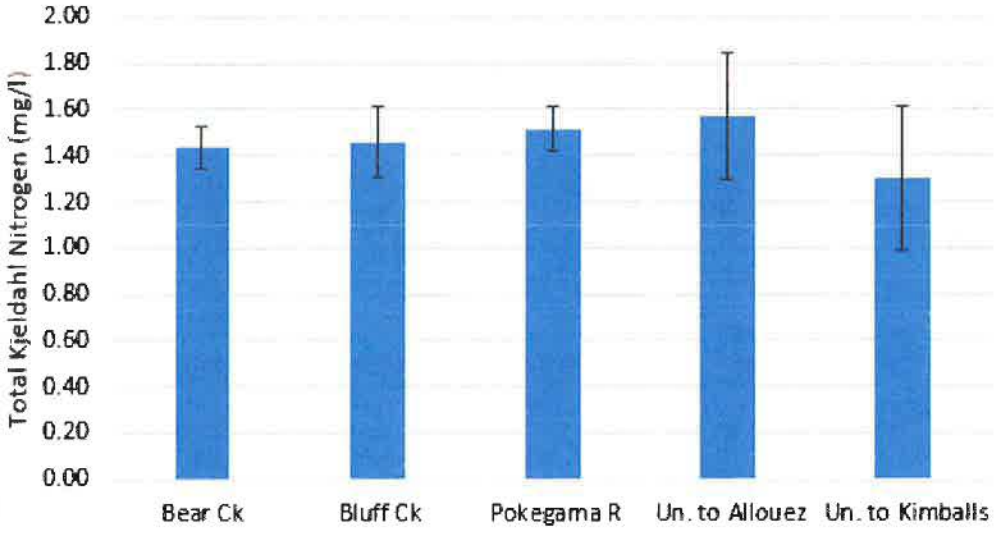


Total Kjeldahl Nitrogen

Mean total Kjeldahl nitrogen concentrations (TKN's) ranged from 1.30 mg/l (unnamed tributary to Kimballs Bay) to 1.57 mg/l (unnamed tributary to Allouez Bay) (Tables 28 and 29). There were no significant differences between site TKN means (Figure 69). TKN was weakly correlated with stream flow (Pokegama R $R^2 = 0.26$, Bear Ck $R^2 = 0.12$, Bluff Ck $R^2 = 0.29$).

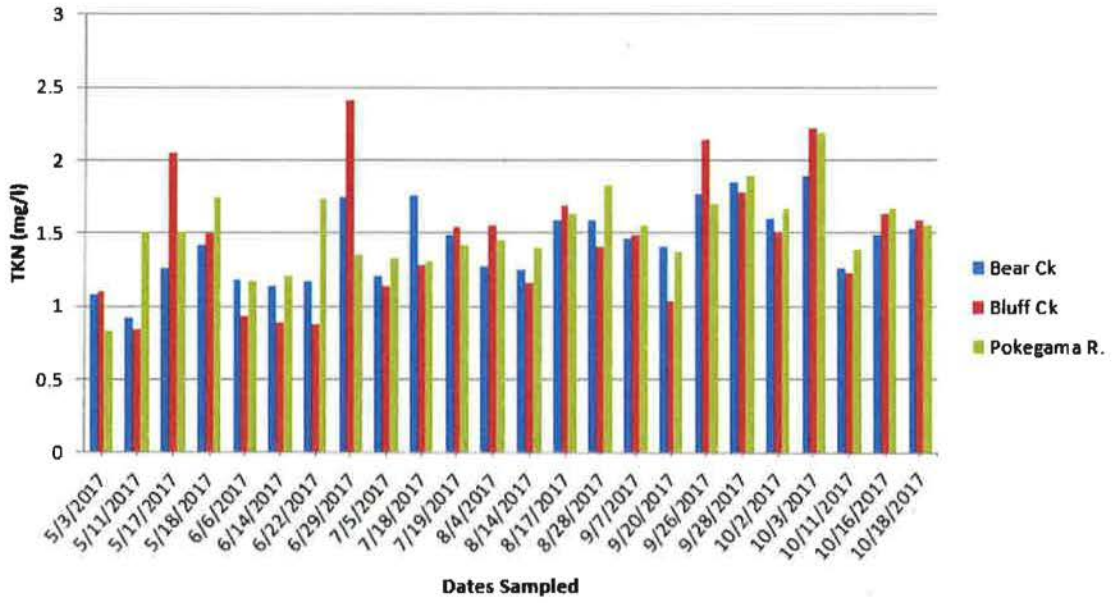
Bluff Creek had four dates when TKN's exceeded 2 mg/l (Figure 70). Stream flow on these dates averaged much higher than the mean flow for all sampled dates.

Figure 69. Stream Total Kjeldahl Nitrogen Concentration Means



Error bars are 90% confidence intervals

Figure 70. Stream Total Kjeldahl Nitrogen Concentrations



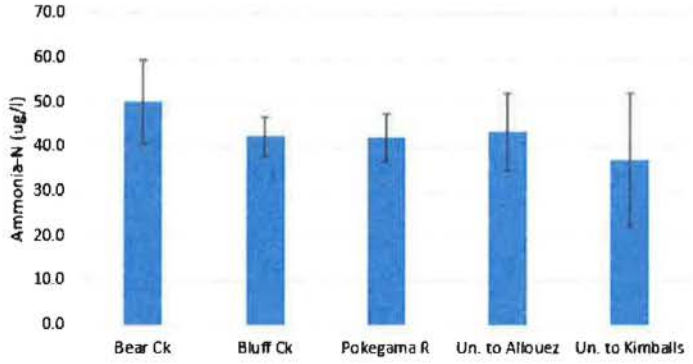
Ammonia Nitrogen

Mean ammonia nitrogen concentrations (NH_3's) ranged from 37.1 ug/l (unnamed tributary to Kimballs Bay) to 50 ug/l (Bear Creek) (Tables 28 and 29). There were no significant differences between site NH_3 means (Figure 71). NH_3's were not significantly correlated with stream flows.

NH_3's > 80 ug/l occurred in Bear Creek on 3 dates (Figure 72). These three dates had low stream flows (1.6 – 2.9 cfs). This suggests that septic systems may have been the source of these elevated NH_3's, as the elevated OP's during low stream flows also suggested.

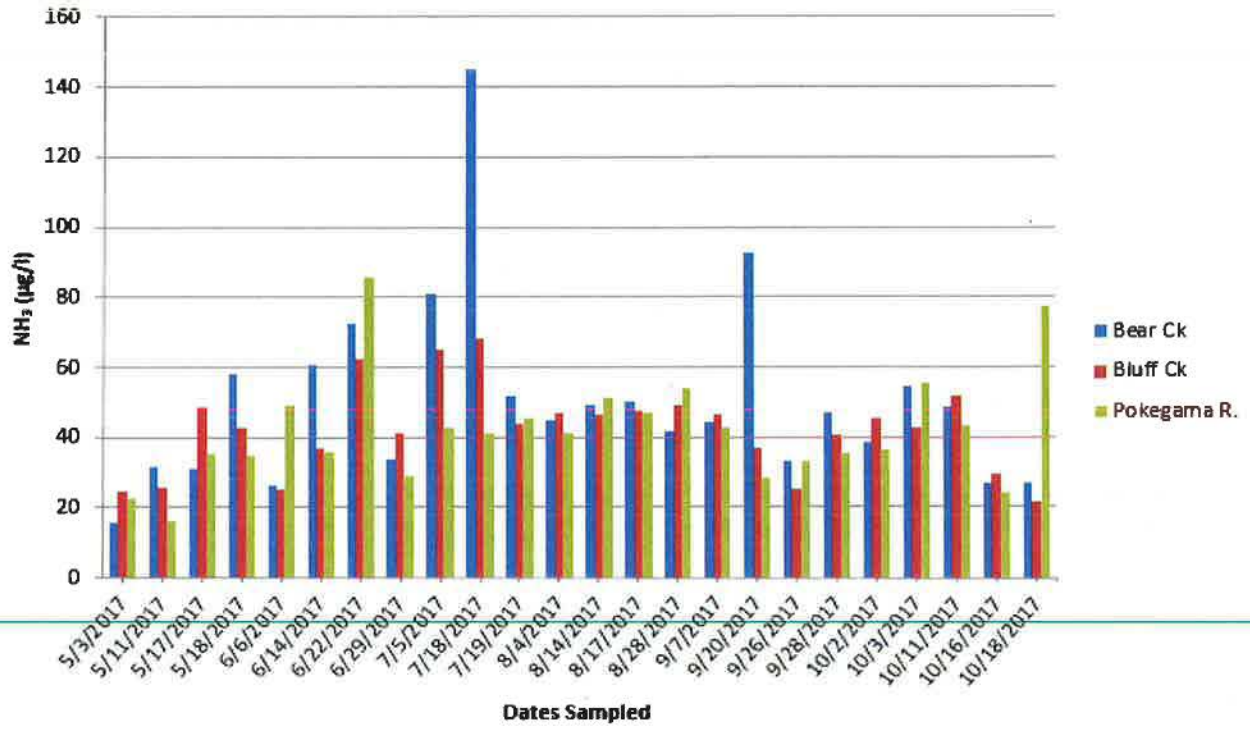
NH_3's > 75 ug/l occurred in the Pokegama River on 2 dates (Figure 72) with relatively low stream flows. The elevated NH_3 on October 18th can be fully accounted for by the Village of Superior wastewater lagoon discharge reported for that time period. Lagoon discharge reportedly was not occurring on June 22nd, the other date with elevated NH_3 in the Pokegama River. NH_3's for Bear and Bluff Creek were also relatively high on that date.

Figure 71. Stream Ammonia-N Concentration Means



Error bars are 90% confidence intervals

Figure 72. Stream Ammonia-N Concentrations

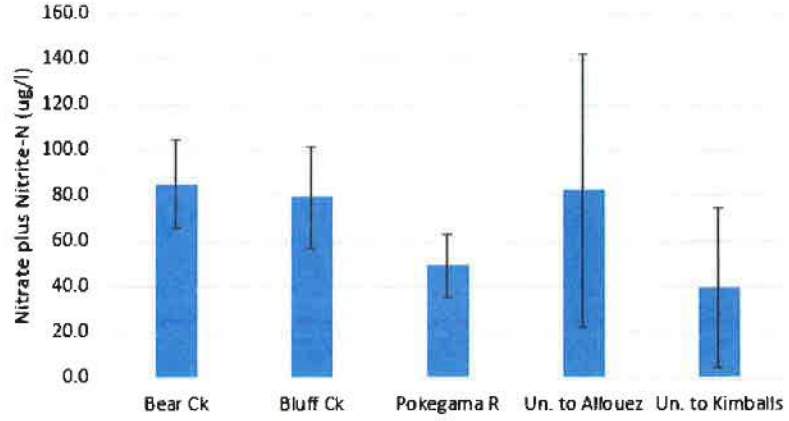


Nitrate plus Nitrite Nitrogen

Mean nitrate plus nitrite nitrogen concentrations (NO_x's) ranged from 39.1 ug/l (unnamed tributary to Kimballs Bay) to 85 ug/l (Bear Creek) (Tables 28 and 29). The mean NO_x for the Pokegama River was significantly lower than for Bear Creek. There were no other significant differences between site NO_x means (Figure 73). NO_x's were not significantly correlated with stream flows.

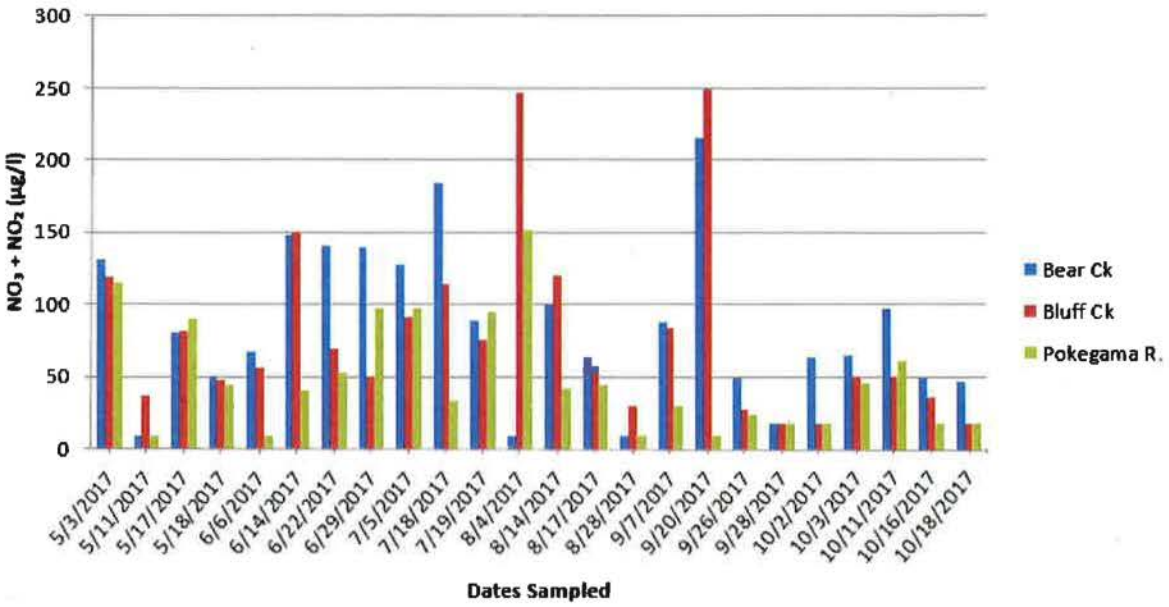
Bluff Creek had NO_x's > 200 ug/l on two dates (Figure 74). Stream flows were low to moderate on those dates and no specific source is suggested. Bear Creek had NO_x's > 175 ug/l on two dates. Both dates had low stream flows and also had high NH_3's, so septic systems are again suggested as a possible source.

Figure 73. Stream Nitrate plus Nitrite-N Concentration Means



Error bars are 90% confidence intervals

Figure 74. Stream Nitrate plus Nitrite-N Concentrations

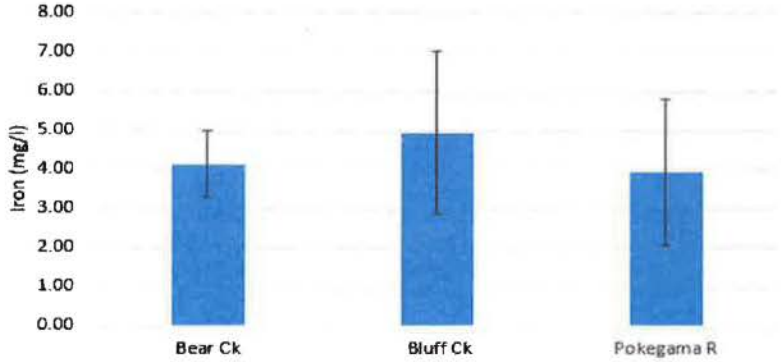


Iron

Mean iron concentrations ranged from 3.9 mg/l (Pokegama River) to 4.9 mg/l (Bluff Creek) (Table 28) (only named streams were tested). There were no significant differences between site means (Figure 75). Iron concentrations were highly correlated with stream flow (Pokegama R $R^2 = 0.98$, Bear Ck $R^2 = 0.90$, Bluff Ck $R^2 = 0.94$). Iron concentrations were also highly correlated with total suspended solids concentrations (Pokegama R $R^2 = 0.98$, Bear Ck $R^2 = 0.83$, Bluff Ck $R^2 = 0.89$). This suggests most iron was derived from soil erosion. Iron as a percent of total suspended solids ranged from 4 to 28%. Red clay contains iron, which produces its red color.

Bluff Creek was observed to be unusually red on September 20th following a ¾ inch rainfall (Figure 77). The iron concentration was 10 mg/l. The railyard for the BNSF taconite storage facility drains to Bluff Creek upstream of the sampling site and was the likely source of the color. The railyard is mostly covered with a layer of spilled taconite pellets. The color observed in Bluff Creek was nearly identical to that observed in runoff puddles within the taconite storage facility (Figure 78). Runoff from the taconite storage facility is captured and treated and does not drain to Bluff Creek.

Figure 75. Stream Iron Concentration Means



Error bars are 90% confidence intervals

Figure 76. Stream Iron Concentrations

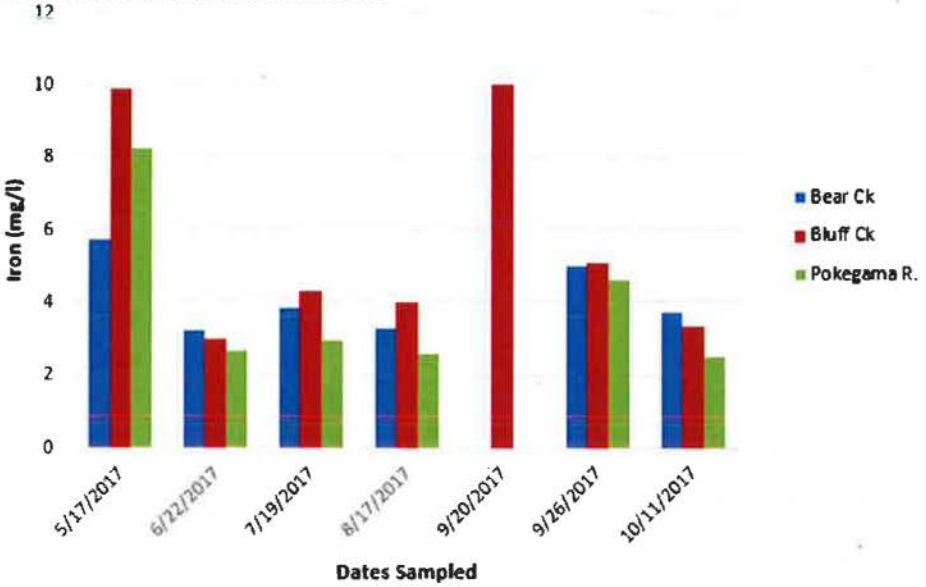


Figure 77. Red water in Bluff Creek on 9/20/17 when Iron Concentration was 10 mg/l

Figure 78. Air Photo of Runoff Puddles at BNSF Taconite Storage Facility

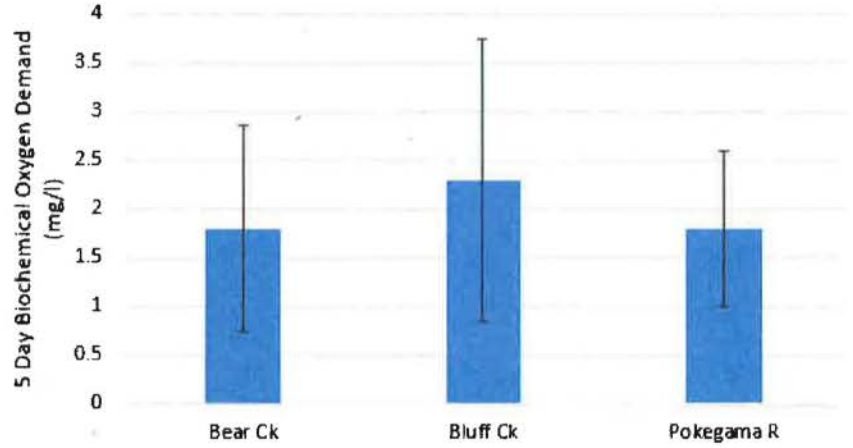
5-Day Biochemical Oxygen Demand

Mean 5-Day Biochemical Oxygen Demand concentrations (BOD's) ranged from 1.8 mg/l (Bear Creek and Pokegama River) to 2.3 mg/l (Bluff Creek) (Table 28) (only named streams were tested). There were no significant differences between site means (Figure 79). BOD's were poorly correlated with stream flow (Pokegama R $R^2 = 0.23$, Bear Ck $R^2 = 0.008$, Bluff Ck $R^2 = 0.10$).

BOD's were highest on June 29th and September 26th. Both days have moderate stream flow spikes (Figure 57).

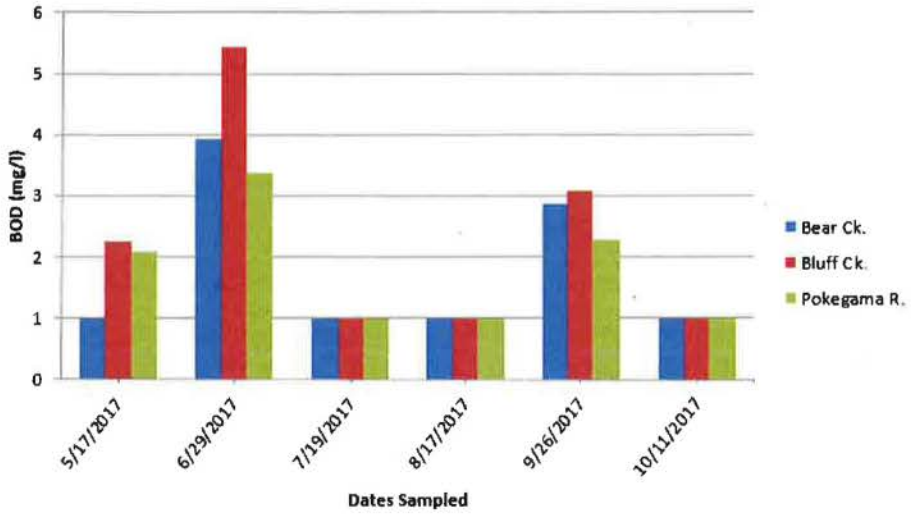
The 2017 May-October BOD load for the Pokegama River was 34,507 kg. The Village of Superior wastewater lagoons discharged 961.4 kg of BOD to the river during that period. The discharge was about 2.8 % of the river's BOD load.

Figure 79. Stream 5-Day Biochemical Oxygen Demand Concentration Means



Error bars are 90% confidence intervals

Figure 80. Stream 5-Day Biochemical Oxygen Demand Concentrations



Level of detection = 2 mg/l; samples less than LOD assumed to be 1 mg/l

Bay Sediment Characteristics

Bay sediment chemistry and grain size data is shown in table 30.

Table 30. Bay Sediment Chemistry and Grain Size

	Site	Solids	Total Organic C	Iron	Phosphorus	TKN	NH3	NO3+NO2	Sand	Silt	Clay
		%	(ug/g)	(mg/kg)	(mg/kg)	(mg/kg)	mg/kg	mg/kg	%	%	%
Allouez Bay	ASD	24.9	50310	39000	791	3480	25.9	<1.04	4	35	61
	ASE	33	35200	15200	360	2210	0.626	<0.763	64	13	23
	ANW	33	35420	35300	765	1660	0.694	<0.748	10	45	45
	AIS-1	52.4	18240	12000	190	941	0.988	<0.489	85	4	12
	AIS-2	26.4	51630	38500	789	3680	1.17	<0.930	11	34	56
	AIS-3	56.1	15780	26200	509	989	0.325	<0.451	61	14	26
	AIS-4	32.5	38240	33000	671	2210	0.777	<0.796	23	30	48
	AIS-5	31	44530	34700	775	2750	0.824	<0.792	13	42	46
Mean:		36.2	36169	29238	606	2240	3.9		34	27	40
Median:		32.8	36830	33850	718	2210	0.8		18	32	46
Min:		24.9	15780	12000	190	941	0.325		4	4	12
Max:		56.1	51630	39000	791	3680	25.9		85	45	61
Kimballs Bay	KND	35.1	32030	30200	775	1770	2.23	<0.694	16	51	33
	KND DUP.	35.9	33220	29300	755	1880	3.46	<0.693	18	51	31
	KND-AV.	35.5	32625	29750	765	1825	2.845	<0.694	17	51	32
	KIS-1	24	38130	41100	799	2100	18.7	<1.06	6	31	63
	KIS-2	32.6	43420	32000	677	2590	4.93	<0.770	14	43	43
Mean:		30.7	38058	34283	747	2172	8.8		12	42	46
Median:		32.6	38130	32000	765	2100	4.9		14	43	43
Min:		24	32625	29750	677	1825	2.8		6	31	32
Max:		35.5	43420	41100	799	2590	18.7		17	51	63
	Site	Solids	Total Organic C	Iron	Phosphorus	TKN	NH3	NO3+NO2	Sand	Silt	Clay
		%	(ug/g)	(mg/kg)	(mg/kg)	(mg/kg)	mg/kg	mg/kg	%	%	%
Pokegama Bay	PND	31.5	21590	39600	763	1460	0.854	<0.806	5	34	62
	PMID	42.8	18820	32300	620	1390	3.07	<0.565	4	49	47
	PMID-DUP.	41.8	23150	31700	633	1150	5.18	<0.576	4	47	49
	PMID-AV.	42.3	20985	32000	626.5	1270	4.125	<0.570	4	48	48
	PS	44	20770	30500	643	1140	3.56	<0.560	7	44	50
	PIS-1	38.5	29080	31700	737	1720	0.754	<0.618	7	56	38
	PIS-2	34.3	19480	38900	721	1220	1.88	<0.711	11	46	34
	PIS-3	32	19820	40500	710	1540	3.9	<0.775	7	47	45
	PIS-4	40.1	18290	30900	611	1370	0.714	<0.617	7	54	40
	PIS-5	55.7	15840	24900	501	737	1.75	<0.445	43	22	36
Mean:		39.8	20732	33625	664	1307	2.19		11	44	44
Median:		39.3	20295	31850	677	1320	1.82		7	47	43
Min:		31.5	15840	24900	501	737	0.714		4	22	34
Max:		55.7	29080	40500	763	1720	4.125		43	56	62

Clay content of sediment (% Clay) was moderately well correlated with phosphorus concentration ($R^2 = 0.75$) and iron concentration ($R^2 = 0.76$). Iron will readily attach to the extensive bonding surfaces of clay particles, and phosphorus will attach to the iron.

Clay content was also moderately inversely correlated with % solids ($R^2 = 0.43$). Clay sediment tends to have a higher water content than coarser grained sediment.

Mean clay content of sediment in all three bays (40 – 46%) was significantly higher than that found in the remainder of the central and lower SLRE, where clay content averaged about 14.7% (NOAA DIVER 2018). This is not surprising given the clay rich soils in the direct watersheds of the bays.

Total organic carbon (TOC) and total Kjeldahl nitrogen (TKN) were highly correlated ($R^2 = 0.92$). Both parameters reflect the organic matter content of the sediment.

Allouez Bay sites had the highest mean, median and maximum % sand. There was an inverse correlation between site depth and % sand for the bay ($R^2 = 0.73$). Sediment scouring by wave action is probably removing finer sediments at shallow sites and leaving more sand. Proximity to sand sources may also be significant. The two sites with the highest % sand (ASE, AIS-1) are near the sandy barrier beach on the north side of the bay. The site with the third highest % sand (AIS-3) is near the mouth of Bluff Creek, where a bed load of sand is likely to enter the bay.

The Pokegama Bay sites had significantly lower TOC and TKN concentrations than the other two bays. This was probably due to the higher rates of inorganic sediment deposition due to the high watershed to bay area ratio (Table 6).

Sediment descriptions and soft sediment thicknesses are shown in Table 31. Soft sediment thickness ranged from 0.9 to 12.9 feet. For all sites, water depth and soft sediment thickness were weakly correlated ($R^2 = 0.29$). Deeper sites tend to favor long term sediment deposition. Site ASD in Allouez Bay was historically dredged, so the soft sediment thickness there has been altered. Water depth and soft sediment thickness showed better correlations for individual bays (Allouez Bay (less site ASD), $R^2 = 0.42$; Pokegama Bay, $R^2 = 0.70$).

Table 31. Bay Sediment Descriptions and Thicknesses

SITE	Water Depth (ft)	Soft Sediment Thickness (ft)	Sediment Description
ASD	17.5	9.5	reddish brown silt
ASE	6.7	6.4	brown silty sand with organic matter
ANW	8.2	4.6	reddish brown silt
AIS-1	5.1	0.9	reddish brown silty sand; sand beneath soft sediment
AIS-2	8.3	7.7	reddish brown silt; hard clay beneath soft sediment
AIS-3	6.9	2.1	reddish brown clayey silt with zebra mussel shells
AIS-4	7.7	1.5	reddish brown silt; sand beneath soft sediment
AIS-5	7.6	10.2	reddish brown silt; woody debris felt while probing
KND	15.8	12.7	medium brown silt
KIS-1	15.8	10	medium brown silt
KIS-2	11.8	5.1	medium brown silt with organic debris; thin sand layer penetrated 2 ft above firm bottom
PND	11	12.9	medium brown to reddish brown silt; sand layer penetrated a few feet above firm bottom
PMID	6.1	5.4	reddish brown silt
PS	3.4	5.2	reddish brown silt
PIS-1	9	9.9	medium brown silt
PIS-2	9.3	11.6	medium brown to reddish brown silt
PIS-3	11.5	10.7	reddish brown silt
PIS-4	4.9	7.1	reddish brown silt
PIS-5	4.2	8.8	reddish brown sandy clay with organic detritus

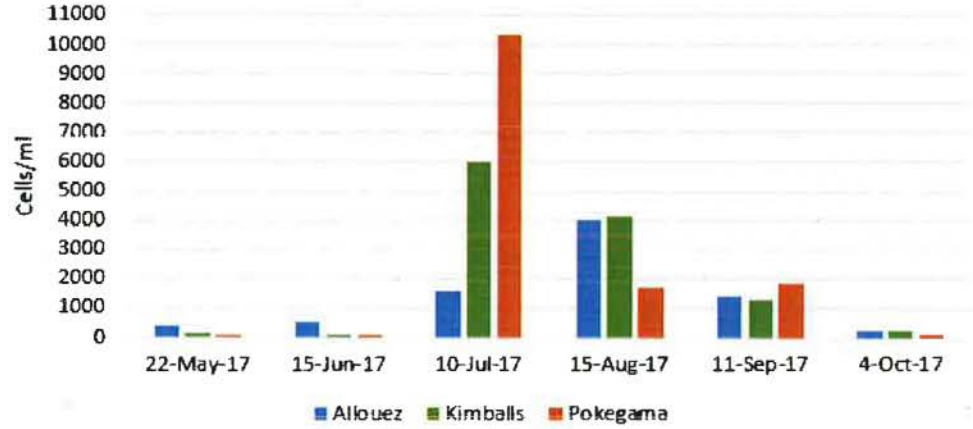
Algae

Identification and enumeration results from monthly algae samples are contained in Appendix 4. Seven phyla and 198 taxa of algae were identified.

All phyla occurred in higher densities in July, August, and September. Correspondingly, chlorophyll a concentrations were also generally higher during these months (Figure 27). Total suspended solids concentrations and turbidity were lower during these months (Figures 47 and 53) which increased light availability for algal growth. Water temperatures were higher during these months (Table 7) which can also promote algal growth.

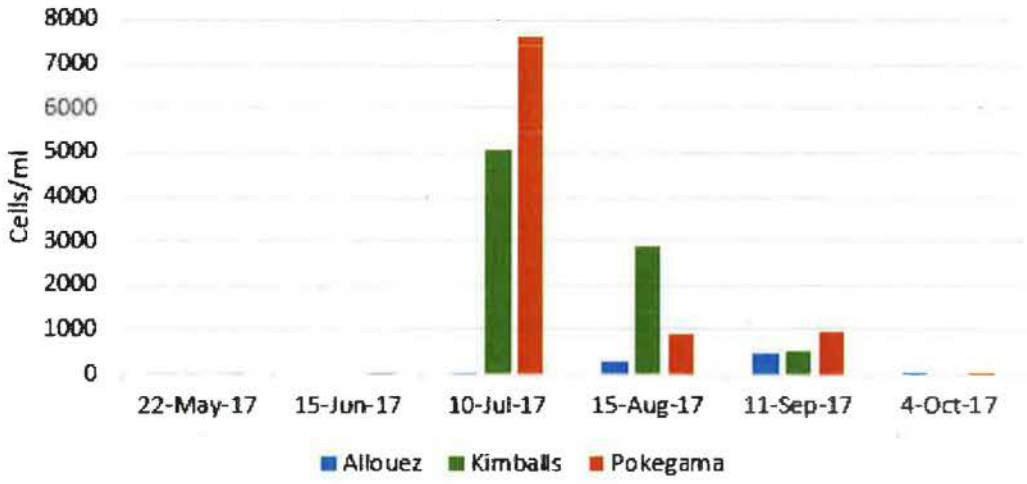
Total algal cell densities were highest in all bays in July, August, and September (Figure 81). Pokegama Bay had the highest total cell density on July 10th (10,343 cells/ml).

Figure 81. Bay Mean Total Algal Cell Densities



Blue-green algae cell densities were highest in all bays in July, August, or September (Figure 82). Pokegama Bay had the highest blue-green algae density on July 10th (7,657 cells/ml). Blue-green algae density was very low in all bays during May, June, and October (< 50 cells/ml). Allouez Bay had the lowest blue-green algae densities on five of the six sampling dates. *Aphanazomenon flos-aquae* was the dominant blue-green algae in ten of the thirteen samples with densities > 500 cells/ml.

Figure 82. Bay Mean Blue-green Algae Cell Density

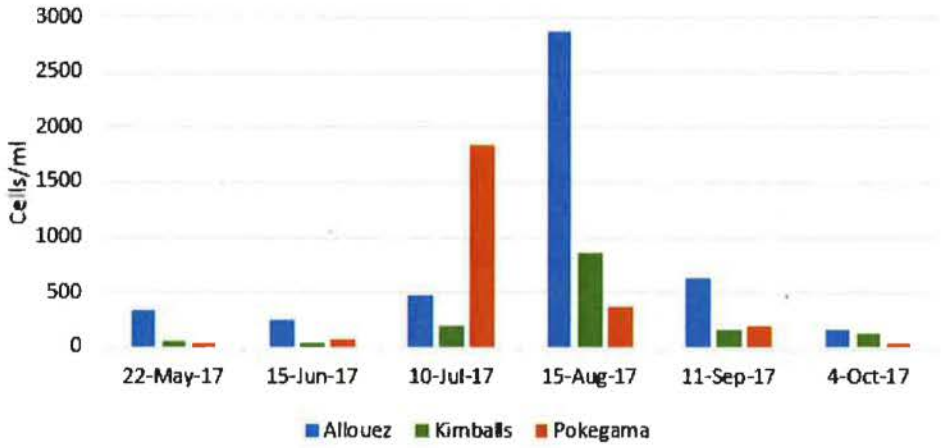


Diatom densities were highest in all bays in July or August (Figure 83). Allouez Bay had the highest diatom density on August 15th (2,875 cells/ml). Allouez Bay had the highest diatom densities on five of the six sampling dates.

Diatoms comprised > 50% of the algal population at most sites during May, June, and October, when total algal cell densities were low. They also comprised > 50% of the algal population at some sites in Allouez Bay during July, August, and September.

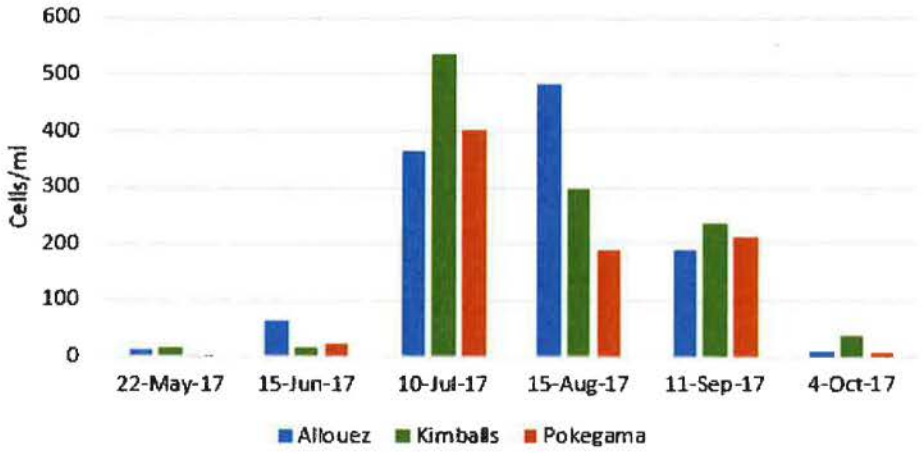
The five most abundant diatom species, in order of abundance, were – *Aulacoseira granulata*, *A. ambigua*, *A. distans*, *A. subarctica*, and *Stephanodiscus oregonicus*. The two most abundant species, *Aulacoseira granulata* and *A. ambigua*, were identified as some of the most common species in the sediment cores collected in Allouez and Pokegama Bays (Reavie 2016).

Figure 83. Bay Mean Diatom Cell Densities



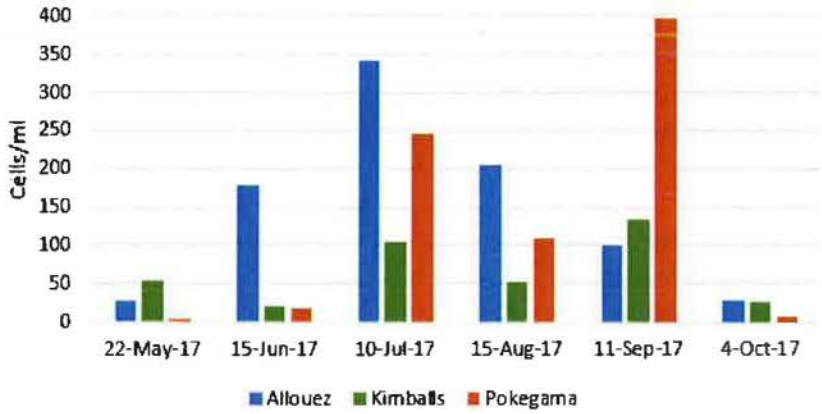
Green algae densities were highest in all bays in July or August (Figure 84). Kimballs Bay had the highest green algae density on July 10th (537 cells/ml).

Figure 84. Bay Mean Green Algae Cell Densities



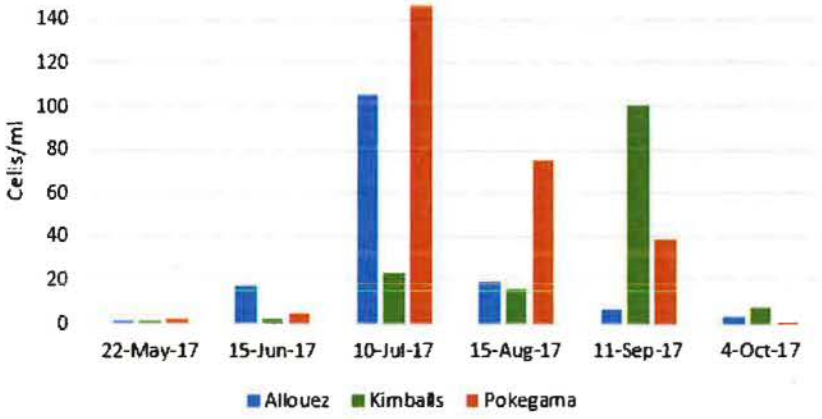
Cryptomonad densities were highest in all bays in July or September (Figure 85). Pokegama Bay had the highest Crypromonad density on September 11th (397 cells/ml).

Figure 85. Bay Mean Cryptomonad Cell Densities



Euglenoid densities were highest in all bays in July or September (Figure 86). Pokegama Bay had the highest Euglenoid density on July 10th (147 cells/ml).

Figure 86. Bay Mean Euglenoid Cell Densities



Yellow-brown algae (Chrysophyta) and dinoflagellates (Pyrrophyta) comprised small components of the algal community with monthly mean densities for all bays < 100 cells/ml.

Four of the phyla present were heterotrophic algae (Cryptomonads, Euglenoids, Chrysophytes, Pyrrhophytes). Heterotrophic algae can also consume bacteria as a food source. These algae were present at higher densities than commonly found (Pillsbury 2018). The mean heterotrophic algae cell counts for all sites comprised more than 10% of total cell counts on all dates and reached 32% for the June 15th samples. Light limitation due to clay turbidity may favor a larger heterotrophic algae population.

Benthic Invertebrates

Benthic invertebrate results from the 19 sites sampled are summarized in Table 32. Complete benthic invertebrate data is contained in appendix 6. The trimetric index (TMI) and the ephemerid (mayfly) density index developed by Angradi et al (2016) for the SLRE were applied to provide a qualitative assessment of the invertebrate communities found. The trimetric and ephemerid density indices were developed for three zones within the SLRE - Superior Bay, St. Louis Bay, and Spirit Lake. Allouez Bay and Pokegama Bay were excluded from the development of these indices since the bays were felt to have distinct water chemistry and substrate characteristics and had been infrequently sampled. Application of these indices to Allouez

and Pokegama Bays is still useful since it provides a comparison to the rest of the SLRE. The indices from the SLRE zone with the most similar mean depth have been selected to apply to each bay.

Table 32. Benthic Invertebrate Trimetric and Ephemerid Density Index Values for Bay Sites

SITE	SCALED TMI	WATER DEPTH M	SUPERIOR BAY* TMI CONDITION	ST LOUIS BAY* TMI CONDITION	SPIRIT LAKE* TMI CONDITION	EPHEMERID DENSITY NO./M2	SUPERIOR BAY* EPHEMERID DENSITY CONDITION	ST LOUIS BAY* EPHEMERID DENSITY CONDITION	SPIRIT LAKE* EPHEMERID DENSITY CONDITION
KIMBALLS BAY									
KND	0.08	5.0	POOR	POOR	POOR	0	POOR	POOR	POOR
KIS-1	0.16	4.4	POOR	POOR	POOR	0	POOR	POOR	POOR
KIS-2	0.39	3.1	GOOD	FAIR	GOOD	0	POOR	POOR	POOR
BAY SITE MEAN DEPTH		4.1							
BAY MEDIAN CONDITION			POOR				POOR		
POKEGAMA BAY									
PND	0.32	3.2	FAIR	POOR	FAIR	130.4	EXCELLENT	EXCELLENT	EXCELLENT
PMID	0.40	1.6	GOOD	FAIR	POOR	434.8	EXCELLENT	EXCELLENT	EXCELLENT
PMID-DUP	0.45	1.6	GOOD	FAIR	FAIR	434.8	EXCELLENT	EXCELLENT	EXCELLENT
PS	0.44	1.1	GOOD	FAIR	FAIR	347.8	EXCELLENT	EXCELLENT	EXCELLENT
PS-1	0.43	2.6	GOOD	FAIR	GOOD	347.8	EXCELLENT	EXCELLENT	EXCELLENT
PS-2	0.32	2.6	FAIR	POOR	POOR	173.9	EXCELLENT	EXCELLENT	EXCELLENT
PS-3	0.19	3.2	POOR	POOR	POOR	0	POOR	POOR	POOR
PS-4	0.38	1.4	FAIR	POOR	POOR	43.5	FAIR	GOOD	FAIR
PS-5	0.54	1.1	GOOD	GOOD	GOOD	347.8	GOOD	EXCELLENT	EXCELLENT
BAY SITE MEAN DEPTH		2.1							
BAY MEDIAN CONDITION					FAIR				EXCELLENT
ALLOUEZ BAY									
ASD	0.12	5.2	POOR	POOR	POOR	0	POOR	POOR	POOR
ASE	0.51	1.8	GOOD	GOOD	GOOD	1913.1	EXCELLENT	EXCELLENT	EXCELLENT
ANW	0.19	2.4	POOR	POOR	POOR	43.5	GOOD	GOOD	GOOD
ANW-DUP	0.28	2.4	POOR	POOR	POOR	173.9	EXCELLENT	EXCELLENT	EXCELLENT
AIS-1	0.39	1.5	FAIR	POOR	POOR	130.4	GOOD	GOOD	GOOD
AIS-2	0.20	2.4	POOR	POOR	POOR	0	POOR	POOR	POOR
AIS-3	0.46	2.1	GOOD	FAIR	GOOD	695.7	EXCELLENT	EXCELLENT	EXCELLENT
AIS-4	0.42	2.2	GOOD	FAIR	FAIR	173.9	GOOD	EXCELLENT	EXCELLENT
AIS-5	0.23	2.2	POOR	POOR	POOR	87	GOOD	GOOD	GOOD
BAY SITE MEAN DEPTH		2.5							
BAY MEDIAN CONDITION				POOR				GOOD	

**mean zone depths for index development sites: Superior Bay - 5.0 m, St. Louis Bay – 2.8 m, Spirit Lake – 1.8 m. The indices from the SLRE zone with the most similar mean depth are highlighted in color in the table.*

The three deepest (≥ 4.4 m) sites (ASD, KND, KIS-1) all had poor TMI and ephemerid density conditions. Periods of anoxia probably occur at these sites, which limits the invertebrate community. Profile data from site ASD suggests occasional anoxia occurred (see bay water quality discussion section). Profile data from site KND indicated extended periods of anoxia occurred, and it seems likely this is the case for site KIS-1, as well.

The shallowest site in Kimballs Bay had a good TMI condition and a poor ephemerid density condition (No ephemerids were present). The median TMI condition for the bay was poor. The median ephemerid density condition for the bay was also poor. The occurrence of anoxia at two of the three sites sampled in the bay probably accounted for this.

Pokegama Bay TMI conditions ranged from poor to good. The median TMI condition for the bay was fair. Pokegama Bay ephemerid density conditions ranged from poor to excellent. The median ephemerid density condition for the bay was excellent. Allouez Bay TMI conditions ranged from poor to good. Six of the nine samples (including duplicate) were poor, two were fair, and one was good. The median TMI condition for

the bay was poor. Allouez Bay ephemerid density conditions ranged from poor to excellent. The median ephemerid density condition for the bay was good.

Both Pokegama and Allouez Bay had higher total suspended solids concentrations and turbidity, and probably higher rates of inorganic sediment deposition than the remainder of the SLRE where the TMI and ephemerid density condition indices were developed. Clay content of sediment in all three bays was also substantially higher than in the remainder of the SLRE (NOAA DIVER 2018). The bay water quality along with the physical characteristics of sediment with high clay content (and corresponding high water content) may be restrictive to some benthic invertebrates and result in poorer TMI conditions. Ephemerid mayflies do not appear to be affected by these water and sediment characteristics.

Aquatic Macrophytes

Aquatic vegetation data from the St. Louis River Estuary Vegetation Database (Danz et al. 2017) was reviewed for each of the bays to assess their plant communities. Data includes several different projects over a span of eleven years (2004-2015) within the St. Louis River Estuary. Emergent, submergent, and floating leaf vegetation are included in the database. Number of species, average species per plot, and average mean Coefficient of Conservatism (mean C) were calculated from the database by project.

Mean C has a range of 0 to 10, ten being the highest quality sites with species that have a low tolerance of disturbance and are restricted to certain plant communities. Conversely, a mean C value of 0 indicates a site with species that are very tolerant of disturbance and found in a wide variety of community types. Project values were averaged for each bay and are summarized in Table 33. Species lists for the bays are contained in appendix 7.

Table 33. Aquatic Macrophyte Survey Data for Bays

	Allouez Bay	Kimballs Bay	Pokegama Bay	All SLRE surveys
Number of species	155	74	148	NC**
Species per plot	8.8	5.0	5.8	NC**
Mean C* value	5.6	3.6	5.4	5.06

*C = coefficient of conservatism, an index of tolerance to disturbance. **NC = not comparable; number of species and species per plot are influenced by size of area surveyed and survey methods, so do not offer a simple means of comparison.

Wetland vegetation data for the three bays from the Great Lakes Coastal Wetland Monitoring Program (Brady 2018) was also reviewed. Data is from surveys conducted during 2011 through 2017. IBI (index of biotic integrity) scores and ratings for the three bays were compared to survey sites that are not clay influenced. This is summarized in Table 34.

Table 34. Wetland Vegetation Biological Community Indicator Summary for Bays

BIOLOGICAL COMMUNITY	INDICATOR	ALLOUEZ BAY	KIMBALLS BAY	POKEGAMA BAY
Wetland Vegetation	Wetland vegetation IBI[1]	2011-2017 median = moderately impacted = median for non-clay influenced SLRE surveys	2014, 2016 = moderately degraded, which is poorer than the median for non-clay influenced SLRE surveys (moderately impacted).	2011, 2012, 2016 median = moderately impacted = median for non-clay influenced SLRE surveys

[1]Uzarski, DG, et al. 2017. Standardized measures of coastal wetland condition: implementation at a Laurentian Great Lakes basin-wide scale. Wetlands (37:15).

Allouez Bay

Allouez Bay had 155 plant species reported, the highest of the three bays. Two species of special concern, *Nuphar advena,* yellow water lily, and *Schoenoplectus torreyi,* Torrey's bulrush, were found. The bay had the highest average species per plot at 8.8, and the highest mean C value per plot at 5.6. This mean C value indicates that species tolerate moderate disturbance. It is better than the mean C value of 5.1 for all SLRE aquatic vegetation surveys.

Eight wetland vegetation surveys from Allouez Bay had a median IBI score of 2.7 (rating = moderately impacted). This IBI score is slightly poorer than, the median IBI score of 2.85 for 16 site surveys in SLRE locations that are not clay influenced. However, the median IBI rating for the Allouez Bay surveys was the same as the median rating for the 16 site surveys in SLRE locations that are not clay influenced (rating = moderately impacted).

Kimballs Bay

Kimballs Bay had the lowest number of species, with 74 plant species reported, and an average species per plot of 5.0. Mean C value was 3.6, the lowest value for the three bays. This value indicates a plant community of generalists that are tolerant of disturbance. This is substantially poorer than the mean C value of 5.1 for all SLRE aquatic vegetation surveys. Only three projects in the SLRE vegetation database had data for Kimball's Bay, which may have influenced these numbers.

Two wetland vegetation surveys from Kimballs Bay had a median IBI score of 2.05 (rating = moderately degraded). This is substantially poorer than the median IBI score of 2.85 (rating = moderately impacted) for 16 site surveys in SLRE locations that are not clay influenced.

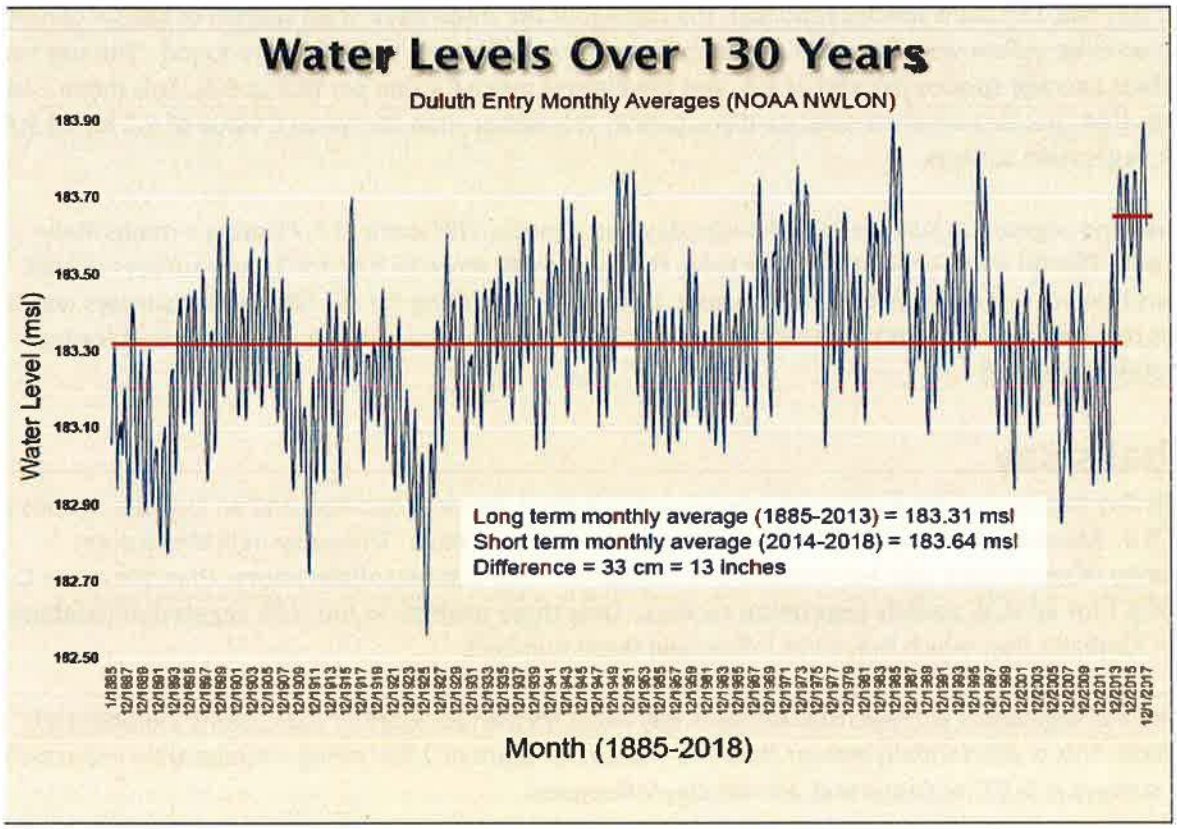
Pokegama Bay

Pokegama Bay had 148 species reported. This includes one species of special concern, *Schoenoplectus torreyi,* Torrey's bulrush. Average species per plot was 5.8. Mean C value was 5.4, indicating species that tolerate moderate disturbance. This is slightly better than the mean C value of 5.1 for all SLRE aquatic vegetation surveys.

Three wetland vegetation surveys from Pokegama Bay had a median IBI score of 2.8 (rating = moderately impacted). This is very similar to the median IBI score of 2.85 (rating = moderately impacted) for 16 site surveys in SLRE locations that are not clay influenced.

With the recent rise in Lake Superior's water level (Figure 87), a shift in Pokegama Bay's vegetation community from more emergent wetland plants to more submergent aquatic plants has been observed (Schooler 2018). Similar shifts are likely to have occurred in the other bays. Shoreward shifts of emergent plant zone boundaries and die-off of speckled alders due to rising water levels have been observed elsewhere in the SLRE (Roesler 2017).

Figure 87. Monthly Average Water Levels at the Duluth Entry of the St. Louis River (Schooler 2018)



Wetlands

Wetland Data Sources

The Great Lakes Coastal Wetland Monitoring Program monitored several sites in the St. Louis River Estuary. Three monitored sites are in the bays of this study: one at the upstream end of Kimball's Bay, one in the upstream end of Pokegama Bay, and one covering most of the wetlands in Allouez Bay (Figure 88). A suite of data was collected in these wetlands during 2011-2017, including vegetation, water quality, macroinvertebrates, and fish (http://www.greatlakeswetlands.org/Home.vbhtml). A full list of parameters and protocols for this monitoring program are available in Uzarski 2016. Vegetation data from the wetland monitoring program is included in the SLRE Aquatic Vegetation Database (Danz et al. 2017) and is summarized with other aquatic plant monitoring data for the clay-influenced bays in the preceding "Aquatic Macrophytes" section.

Figure 88. Coastal Wetland Monitoring Program Sites in Study Bays

Wetland Water Quality Data

Wetland water quality data for the three bays is summarized with mean parameter values in Table 35. Allouez Bay had the lowest total phosphorus concentration (TP) (67 ug/l), the lowest total nitrogen concentration (TN) (0.709 mg/l), and the highest chlorophyll a concentration (CHL) (5.4 ug/l). Kimball's Bay had the highest TP (126 ug/l), the lowest turbidity (13.8 NTU) and lowest CHL (2.9 ppb). Pokegama Bay had the highest TN (1.16 ppm) and the highest turbidity (36.2 NTU). Mean concentrations for the four parameters were generally within the range of open water values found at nearby sites in the respective bays in July and August of 2017 (Table 36). One exception was Kimballs Bay where the wetland mean CHL was lower than the range of open water bay values. Also, the wetland mean TP was about double that found at the open water site. The CHL difference may be due to annual variability. The TP difference again suggests wetland phosphorus release is occurring in Kimballs Bay.

Dissolved oxygen concentrations (D.O.'s) were measured at the wetland monitoring sites in areas with submergent vegetation and areas with emergent vegetation. Daytime D.O.'s were less than 5 mg/l for a significant percentage of measurements (24% - Allouez Bay, 14% - Pokegama Bay, 83% - Kimballs Bay). Daytime D.O.'s were also less than 3 mg/l for a significant percentage of measurements (12% - Allouez Bay, 5% - Pokegama Bay, 25% - Kimballs Bay). Nighttime D.O.'s would be lower, and sediment surface D. O's at night may be low enough to allow sediment phosphorus release to occur at times. Kimballs Bay has the highest frequency of low daytime D.O.'s, which again suggests higher rates of sediment or wetland phosphorus release.

Table 35. Mean Water Quality Values for All Vegetation Zones from Coastal Wetland Monitoring Data (data collected during July or August of 2011 – 2017; years with data varies between bays)

Site	Turbidity (ntu)	Chlorophyll a (ug/l)	Total Phosphorus (ug/l)	Total Nitrogen (mg/l)
Allouez Bay	31.2	5.4	67	0.709
Kimball's Bay	13.8	2.9	126	0.929
Pokegama Bay	36.2	5.1	112	1.160

Table 36. Ranges of July and August 2017 Water Quality Values for Open Water Bay Site(s) Closest to Wetland Monitoring Zones.

Site	Turbidity (ntu)	Chlorophyll a (ug/l)	Total Phosphorus (ug/l)	Total Nitrogen (mg/l)
Allouez Bay (site ASE)	28 - 58	2.7 - 15.5	46 - 82	0.72 - 0.84
Kimball's Bay (site KND)	9 - 13	7.7 - 20.7	56 - 63	0.85 - 1.01
Pokegama Bay (sites PS and PMID)	32 - 123	2.7 - 23.4	77 - 156	1.0 - 1.6

Wetland Macroinvertebrates

Allouez Bay wetlands had the highest macroinvertebrate taxa richness with 88 taxa found. Kimball's Bay had the lowest macroinvertebrate taxa richness with 54 taxa found. Pokegama Bay had 77 macroinvertebrate taxa found. Wetland macroinvertebrate IBIs were available for Allouez Bay and Pokegama Bay and are summarized in Table 37.

Table 37. Wetland Macroinvertebrate Biological Community Indicators

BIOLOGICAL COMMUNITY	INDICATOR	ALLOUEZ BAY	KIMBALLS BAY	POKEGAMA BAY
Wetland Macroinvertebrates	Wetland macroinvertebrate IBI[1]	2011, 2012 = moderately impacted; not enough non-clay influenced SLRE surveys to allow comparison.	IBI not available	2011, 2012 median = mildly impacted; not enough non-clay influenced SLRE surveys to allow comparison.

[1]Uzarski, DG, et al. 2017. Standardized measures of coastal wetland condition: implementation at a Laurentian Great Lakes basin-wide scale. Wetlands (37:15).

Allouez Bay had a score of 134 in 2011 and 130 in 2012. Both scores are considered moderately impacted. Pokegama Bay had a score of 136 in 2011, which is rated as moderately impacted, and a score of 162 in 2012, which is considered a reference condition or most pristine. There were not enough SLRE survey sites from locations not influenced by clay to allow a comparison.

Wetland Birds and Frogs

Wetland bird and frog survey results (2012-2013) are available for Allouez and Pokegama Bays (Tozer 2014). Additional wetland bird and frog survey result are available for one or more years during 2014 -2017 for all three bays (Brady 2018). The 2014-17 survey results are compared to survey results from SLRE survey sites not influenced by clay turbidity. Results from both data sets are summarized in Table 38.

Table 38. Wetland Bird and Frog Biological Community Indicators

BIOLOGICAL COMMUNITY	INDICATOR	ALLOUEZ BAY	KIMBALLS BAY	POKEGAMA BAY
Wetland Birds	Bird IBI[1]	2012-13 IBI = 31.8; fair - slightly poorer than the median value of 33.3 found for 14 Lake Superior coastal wetlands, mostly	no data	2012-13 IBI = 34.0; fair - slightly better than the median value of 33.3 found for 14 Lake Superior coastal wetlands, mostly outside of SLRE
Wetland Birds	Bird IEC[2]	2014 2016, 2017 median = high quality, which is better than the median for non-clay influenced SLRE surveys (moderately impacted)	2016 = degraded, which is poorer than the median for non-clay influenced SLRE surveys (moderately impacted)	2016 = mildly impacted, which is better than the median for non-clay influenced SLRE surveys (moderately impacted)
Wetland Frogs	Frog IBI[1]	2012-13 IBI = 60.0; good - poorer than the median value of 86.5 found for 13 Lake Superior coastal wetlands, mostly outside of SLRE	no data	2012-13 IBI = 70.3; very good - poorer than the median value of 86.5 found for 13 Lake Superior coastal wetlands, mostly outside of SLRE
Wetland Frogs	Frog IEC[2]	2014 2016, 2017 median = reference condition, which is better than the median for non-clay influenced SLRE surveys (mildly impacted)	2016 = moderately degraded, which is poorer than the median for non-clay influenced SLRE surveys (mildly impacted)	2016 = moderately impacted, which is poorer than the median for non-clay influenced SLRE surveys (mildly impacted)

[1]Tozer, D. 2014. LSRI nearshore monitoring project: 2012-2013 bird and frog indices of biotic integrity. EPA assistance no. GL00E00500-0.

[2]Uzarski, DG, et al. 2017. Standardized measures of coastal wetland condition: implementation at a Laurentian Great Lakes basin-wide scale. Wetlands (37:15).

For the 2012-13 bird surveys, Allouez Bay had an index of biotic integrity (IBI) score of 31.8 (rated fair) which is slightly poorer than the median score of 33.3 found for 14 Lake Superior coastal wetlands (mostly outside of the SLRE). Pokegama Bay had a score of 34.0 (rated fair) which is slightly better than that median score. For Allouez Bay, three wetland bird surveys (2014,2016,2017) had a median index of ecological condition (IEC) rating of high quality, which is better than the median for SLRE survey sites in locations not influenced by clay (moderately impacted). A Kimballs Bay survey (2016) had an IEC rating of degraded, which is poorer than the median for SLRE survey sites in locations not influenced by clay. A Pokegama Bay survey (2016) had an IEC of mildly impacted, which is better than the median for SLRE survey sites in locations not influenced by clay.

For the 2012-13 frog surveys, Allouez Bay had an IBI score of 60.0 (rated good) which is poorer the median score of 86.5 found for 13 Lake Superior coastal wetlands (mostly outside of the SLRE). Pokegama Bay had a score of 70.3 (rated very good) which is also poorer than that median score.
For Allouez Bay, three wetland frog surveys (2014,2016,2017) had a median IEC rating of reference condition, which is better than the median for SLRE survey sites in locations not influenced by clay (mildly impacted). A Kimballs Bay survey (2016) had an IEC rating of moderately degraded, which is poorer than the median for SLRE survey sites in locations not influenced by clay. A Pokegama Bay survey (2016) had an

EIC rating of moderately impacted, which is poorer than the median for SLRE survey sites in locations not influenced by clay.

Wetland Fish

Allouez Bay had 33 species of fish captured (Table 40) and of those 6 were invasive species: alewife, common carp, Eurasian ruffe, tubenose goby, round goby, and white perch. Kimballs Bay had the lowest number of fish species captured, 17, (Table 41) two of which were invasive (Eurasian ruffe and tubenose goby). Pokegama Bay had 19 fish species captured (Table 42) with two species being invasive (common carp and Eurasian ruffe). The more common species found in each bay's wetlands are listed in Table 39. More surveys were done in Allouez Bay, which may partially account for the larger number of common species found there.

Allouez Bay has site level wetland fish IBI's available for five survey years. The IBI ratings range from moderately degraded (2011, 2013, 2015) to mildly impacted (2016). Kimballs Bay has an IBI rating of moderately degraded for a single 2014 survey. Pokegama Bay has an IBI rating of mildly impacted from a single 2012 survey.

Table 39. Common Wetland Fish Species Found

	Fish species with vegetation zone average CPUE's >10
Allouez Bay	
2011,2013,2015,2016,2017 surveys	
	Yellow perch, yellow perch YOY
	Central mudminnow
	Black crappie YOY
	Emerald shiner
	Spottail shiner, spottail shiner YOY
	Round goby
	Bluegill or pumpkinseed, bluegill or pumpkinseed YOY
	Golden shiner YOY
Kimballs Bay	
2014, 2016 surveys	
	Yellow perch YOY
	Golden shiner YOY
	Pumpkinseed
	Black crappie YOY
Pokegama Bay	
2011, 2012 surveys	
	Black crappie YOY
	Bluegill or pumpkinseed YOY
	Black or brown bullhead YOY
	Golden shiner, golden shiner YOY
CPUE = catch per unit effort	
YOY = young of year	

Table 40. Allouez Bay Wetland Fish Species

(invasive species are highlighted in orange)

Common Name	Scientific Name
Alewife	Alosa pseudoharengus
Black Bullhead	Ameiurus melas
Black Crappie	Pomoxis nigromaculatus
Blacknose Shiner	Notropis heterolepis
Brook Silverside	Labidesthes sicculus
Brown Bullhead	Ameiurus nebulosus
Central Mudminnow	Umbra limi
Channel Catfish	Ictalurus punctatus
Common Carp	Cyprinus carpio
Common Shiner	Luxilus cornutus
Emerald Shiner	Notropis atherinoides
Eurasian Ruffe	Gymnocephalus cernua
Fathead Minnow	Pimephales promelas
Freshwater Drum	Aplodinotus grunniens
Tubenose Goby	Proterorhinus semilunaris
Golden Shiner	Notemigonus crysoleucas
Johnny Darter	Etheostoma nigrum
Logperch	Percina caprodes
Mimic Shiner	Notropis volucellus
Northern Pike	Esox lucius
Pumpkinseed	Lepomis gibbosus
Rock Bass	Ambloplites rupestris
Round Goby	Neogobius melanostomus
Sand Shiner	Notropis stramineus
Shorthead Redhorse	Moxostoma macrolepidotum
Silver Redhorse	Moxostoma anisurum
Smallmouth Bass	Micropterus dolomieu
Spottail Shiner	Notropis hudsonius
Tadpole Madtom	Noturus gyrinus
Trout-perch	Percopsis omiscomaycus
White Perch	Morone americana
White Sucker	Catostomus commersonii
Yellow Perch	Perca flavescens

Table 41. Kimballs Bay Wetland Fish Species

(invasive species are highlighted in orange)

Common Name	Scientific Name
Black Crappie	Pomoxis nigromaculatus
Bluegill	Lepomis macrochirus
Brown Bullhead	Ameiurus nebulosus
Eurasian Ruffe	Gymnocephalus cernua
Fathead Minnow	Pimephales promelas
Freshwater Tubenose Goby	Proterorhinus semilunaris
Golden Shiner	Notemigonus crysoleucas
Johnny Darter	Etheostoma nigrum
Largemouth Bass	Micropterus salmoides
Pumpkinseed	Lepomis gibbosus
Rock Bass	Ambloplites rupestris
Shorthead Redhorse	Moxostoma macrolepidotum
Tadpole Madtom	Noturus gyrinus
Walleye	Sander vitreus
White Sucker	Catostomus commersonii
Yellow Bullhead	Ameiurus natalis
Yellow Perch	Perca flavescens

Table 42. Pokegama Bay Wetland Fish Species

(invasive species are highlighted in orange)

Common Name	Scientific Name
Black Bullhead	Ameiurus melas
Black Crappie	Pomoxis nigromaculatus
Bluegill	Lepomis macrochirus
Brown Bullhead	Ameiurus nebulosus
Channel Catfish	Ictalurus punctatus
Common Carp	Cyprinus carpio
Emerald Shiner	Notropis atherinoides
Eurasian Ruffe	Gymnocephalus cernua
Golden Shiner	Notemigonus crysoleucas
Johnny Darter	Etheostoma nigrum
Logperch	Percina caprodes
Northern Pike	Esox lucius
Pumpkinseed	Lepomis gibbosus
Spottail Shiner	Notropis hudsonius
Tadpole Madtom	Noturus gyrinus
Trout-perch	Percopsis omiscomaycus
White Perch	Morone americana
White Sucker	Catostomus commersonii
Yellow Perch	Perca flavescens

Bay Fish Communities

A bay fish assessment was done as a companion project to the SLRE Clay-Influenced Bay Assessment project. The fish assessment project was conducted by Wisconsin DNR fish management staff. The complete project report (Nelson 2018) is available elsewhere and is summarized here.

Bay fisheries were monitored during 2017 using gill nets and shoreline electrofishing. Results are summarized and compared to Minnesota DNR 2017 gill netting results In Table 43 below:

Table 43. Bay Fish Data Summary with Comparison to MN DNR Gill Net Data

Gill Net Data	Allouez Bay	Kimballs Bay	Pokegama Bay	21 MN SLRE gill net sites
Total number of species	12	6	9	19
Median number of species/net lift	9	3	9	8
Mean fish/net lift	39.9	3.6	19.3	27.5
Mean kg fish/net lift	21.9	1.3	8.3	13.0
Gill Net plus Electrofishing Data				
Total number of species	22	15	21	not applicable
Number of native species	18	14	16	not applicable
Number of non-native species	4	1	5	not applicable
Number of intolerant species	4	4	3	not applicable

Allouez and Pokegama Bays gill net data is generally similar to data collected by the Minnesota DNR during 2017 from 21 SLRE gill net sites for median number of species/net lift, mean fish/net lift, and mean kg of fish/net lift. Kimballs Bay gill net data is substantially lower than the Minnesota DNR data for those parameters. The total number of species from the 21 Minnesota gill net sites is higher than in the three bays. Only one site in each bay was netted, so these values are not comparable.

Total number of fish species captured ranged from 15 in Kimballs Bay to 22 in Allouez Bay. One to five non-native species were found in each bay. Three to four intolerant species were found in each bay.
A list of fish species found and the catch totals for gill netting and electrofishing combined are shown in Table 44.

Fish considered at least moderately tolerant of turbid conditions made up 85% of the catch in Allouez Bay, 97% in Kimballs Bay, and 94% in Pokegama Bay. Kimballs Bay, with the highest percent turbidity tolerant fish, has substantially lower turbidity than Allouez and Pokegama Bays. Additional data from throughout the SLRE would need to be assessed to determine if there is a relationship between turbidity tolerant fish and local water turbidity.

Only one fish, a northern pike in Allouez Bay with an open lesion, was found with a visible DELT (deformities, eroded fins, lesions, or tumors). That fish accounted for 0.6% of the total catch for the bay.

Conclusions of the fishery survey report included, "Despite turbid conditions that may lead to the perception of poor water quality or habitat, locally popular sport fish species like walleye, northern pike, black crappie, and yellow perch were well represented in both Allouez and Pokegama Bays. Other species of interest to anglers and state fisheries management agencies were also found in these bays including lake sturgeon, muskellunge, bluegill, and channel catfish. While Increased turbidity in Allouez and Pokegama Bays may influence the presence or abundance of specific species, it has not diminished the fishery value or eliminated desirable gamefish species from these areas."

Table 44. Combined Gill Netting and Electrofishing Catch

	COMBINED GILL NETTING AND ELECTROFISHING CATCH BY BAY		
FISH SPECIES	ALLOUEZ	KIMBALLS	POKEGAMA
Black Crappie	10	1	8
Bluegill	0	12	9
Brown Bullhead	1	1	0
Channel Catfish	35	0	8
Common Carp	0	0	2
Emerald Shiner	7	0	1
Eurasian Ruffe	2	1	3
Freshwater Drum	4	0	1
Golden Shiner	0	14	10
Lake Sturgeon	2	0	0
Largemouth Bass	0	4	2
Log Perch	2	0	3
Mimic Shiner	4	0	0
Muskellunge	1	2	0
Northern Pike	10	5	6
Pumpkinseed	1	5	7
Rock Bass	2	1	1
Shorthead Redhorse	12	1	0
Silver Redhorse	7	0	4
Smallmouth Bass	1	1	1
Spottail Shiner	7	1	2
Walleye	20	3	16
White Bass	5	0	5
White Perch	3	0	15
White Sucker	5	0	3
Yellow Perch	37	15	18
Total Individuals	178	67	125

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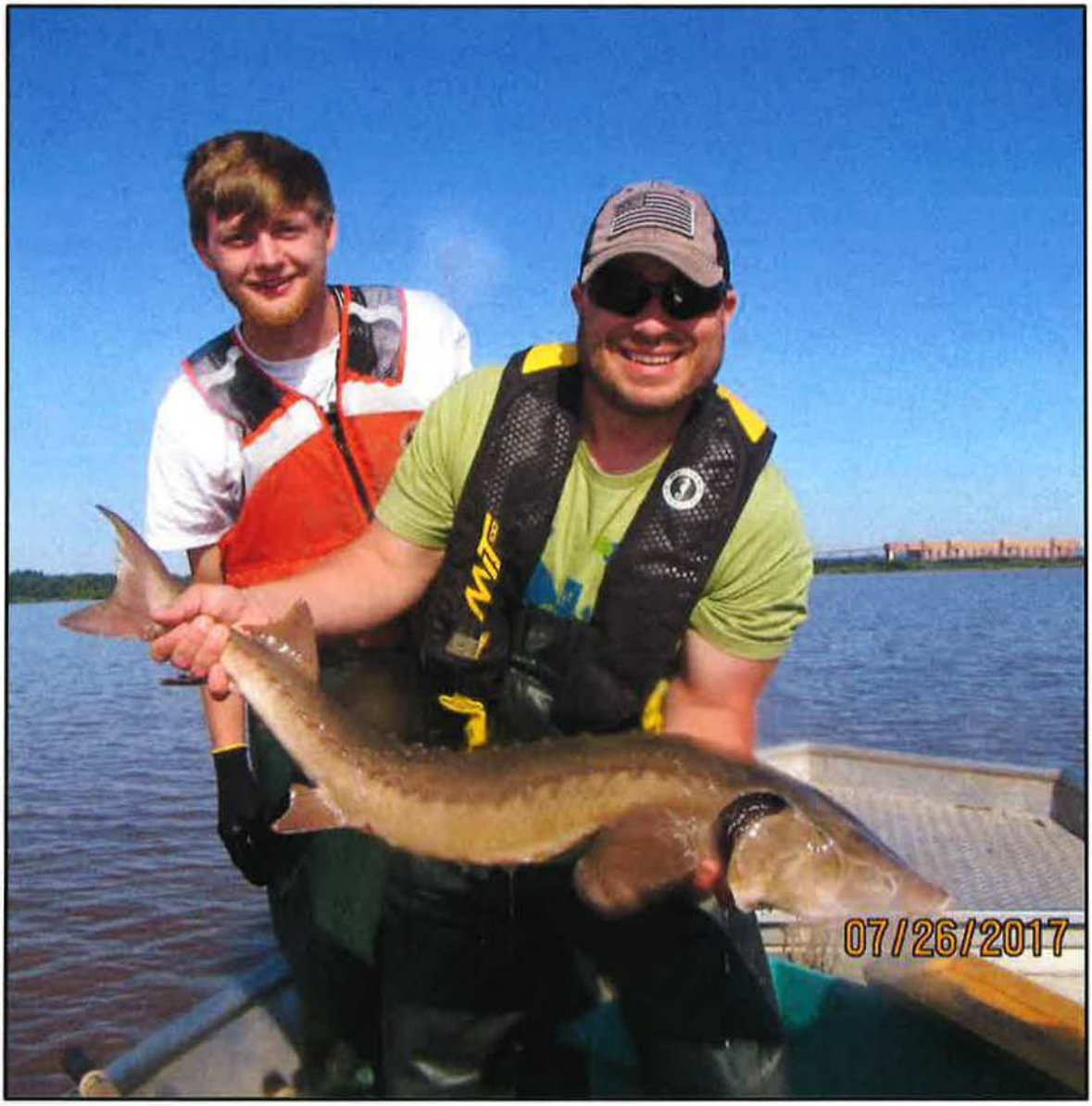
St Louis River Bays – Douglas County
2017 Fish Community Survey



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Cover photograph: Jeremy Bates, Lake Superior Basin Invasive Species Coordinator and McKenzie Schwartz, Fisheries Technician LTE, with a lake sturgeon captured in a gill net set in Allouez Bay.

2017 St Louis River Bays Fish Community Survey, Douglas County, WI
Lake Superior Fisheries Unit – Superior Field Office
Aaron Nelson – Fisheries Technician

1. Introduction

The 1992 St. Louis River Remedial Action Plan (RAP) states there are high levels of phosphorus in the St. Louis River Area of Concern (AOC) waters, however there remains little evidence of water quality problems associated with eutrophication. There is still concern that excess sediment and nutrient loading may impact the aquatic community and habitats of Lake Superior's nearshore areas, so Excessive Loading of Sediments and Nutrients remains listed as a Beneficial Use Impairment (BUI) in the St. Louis River Area of Concern RAP. This report will summarize survey work completed by Wisconsin DNR Fisheries Management staff to assess the fish community present in Allouez, Pokegama, and Kimball's Bays. This assessment work was completed as part of a cooperative study with WDNR Water Resources staff to assess the water quality and biological communities in two bays in the St. Louis River Estuary that experience excessive loading of sediment and nutrients.

2. Site Description

Pokegama and Kimball's Bays are located on the west side of the City of Superior, and Allouez Bay is located on the east side of the City of Superior (Figure 1). Pokegama Bay covers an area of approximately 390 acres with a shoreline of 15.5 miles (GIS derived) and is fed primarily by the Pokegama River and two small unnamed streams which drain a watershed of approximately 32-square miles. Allouez Bay covers an area of 990 acres with 8.3 miles of shoreline; Bluff Creek, Bear Creek and one unnamed stream directly drain to Allouez Bay from a 31-square mile watershed. Kimball's Bay covers an area of approximately 55 acres with a shoreline of 3.4 miles (GIS derived) and receives water from one unnamed stream that drains from a watershed of only 1.75 square miles.

The underlying geology of the Wisconsin side of Lake Superior and its watershed lie within the glacial deposits of red clay, silt and sand that were exposed as glacial Lake Duluth receded to the current level of Lake Superior. The upper Nemadji River watershed and lower reaches of the St. Louis River and its tributaries also drain these same glacial deposits on the Minnesota side of the Lake Superior basin. Erosion of this sediment from these stream drainages leaves much of the nearshore waters on Wisconsin's South Shore turbid with a characteristic red color. Due to the sheltered nature of Pokegama Bay and the seiche effect of Lake Superior on Allouez Bay, both areas remain turbid, with the continual input of eroded red clay from the tributaries. Kimball's Bay is sheltered similar to Pokegama Bay, but remains relatively clear, likely the result of a smaller watershed relative to the other bays and a relatively narrow embayment that may result in higher water velocities that moves eroded sediment out into the St. Louis River Estuary.

Since the bays are part of the estuary, sampling boundaries were set for each bay. For Allouez Bay, boundaries were based on the description of the bay as provided in the 2016 AOC RAP. A west boundary for Allouez Bay runs north off the north-eastern corner of the Duluth Power Squadron dock up to the end of a sand peninsula extending off Wisconsin Point. The confluence area with Bluff Creek was also included as part of Allouez Bay with a boundary at the U.S. Highway 2 bridge. The north boundaries for both Kimball's and Pokegama Bay were based on the Surface Water Data Viewer boundary between each bay and the St. Louis River. Southern boundaries for Kimball's and Pokegama Bay to exclude the riverine portion of the bay were based on best professional judgment using presence of emergent vegetation as visible on 2013 NAIP aerial photos.

3. Methods

3.1 Field

The fish community was surveyed using the two types of gear described in the Quality Assurance Project Plan (QAPP) developed for the St Louis River Biological and Water Quality Monitoring project.

"Fish surveys will be conducted in Allouez Bay, Pokegama Bay, and Kimball's Bay. Game and nongame fish will be surveyed once in each bay with a single, one to four-mile-long electrofishing pass along the shoreline in July or August. Single or multi-mesh gill nets may also be used to capture fish, particularly to address potential sampling bias in the known turbid waters of the three bays. The summer electrofishing survey will assess gamefish, panfish, and non-game fish, with data collection to include length and weight, as well as total fish counts. Fish scales may also be collected to document the fish community's age structure, particularly smallmouth bass, largemouth bass, northern pike, black crappie, bluegill, and yellow perch. Non-game fish and other sport fish will be counted. Prior to field data collection, the spring 2017 trap net data collected cooperatively between Minnesota and Wisconsin DNR will be reviewed to determine the extent to which length, weight, and age data need to be collected."

3.1.1 Gill Netting

Gill netting was completed using a Minnesota Department of Natural Resources (MNDNR) style lake survey gill net consisting of a net 250-feet long by 6-feet high with five 50-foot long panels of mesh measuring ¾-inch, 1-inch, 1 ¼-inch, 1 ½-inch, and 2-inches, respectively. The ends of the gill net lines are looped and attached with a carabiner to a 5-foot long 2" x 2" wooden brail that ensures the net panels stay open during deployment. An anchor was clipped to the bottom of each brail to provide the necessary weight to sink the net, and a rope was connected from the anchor to a mast buoy to mark each end of the net; additional floats were added along the float line of nets set in shallow water as a visual signal to boaters to avoid navigating through the area. Each gill net set was retrieved and reset for three consecutive net nights.

3.1.2 Shoreline Electrofishing

Shoreline electrofishing stations were surveyed with a Wisconsin-style mini-boom shocker comprised of an outboard motor propelled aluminum johnboat with a pulsed DC electrofishing unit powered by a 3500-watt AC generator. An anode consisting of a standard aluminum "Wisconsin ring" with 16 cylindrical, 17-mm diameter stainless steel droppers was used to deliver electrical current to the water. Pulsed DC settings were set at a pulse rate of 60-hertz and 25% duty cycle; the voltage and amperage were set to maintain electrical output to the anode as close to 3000 watts as possible. This electrofishing unit requires a two-person crew consisting of a driver/operator at the stern to run the outboard, generator and electrofishing system and a "dipper" is seated at the bow of the boat with a fiberglass long-handled dip net to capture fish as they are stunned. Fish were drawn to the anode end of the electrical field and any fish captured were transferred to an aerated, on-board stock tank.

The non-wadable guidelines to derive an Index of Biotic Integrity (IBI) score and rating were developed for riverine sampling (Lyons, et al., 2001). These guidelines were used for sampling the bays on an experimental basis since the bays are still considered part of a flowing river system, but function more like the shoreline of a lake and lack the morphology changes and habitat features that would be found in a river channel. The shoreline for each bay was measured in one-mile segments to assign one-mile non-wadable stations; the first stations for each bay were selected by coin flip to randomize choice, subsequent stations were selected to expand coverage within each bay. Electrofishing commenced at one end of the station and the mini-boom shocker was run at idle speed along the shoreline for one-mile as determined by a hand-held GPS unit. Extra sampling effort beyond a simple shoreline pass was used to capture fish holding near large woody cover, aquatic vegetation, or other habitat features as they occurred along the shoreline. All the stations were surveyed as single-run fish community (all species collected) catch-per-effort only.

3.1.3 Fish Processing / Data Collection

All fish captured were identified by species, measured to the nearest tenth of an inch and weighed. Species of management interest required additional processing and data collection. Muskellunge that were captured and fit to release were scanned for a PIT tag and tagged with a PIT tag if they did not have one, a scale sample was taken for aging, and a fin clip taken for stable isotope analysis in accordance with MNDNR musky sampling guidelines for the St. Louis River. Lake sturgeon that were fit to release were also scanned for a PIT tag and tagged with a PIT tag if they did not have one, pectoral girth was measured with a soft tape, and a fin clip was retained for genetic analysis to determine genetic strain.

Any dead fish captured in the gill nets were sunk to the bottom by puncturing the air bladder. Several walleye and northern pike from Allouez Bay were retained for contaminant analysis at the request U.S. EPA in Duluth.

All fish captured in the electrofishing survey were released except for any voucher species that were retained to confirm species identification.

3.2 Data Analysis

3.2.1 Gill Netting

The gill net configuration was chosen to allow for comparison of data from each bay as well as data collected by Minnesota DNR during their annual gill net assessment of the St. Louis River which includes 21 different locations on the river, estuary, and harbor from North Bay downstream to the eastern tip of Minnesota Point. Metrics computed from the data will include number of fish per lift, number of species represented, and total weight of fish caught.

3.2.2 Shoreline Electrofishing

The number of fish collected, number of species represented, and total weight of fish caught from the shoreline electrofishing stations will be used for comparison between each bay.

3.2.3 Fish Assemblage from Gear Combined – IBI Metrics

Several states, including Wisconsin, have developed IBI scoring methods for rivers and streams but the metrics and scoring criteria are calibrated for wadable streams or non-wadable large rivers within a defined channel. Therefore, common IBI metrics and computations were used experimentally to describe the fish community. This allowed for a broad comparison among the fish assemblages in each bay. Common metrics in Wisconsin based IBI warmwater and non-wadable river rating systems as well as other agency based IBI rating systems include species richness, the number or percentage of intolerant or tolerant species sampled, number of representatives from specific taxonomic groups, trophic classification or feeding guild, and number of fish with DELT (externally visible deformities, eroded fins, lesions, or tumors). These metrics will be used to generate a percentage that will be used for the basis of comparing the fish assemblages documented in each bay.

4. Results and Discussion

4.1 Catch Per Effort

4.1.1 Gill Netting

A total of 189 fish with a combined weight of 94.5 kilograms was captured among all bays during the entire gill netting effort; 15 species were represented in the catch.

Allouez Bay yielded a total catch of 120 fish weighing 65.59 kilograms represented by 12 species with a 3-day average of 9.0 species present, 39.9 fish captured weighing 21.86 kilograms (Table 1).

Kimball's Bay yielded a total catch of 11 fish weighing 3.96 kilograms represented by 6 species, with a 3-day average of 3 species present, 3.6 fish captured weighing 1.32 kilograms (Table 2).

Pokegama Bay yielded a total catch of 58 fish weighing 24.98 kilograms represented by 9 species with a 3-day average of 8.3 species present, 19.3 fish captured weighing 8.33 kilograms (Table 3).

For comparison, the 2017 MNDNR index gill netting throughout the St. Louis River Estuary yielded a total of 578 fish with a combined weight of 275.66 kilograms for 21 net lifts; 19 species were represented in their catch. Their average catch per net lift was 7.25 fish species, 27.52 fish and weight of 13.01 kilograms.

4.1.2 Shoreline Electrofishing

A total of 177 fish with a combined weight of 30.5 kilograms were captured among all bays during the entire shoreline electrofishing effort; 22 species were represented in the catch.

Three stations sampled in Allouez Bay yielded a total catch of 53 fish weighing 14.9 kilograms represented by 15 species with an average of 7.3 fish species, 17.7 fish and weight of 4.98 kilograms per one-mile transect. (Table 6).

Two stations sampled in Kimball's Bay yielded a total catch of 56 fish weighing 4.4 kilograms represented by 12 species with an average of 8.5 species, 28 fish and weight of 2.27 kilograms per one-mile transect (Table 7).

The three stations sampled in Pokegama Bay yielded a total catch of 67 fish weighing 11.2 kilograms represented by 18 species with an average of 9 fish species, 22.3 fish and weight of 4.94 kilograms per one-mile transect (Table 8).

4.2 Fish Assemblage from Gear Combined – IBI Metrics

4.2.1 Species Richness and Taxonomic Groups

Location and size of warmwater streams are two important factors that influence fish community structure and function; these factors in turn influence fish species richness metrics (Lyons, 1992). Due to the complex nature of the St. Louis River estuary with tributary streams that drain to each of the bays and the bays themselves being part of a large warmwater estuary and tributary to Lake Superior, a wide variety of fish species may use these bays depending on life history and habitat preferences. A combined total of 88 native and non-native species of fish are considered present in the Lake Superior (Minnesota Sea Grant) basin. The presence of 69 fish species has been documented through sampling by Wisconsin DNR or other agencies (WDNR file information), so overall species richness will be based on the maximum number of fish species that have been documented in the estuary regardless of perceived abundance, residency, or known seasonal migrations.

Allouez Bay had the highest species richness with 22 fish species present, followed by Pokegama Bay with 21 species present and Kimball's Bay had 15 species present. For comparison, the fish community present in each bay will be categorized and discussed as native and non-native species, taxonomic groups present and trophic level to locate potential differences in each of the bays. Several taxonomic groups of native fish are considered indicators of environmental health due to relative intolerance of environmental degradation and need for specific habitat or diet (Lyons, 1992). Presence of individuals from three specific groups were identified for use by the wadable stream warmwater IBI; centrarchid panfish, suckers, and darters.

Native Species

Native species were well represented and represented the majority of fish captured in all three of the bays. Fourteen native species comprised 99-percent of the fish captured in Kimball's Bay, eighteen native fish species comprised 92-percent of the fish captured in Allouez Bay, and sixteen native species comprised 79-percent of the fish captured in Pokegama Bay.

Non-Native Species

Presence of non-native fish species was documented in each bay, but at different abundance levels. Four non-native species were found in Allouez Bay; Eurasian ruffe, white perch, white bass, and freshwater drum, representing eight-percent of the total catch. Five non-native species were found in Pokegama Bay; Eurasian ruffe, white perch, white bass, freshwater drum, and common carp, representing 21-percent of the total catch. One Eurasian ruffe, representing one-percent of the total catch, was captured in Kimball's Bay. A total of 18 non-native fish species have established self-sustaining populations in the Lake Superior basin (Minnesota Sea Grant) that originated from intentional introduction of gamefish for sport fishing, migration into the upper Great Lakes after natural barriers were by-passed for the shipping industry, or ballast water discharge from cargo ships arriving from overseas ports. Seventeen of these species have been documented in the St. Louis River Estuary (WDNR Fisheries Management file information).

Sunfish & Yellow Perch

Sunfish and yellow perch abundance were variable among the bays. Bluegill, rock bass, pumpkinseed, and black crappie are the sunfish (centrarchid panfish) species established in the St. Louis River Estuary (WDNR Fisheries Management file information). Yellow perch represented 74% of the panfish catch in Allouez Bay, while representing only 44% of the panfish catch in Kimballs Bay and 42% of the panfish catch in Pokegama Bay. Both bluegill and pumpkinseed abundance in the panfish catch were higher in Pokegama Bay (bluegill –21%; pumpkinseed – 16%) and Kimballs Bay (bluegill – 35%; pumpkinseed – 15%) than in Allouez Bay (bluegill – 0%; pumpkinseed – 2%). Black crappie also showed an interesting trend in relative abundance; black crappie represented only 3% of the panfish catch in Kimballs Bay, but in the two turbid bays, Allouez and Pokegama Bays, crappie represented 20% and 19% of the catch, respectively. Rock bass were the only species with similar abundance in all three bays and would appear to be relatively low; rock bass represented between two-percent (Pokegama Bay) to four-percent (Allouez Bay) of the panfish catch. The only difference in presence of these species among the three bays sampled was that bluegill were not represented in Allouez Bay.

Suckers

White suckers, shorthead redhorse and silver redhorse were all captured during the 2017 assessment, however abundance and distribution varied among bays. Allouez Bay had the highest electrofishing and gill net catch rates for all three sucker species, followed by Pokegama Bay and Kimball's Bay had the lowest catch of sucker species with only one shorthead redhorse captured with electrofishing gear.

Longnose suckers and quillback are two other sucker species considered present in the St. Louis River Estuary, however they were not captured during any of the sampling. Both species appear to have a low relative abundance in the estuary, so their absence in the survey may have been expected and is not a function of water quality in any of the bays. Longnose suckers appear to be highly migratory, although they are commonly encountered below Fond du Lac dam during WI and MN DNR spring walleye and sturgeon assessment work. This assessment work coincides the longnose sucker spawning period but adult longnose suckers appear to return to Lake Superior and are rarely encountered in estuarine fisheries surveys. Quillback are non-native to the St. Louis River and appear to have very low abundance in the river and estuary. Data from long term sampling by 1854 Treaty Authority and U. S. Geological Survey (bottom trawling), U.S. Fish and Wildlife Service (shoreline electrofishing and trap netting) and Wisconsin DNR (shoreline seining) seem to indicate very low abundance of both species in the St. Louis River Estuary.

Darters, Madtoms, and Sculpins

Despite known presence in the estuary, native small benthic dwelling fish were sparsely represented in this survey. Log perch were the only darter species captured from Allouez and Pokegama bays and no sculpins or madtoms were captured. Data from long term sampling by 1854 Treaty Authority and U. S. Geological Survey (bottom trawling), U.S. Fish and Wildlife Service (shoreline electrofishing and trap netting) and Wisconsin DNR (shoreline seining) suggests that species like Johnny darter, log perch and tadpole madtoms should have been encountered at a higher rate what was captured in our sampling.

Two species of sculpins are considered present in the St. Louis River Estuary. These same data sources suggest low abundance and a limited range in the St. Louis River Estuary for sculpins with the only documented occurrences at areas near the entries to the harbor, likely an indicator of thermal preferences. Stonecats are also considered present, but not have been captured in any of the long-term agency sampling efforts. The absence of stonecats in any of this sampling may be linked to their distribution in the St. Louis River Estuary, most likely limited to the upper most reaches of the Estuary because of habitat preferences for moderate to fast current and gravel and rubble substrates.

The absence of small benthic dwelling fish species or groups in our sampling is probably not linked to water quality or turbid conditions, rather their absence is most likely a result a combination of being missed by the gear used because of their relatively small size or the respective habitat or thermal preferences for some species. Use of different gear like shoreline seining or fine-mesh fyke nets could increase capture of small benthic dwelling species and could also increase the catch of juvenile gamefish, minnows or other small non-game fish species.

4.2.2 Intolerant Species

Presence of intolerant fish is considered an indicator of good water quality, but as degradation of habitat and water quality occur, species diversity declines and intolerant fish species are replaced by species tolerant of degraded conditions (Lyons, 1992). Allouez and Kimball's bays each had four intolerant species represented by muskellunge, smallmouth bass, rock bass, and spottail shiner. Pokegama Bay had three of those same four species; muskellunge was the intolerant species not captured, however only one individual was captured in Allouez Bay and two were captured in Kimball's Bay which may suggest overall low abundance, not necessarily linked to excessive sedimentation and nutrients. Relatively low percentages of the total catch in each bay were species intolerant of low dissolved oxygen or degraded habitat: six percent of the species captured in Allouez Bay, seven percent in Kimball's Bay, and three percent in Pokegama Bay were considered intolerant.

4.2.3 Tolerant Species

Presence of fish species considered tolerant of either low dissolved oxygen or degraded habitat was also documented in each of the bays. Although native to the St. Louis River estuary, white suckers are considered tolerant of disturbed habitat and were found in Pokegama and Allouez Bays, but not in Kimball's Bay. White suckers comprised nine percent of the forage fish captured in Pokegama Bay and seven percent of the forage fish captured in Allouez Bay; white suckers comprised three percent or less of the fish assemblage in both bays. If chronic low dissolved oxygen levels were a problem in either of these bays, other low dissolved oxygen tolerant fish species like common carp, central mudminnows, fathead minnows and yellow or black bullheads would have been expected in higher abundance.

Only two species tolerant of low dissolved oxygen conditions, golden shiners and common carp, were captured in all the sampling. Golden shiners were captured in both Pokegama and Kimball's Bays and common carp were captured in Pokegama Bay. Low dissolved oxygen tolerant forage fish species represented 82-percent of the forage fish or 20.9-percent of the fish assemblage in Kimball's Bay and 27-percent of the forage fish or 9.6-percent of the fish assemblage in Pokegama Bay. The proportion of low dissolved oxygen tolerant fish would appear high for both Kimball's and Pokegama bays, however this is due primarily to the high catch of golden shiners.

4.2.4 Turbidity Tolerance

Species considered more tolerant of turbid conditions, either by sustained occurrence across a gradient of turbidity levels or no decline in relative abundance at turbidity levels above 50 NTU, represented a larger percent of the fish community in both Allouez (55-percent) and Pokegama Bay (42-percent), compared to Kimball's Bay (24-percent). Including both native and non-native fish species, fish considered at least moderately tolerant of turbid conditions represented 94-percent of the fish captured in Pokegama Bay, 85-percent of the fish captured in Allouez Bay and 97-percent of the fish captured in Kimball's Bay. There were no fish considered intolerant (found at turbidity levels <10 NTU) of turbidity found in any of the

bays. The limited data collected in this survey is consistent with research on coastal wetlands throughout the Great Lakes (Trebitz, et al., 2007, Minns, et al., 1994), where turbidity levels may directly influence the fish community present and abundance of both native and non-native species in areas of the St. Louis River Estuary. The extent to which turbidity is a function of natural vs. anthropogenic sources would require additional surveys. Focus would be on sampling throughout the St. Louis River Estuary to determine the abundance of turbidity tolerant fish species in less turbid areas relative to Allouez and Pokegama bays.

4.2.5 Trophic Level / Feeding Guild

Presence and abundance of primarily insectivorous or carnivorous fishes is often indicative of a high-quality stream environment. Omnivores can subsist on a relatively broad range of food items and are relatively insensitive to changes in the food base of a stream caused by environmental degradation (Lyons, 1992).

White suckers were the only omnivore species captured in Allouez Bay and they represented 3% of the fish assemblage. Golden shiners were captured in Kimball's Bay and were the only omnivore species captured, representing 21% of the fish assemblage. Pokegama Bay had 3 omnivore species present; golden shiners, white suckers and common carp, representing 12% of the fish assemblage.

Top carnivores and insectivores were well represented in the Allouez and Pokegama Bays. In Allouez Bay, 47% of the fish assemblage was represented by carnivore species and the remaining 50% of the fish assemblage was represented by insectivores. In Pokegama Bay, 38% of the fish assemblage was represented by carnivores and 50% of the fish assemblage was represented by insectivores. Despite capture of fewer fish in Kimball's Bay, insectivore and carnivore species were still represented. Twenty-five percent of the fish assemblage were carnivores and 54% of the fish assemblage were insectivores.

4.2.6 Deformities, Eroded Fins, Lesions or Tumors (DELT)

The inclusion of a metric for deformities, eroded fins, lesions and tumors was used based on it being one of the metrics for both wadable and non-wadable stream IBI in Wisconsin. This should not be confused with the tumors and deformities in fish BUI that uses internal indicators as examined by U.S. Environmental Protection Agency. Rather, this metric was retained for IBI ratings as an added measure of sensitivity to untreated point source discharge and highly degraded sites. Only one fish, a northern pike with an open lesion, was captured in Allouez Bay representing 0.6-percent of the Allouez Bay catch with a DELT feature; no fish with DELT features were captured in either Pokegama or Kimball's bays. The overall lack of visible DELT features on fish from the three bays suggests neither large-scale industrial or sewage discharge nor excessive sediment and nutrients to these waters is a concern.

5. Conclusion

Erosion of red clay sediment within the watersheds that drain to the St. Louis River Estuary, including Allouez and Pokegama bays has led to turbid conditions in both the tributary streams and their receiving bays. Historical data inferred from sediment core sampling suggests that turbid conditions may be inherent to these watersheds, but sediment loading has increased because of human settlement, development, and changes in land use within those watersheds. Despite turbid conditions that may lead to the perception of poor water quality or habitat, locally popular and desirable sport fish species like walleye, northern pike, black crappie, and yellow perch were well represented in both Allouez and Pokegama Bays. Furthermore, both bays are known locally for seasonal fishing opportunities for several of these species, specifically, late spring/summer walleye fishing in Allouez Bay and winter/early spring black crappie and yellow perch fishing in Pokegama Bay. Other species of interest to anglers and state fisheries management agencies including lake sturgeon, muskellunge, bluegill and channel catfish were also found in these bays. Increased turbidity in Allouez and Pokegama Bays may influence the presence or abundance of specific species, however, it has not diminished the fishery value or eliminated desirable gamefish species from these areas.

6. Literature and Data Cited

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Table 1. Gill net catch from Allouez Bay

Wisconsin Department of Natural Resources

NON-GAME FISH NETTING DATA COLLECTION SHEET

Form 3600-NON Rev. 6-94

Lake: St. Louis River – Allouez Bay MWB Code: 2751220 Dates: 7/24 - 7/27/17 County: Douglas Collecter: Piszczek, et al

Target Fish: All Species Survey Type: Fish Community/Inventory Mark Given: None Adverse Conditions: None

Station: ¾" Mesh - N46.68870, W-91.99805, 2" Mesh – N46.68837, W-91.99805 Net Type: MNDNR Lake Survey Gill Net

Length/Frame: 250'x6'- 5 Panels Bar Mesh: 50' panels (¾", 1", 1 ½", 1 ½", 2") Color: White Mesh Type: Nylon Net Nights: 3

Species:	7/25/2017		7/26/2017		7/27/2017		Average Per Net Lift	
	# Caught	Weight (g)	# Caught	Weight (g)	# Caught	Weight (g)	# Caught	Weight (g)
Black Crappie	2	624	1	624	3	850	2.00	699.33
Yellow Perch	7	1,163	9	1,134	12	2,098	9.33	1,465.00
Channel Catfish	12	2,240	14	3,794	9	1,560	11.66	2,531.33
Shorthead Redhorse	2	1,190	3	1,616	3	1,247	2.66	1,351.00
Eurasian Ruffe	2	142	0	0	0	0	0.66	47.33
Walleye	9	6,010	7	6,322	4	3,913	6.66	5,415.00
Northern Pike	1	992	2	1,530	3	2,438	2.00	1,653.33
Silver Redhorse	2	2,013	0	0	0	0	0.66	671.00
Lake Sturgeon	0	0	1	7,824	1	11,907	0.66	6,577.00
Freshwater Drum	0	0	1	1,134	1	794	0.66	642.66
White Bass	0	0	3	426	2	284	1.66	236.66
White Sucker	0	0	4	1,730	0	0	1.33	576.66
Totals:	37	14,374	45	26,134	38	25,091	39.94	21,866.30

Comments:

Table 2. Gill net catch from Kimballs Bay.

Wisconsin Department of Natural Resources

NON-GAME FISH NETTING DATA COLLECTION SHEET

Form 3600-NON Rev. 6-94

Lake: St. Louis River – Kimballs Bay MWB Code: 2843900 Dates: 7/24 - 7/27/17 County: Douglas Collecter: Piszczek, et al

Target Fish: All Species Survey Type: Fish Community/Inventory Mark Given: None Adverse Conditions: None

Station: ¾" Mesh - N46.69472, W-92.15717, 2" Mesh – N46.69422, W-92.15652 Net Type: MNDNR Lake Survey Gill Net

Length/Frame: 250'x6' - 5 Panels Bar Mesh: 50' panels (¾", 1", 1 ½", 1 ¾", 2") Color: White Mesh Type: Nylon Net Nights: 3

Species	7/25/2017		7/26/2017		7/27/2017		Average Per Net Lift	
	# Caught	Weight (g)	# Caught	Weight (g)	# Caught	Weight (g)	# Caught	Weight (g)
Brown Bullhead	1	255	0	0	0	0	0.33	85.00
Walleye	1	227	1	425	0	0	0.66	217.33
Northern Pike	1	850	0	0	1	1,021	0.66	623.66
Yellow Perch	0	0	1	85	3	312	1.33	132.33
Eurasian Ruffe	0	0	1	57	0	0	0.33	19.00
Smallmouth Bass	0	0	0	0	1	737	0.33	245.66
Totals:	3	1,332	3	567	5	2,070	3.64	1,322.99

Comments:

Table 3. Gill net catch from Pokegama Bay.

Wisconsin Department of Natural Resources

NON-GAME FISH NETTING DATA COLLECTION SHEET

Form 3600-NON Rev. 6-94

Lake: St. Louis River – Pokegama Bay MWB Code: 2844000 Dates: 7/24 - 7/27/17 County: Douglas Collecter: Piszczek, et al.

Target Fish: All Species Survey Type: Fish Community/Inventory Mark Given: None Adverse Conditions: None

Station: ¾" Mesh - N46.68982, W-92.16658, 2" Mesh – N46.68920, W-92.16579 Net Type: MNDNR Lake Survey Gill Net

Length/Frame: 250'x6' - 5 Panels Bar Mesh: 50' panels (¾", 1", 1 ¼", 1 ½", 2") Color: White Mesh Type: Nylon Net Nights: 3

Species:	7/25/2017		7/26/2017		7/27/2017		Average Per Net Lift	
	# Caught	Weight (g)	# Caught	Weight (g)	# Caught	Weight (g)	# Caught	Weight (g)
Yellow Perch	1	170	2	227	3	311	2.00	236
Channel Catfish	3	2,041	2	624	3	1,615	2.66	1,426.66
Walleye	5	2,537	6	4,877	4	1,588	5.00	3,000.66
Golden Shiner	3	170	3	198	2	113	2.66	160.33
Eurasian Ruffe	1	28	1	113	1	28	1	56.33
Northern Pike	2	1,190	2	1,984	2	4,706	2	2,626.66
White Bass	1	113	1	142	2	538	1.33	264.33
White Sucker	0	0	1	113	1	283	.66	132.00
Black Crappie	0	0	3	424	3	851	2.00	425.00
Totals:	16	6,249	21	8,702	21	10,033	19.31	8,327.97

Comments:

Table 4. Gill net catch per effort summary for AOC bays and MNDNR index gill netting on St Louis River Estuary.

	Allouez Bay		Pokegama Bay		Kimball's Bay		MNDNR Index Nets	
	Total Fish	Avg per set	Total Fish	Avg per set	Total Fish	Avg per set	Total Fish	Avg per set
Alewife	0.00	0.00	0.00	0.00	0.00	0.00	10.00	0.48
Black Crappie	6.00	2.00	6.00	2.00	0.00	0.00	14.00	0.67
Bluegill	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.05
Brown Bullhead	0.00	0.00	0.00	0.00	1.00	0.33	0.00	0.00
Channel Catfish	35.00	11.66	8.00	2.66	0.00	0.00	99.00	4.71
Freshwater Drum	2.00	0.66	0.00	0.00	0.00	0.00	0.00	0.00
Golden Shiner	0.00	0.00	8.00	2.66	0.00	0.00	0.00	0.00
Lake Sturgeon	2.00	0.66	0.00	0.00	0.00	0.00	3.00	0.14
Muskellunge	0.00	0.00	0.00	0.00	0.00	0.00	3.00	0.14
Northern Pike	6.00	2.00	6.00	2.00	2.00	0.66	35.00	1.67
Pumpkinseed	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.05
River/Eurasian Ruffe	2.00	0.66	3.00	1.00	1.00	0.33	21.00	1.00
Rock Bass	0.00	0.00	0.00	0.00	0.00	0.00	23.00	1.10
Shorthead Redhorse	8.00	2.66	0.00	0.00	0.00	0.00	62.00	2.95
Silver Redhorse	2.00	0.66	0.00	0.00	0.00	0.00	4.00	0.19
Smallmouth Bass	0.00	0.00	0.00	0.00	1.00	0.33	2.00	0.10
Walleye	20.00	6.66	15.00	5.00	2.00	0.66	203.00	9.67
White Bass	5.00	1.66	4.00	1.33	0.00	0.00	2.00	0.10
White Perch	0.00	0.00	0.00	0.00	0.00	0.00	13.00	0.62
White Sucker	4.00	1.33	2.00	0.66	0.00	0.00	30.00	1.43
Yellow Bullhead	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.05
Yellow Perch	28.00	9.33	6.00	2.00	4.00	1.33	51.00	2.43
Total Fish Per Set		39.94		19.31		3.64		27.55

Table 5. Gill net catch by weight summary for AOC bays and MNDNR index gill netting on St Louis River Estuary

	Allouez Bay		Pokegama Bay		Kimball's Bay		MNDNR Index Nets	
	Total Weight (lb)	Pounds Per Set	Total Weight (lb)	Pounds Per Set	Total Weight (lb)	Pounds Per Set	Total Weight (lb)	Pounds Per Set
Alewife	0.00	0.00	0.00	0.00	0.00	0.00	1.24	0.06
Black Crappie	4.63	1.54	2.81	0.94	0.00	0.00	4.43	0.21
Bluegill	0.00	0.00	0.00	0.00	0.00	0.00	0.92	0.04
Brown Bullhead	0.00	0.00	0.00	0.00	0.56	0.19	0.00	0.00
Channel Catfish	16.74	5.58	9.44	3.15	0.00	0.00	78.53	3.74
Freshwater Drum	4.25	1.42	0.00	0.00	0.00	0.00	0.00	0.00
Golden Shiner	0.00	0.00	1.06	0.35	0.00	0.00	0.00	0.00
Lake Sturgeon	43.50	14.50	0.00	0.00	0.00	0.00	30.78	1.47
Muskellunge	0.00	0.00	0.00	0.00	0.00	0.00	13.51	0.64
Northern Pike	10.93	3.64	17.37	5.79	4.12	1.37	85.49	4.07
Pumpkinseed	0.00	0.00	0.00	0.00	0.00	0.00	0.26	0.01
River/Eurasian Ruffe	0.31	0.10	0.37	0.12	0.13	0.04	1.79	0.09
Rock Bass	0.00	0.00	0.00	0.00	0.00	0.00	8.86	0.42
Shorthead Redhorse	8.94	2.98	0.00	0.00	0.00	0.00	118.39	5.67
Silver Redhorse	4.44	1.48	0.00	0.00	0.00	0.00	12.00	0.57
Smallmouth Bass	0.00	0.00	0.00	0.00	1.62	0.54	2.55	0.12
Walleye	35.81	11.94	19.85	6.62	1.44	0.48	202.29	9.63
White Bass	1.57	0.52	1.75	0.58	0.00	0.00	1.18	0.06
White Perch	0.00	0.00	0.00	0.00	0.00	0.00	5.59	0.27
White Sucker	3.81	1.27	0.87	0.29	0.00	0.00	26.97	1.28
Yellow Bullhead	0.00	0.00	0.00	0.00	0.00	0.00	0.58	0.03
Yellow Perch	9.69	3.23	1.56	0.52	0.88	0.29	12.38	0.59
Total Pounds Fish/Set		48.21		18.36		2.92		28.97

Table 6. Electrofishing catch from Allouez Bay.

NON-WADABLE RIVER FISH COMMUNITY SUMMARY

WATERS: Allouez Bay
WBIC: 2751220 (considered part of Lk Superior)
COUNTY: Douglas
GEAR: 240V 3000W DCP Mini-Boom
16 Droppers, 1 Dipper

STATION:	DISTANCE SHOCKED (miles):
1	1.00
2	1.00
3	1.00

TAXONOMIC FAMILY / FISH SPECIES	Station 1 Total Caught	Station 1 Total Weight	Station 2 Total Caught	Station 2 Total Weight	Station 3 Total Caught	Station 3 Total Weight	Average Caught	Average Weight
Esocidae								
MUSKELLUNGE	0	0	1	124	0	0	0.3	41.3
NORTHERN PIKE	3	2059	0	0	1	2	1.3	687.0
Percidae								
WALLEYE	0	0	0	0	0	0	0.0	0.0
YELLOW PERCH	5	122	3	97.5	1	104	3.0	107.8
JOHNNY DARTER	0	0	0	0	0	0	0.0	0.0
LOG PERCH	0	0	2	7	0	0	0.7	2.3
Centrarchidae								
SM BASS	0	0	1	76	0	0	0.3	25.3
LM BASS	0	0	0	0	0	0	0.0	0.0
BLACK CRAPPIE	0	0	0	0	0	0	0.0	0.0
BLUEGILL	0	0	0	0	0	0	0.0	0.0
PUMPKINSEED	1	158	0	0	0	0	0.3	52.7
ROCK BASS	2	532	0	0	0	0	0.7	177.3
Cyprinidae								
EMERALD SHINER	1	5	6	25	0	0	2.3	10.0
COMMON SHINER	0	0	0	0	0	0	0.0	0.0
GOLDEN SHINER	0	0	0	0	0	0	0.0	0.0
SPOTTAIL SHINER	1	2	6	25	0	0	2.3	9.0
BLUNTNOSE MINNOW	0	0	0	0	0	0	0.0	0.0
COMMON CARP	0	0	0	0	0	0	0.0	0.0
HORNYHEAD CHUB	0	0	0	0	0	0	0.0	0.0
MIMIC SHINER	0	0	4	9.5	0	0	1.3	3.2
Ictaluridae								
CHANNEL CATFISH	0	0	0	0	0	0	0.0	0.0
Catostomidae								
WHITE SUCKER	1	28	0	0	0	0	0.3	9.3
SILVER REDHORSE	3	5641	2	204	0	0	1.7	1948.3
SHORTHEAD REDHORSE	3	2495	0	0	1	1301	1.3	1265.3
Moronidae								
WHITE PERCH	0	0	3	136	0	0	1.0	45.3
WHITE BASS	0	0	0	0	0	0	0.0	0.0
Scianidae								
FRESHWATER DRUM	0	0	0	0	2	1779	0.7	593.0
Station Total	20	11042	28	704	5	3186	17.7	4977.3

COMMENTS: Weights recorded in grams

Table 7. Electrofishing catch from Kimball's Bay.

NON-WADABLE RIVER FISH COMMUNITY SUMMARY

WATERS: Kimball's Bay
WBIC: 2843900
COUNTY: Douglas
GEAR: 240V 3000W DCP Mini-Boom
16 Droppers, 1 Dipper

STATION:	DISTANCE SHOCKED (miles):
1	1.00
2	1.00

TAXONOMIC FAMILY / FISH SPECIES	Station 1 Total Caught	Station 1 Total Weight	Station 2 Total Caught	Station 2 Total Weight			Average Caught	Average Weight
Esocidae								
MUSKELLUNGE	2	456	0	0			1	228
NORTHERN PIKE	3	1493	0	0			1.5	746.5
Percidae								
WALLEYE	1	385	0	0			0.5	192.5
YELLOW PERCH	5	71	6	134			5.5	102.5
JOHNNY DARTER	0	0	0	0			0	0
LOG PERCH	0	0	0	0			0	0
Centrarchidae								
SM BASS	0	0	0	0			0	0
LM BASS	3	527	1	1			2	264
BLACK CRAPPIE	0	0	1	272			0.5	136
BLUEGILL	11	205	1	122			6	163.5
PUMPKINSEED	3	53	2	128			2.5	90.5
ROCK BASS	1	180	0	0			0.5	90
Cyprinidae								
EMERALD SHINER	0	0	0	0			0	0
COMMON SHINER	0	0	0	0			0	0
GOLDEN SHINER	3	34	11	115			7	74.5
SPOTTAIL SHINER	1	0	0	0			0.5	0
BLUNTNOSE MINNOW	0	0	0	0			0	0
COMMON CARP	0	0	0	0			0	0
HORNYHEAD CHUB	0	0	0	0			0	0
Ictaluridae								
CHANNEL CATFISH	0	0	0	0			0	0
Catostomidae								
WHITE SUCKER	0	0	0	0			0	0
SILVER REDHORSE	0	0	0	0			0	0
SHORTHEAD REDHORSE	0	0	1	369			0.5	184.5
Moronidae								
WHITE PERCH	0	0	0	0			0	0
WHITE BASS	0	0	0	0			0	0
Scianidae								
FRESHWATER DRUM	0	0	0	0			0	0
Station Total	33	3404	23	1141			28	2272.5

COMMENTS: Weights recorded in grams

Table 8. Electrofishing catch from Pokegama Bay.

NON-WADABLE RIVER FISH COMMUNITY SUMMARY

WATERS: Pokegama Bay
WBIC: 2844000
COUNTY: Douglas
GEAR: 240V 3000W DCP Mini-Boom
16 Droppers, 1 Dipper

STATION:	DISTANCE SHOCKED (miles):
1	1.00
2	1.00
3	1.00

TAXONOMIC FAMILY / FISH SPECIES	Station 1 Total Catch	Station 1 Total Weight	Station 2 Total Catch	Station 2 Total Weight	Station 3 Total Catch	Station 3 Total Weight	Average Captured	Average Weight
Esocidae								
MUSKELLUNGE	0	0	0	0	0	0	0.0	0.0
NORTHERN PIKE	0	0	0	0	0	0	0.0	0.0
Percidae								
WALLEYE	0	0	0	0	1	3	0.3	1.0
YELLOW PERCH	3	150	7	367	2	78	4.0	198.3
JOHNNY DARTER	0	0	0	0	0	0	0.0	0.0
LOG PERCH	0	0	3	4.5	0	0	1.0	1.5
Centrarchidae								
SM BASS	0	0	0	0	1	29	0.3	9.7
LM BASS	0	0	2	3	0	0	0.7	1.0
BLACK CRAPPIE	1	178	1	355	0	0	0.7	177.7
BLUEGILL	3	198	5	427	1	32	3.0	219.0
PUMPKINSEED	6	323	1	63	0	0	2.3	128.7
ROCK BASS	1	216	0	0	0	0	0.3	72.0
Cyprinidae								
EMERALD SHINER	0	0	0	0	1	4	0.3	1.3
COMMON SHINER	0	0	0	0	0	0	0.0	0.0
GOLDEN SHINER	0	0	2	14.5	0	0	0.7	4.8
SPOTTAIL SHINER	0	0	0	0	2	10	0.7	3.3
BLUNTNOSE MINNOW	0	0	0	0	0	0	0.0	0.0
COMMON CARP	0	0	2	2438	0	0	0.7	812.7
HORNYHEAD CHUB	0	0	0	0	0	0	0.0	0.0
Ictaluridae								
CHANNEL CATFISH	0	0	0	0	0	0	0.0	0.0
Catostomidae								
WHITE SUCKER	1	1190	0	0	0	0	0.3	396.7
SILVER REDHORSE	2	2834	1	1814	1	1248	1.3	1965.3
SHORTHEAD REDHORSE	0	0	0	0	0	0	0.0	0.0
Moronidae								
WHITE PERCH	1	62	0	0	14	1936	5.0	666.0
WHITE BASS	0	0	0	0	1	58	0.3	19.3
Scianidae								
FRESHWATER DRUM	1	794	0	0	0	0	0.3	264.7
Station Total	19	5945	24	5486	24	3398	22.3	4943

COMMENTS: Weights recorded in grams

Table 9. Fish assemblage found in each bay based on combined catch from electrofishing and gill netting

TAXONOMIC FAMILY FISH SPECIES	Origin / Thermal Guild	Tolerance	Turbidity Tolerance	Feeding	Habitat	Spawning Type	Allouez Bay	Kimballs Bay	Pokegama Bay
Esocidae									
MUSKELLUNGE	NCL	I	LD-MT	TC	O	O	1	2	0
NORTHERN PIKE	NEU	M	MT	TC	O	O	10	5	6
Percidae									
WALLEYE	NEU	M	MT	TC	O	SL	20	3	16
YELLOW PERCH	NEU	M	T-PA	IN	O	O	37	15	18
LOG PERCH	NEU	M	MT	IN	O	SL	2	0	3
EURASIAN RUFFE	EEU	M	MI	IN	O	O	2	1	3
Centrarchidae									
SM BASS	NEU	I	MT	TC	O	O	1	1	1
LM BASS	NEU	M	MT	TC	O	O	0	4	2
BLACK CRAPPIE	NEU	M	MT	TC	O	O	10	1	8
BLUEGILL	NEU	M	MT	IN	O	O	0	12	9
PUMPKINSEED	NEU	M	MT	IN	O	O	1	5	7
ROCK BASS	NEU	I	MT	TC	O	O	2	1	1
Cyprinidae									
EMERALD SHINER	NEU	M	T-PA	IN	L	SL	7	0	1
GOLDEN SHINER	NEU	T-DO	MT	OM	O	O	0	14	10
SPOTTAIL SHINER	NEU	I	T-PA	IN	L	O	7	1	2
COMMON CARP	EEU	T-DO	T-PA	OM	B	O	0	0	2
MIMIC SHINER	NEU	M	MI	IN	R	O	4	0	0
Ictaluridae									
CHANNEL CATFISH	NEU	M	T-NA	TC	B	O	35	0	8
BROWN BULLHEAD	NEU	M	MT	IN	B	O	1	1	0
Catostomidae									
WHITE SUCKER	NEU	T-HB	MT	OM	O	SL	5	0	3
SILVER REDHORSE	NEU	M	MI	IN	R	SL	7	0	4
SHORTHEAD REDHORSE	NEU	M	MI	IN	O	SL	12	1	0
Moronidae									
WHITE PERCH	EEU	M	T-NA	TC	O	O	3	0	15
WHITE BASS*	EEU	M	T-PA	TC	L	O	5	0	5
Scianidae									
FRESHWATER DRUM*	EEU	M	T-NA	IN	L	O	4	0	1
Acipenseridae									
LAKE STURGEON	NEU	M	NC	IN	B	SL	2	0	0

*Native to Wisconsin, but not Lake Superior basin

Origin / Thermal Guild - N - Native, E - Exotic / EU - Eurythermic, CL - Stenothermal Coolwater, CD - Stenothermal Coldwater

Tolerance - I-Intolerant, M-Intermediate, T-DO -Low Dissolved Oxygen Tolerant, T-HB - Degraded Habitat Tolerant

Turbidity Tolerance - T-PA - tolerant; present & abundant, T-NA - tolerant, never abundant, but always present, MT - moderate tolerance - absent or declining at more turbid sites, MI - moderate intolerance - mostly at <25 NTU, I - Intolerant - mostly at <10 NTU, LD-MT - little data - suggest moderately tolerant, LD - little data, suggest not intolerant, ID - insufficient data to determine tolerance , NC - no classification (based on Trebitz et al., 2007.)

Feeding - Fi-Filter Feeder, Ge-Genral Feeder, He-Herbivore, Pa-Parasitic, O-Omnivore, I-Insectivore, TC-Top Carnivore,

Habitat - L-Large River, R-Riverine, O-Other, B-Benthic, Spawning Habitat - SL-Simple Lithophilous, O-Other

Table 10. Computed numbers and percentages for the fish assemblage in each bay based on common Wisconsin stream IBI metrics.

	Allouez Bay	Kimballs Bay	Pokegema Bay
total # of fish	178	67	125
total # of native spp.	18	14	16
total # of non-native spp.	4	1	5
Species Groups			
total # of darter, madtom, and sculpin spp.	1	0	1
total # of sucker spp.	3	1	2
total # of sunfish spp. + yellow perch	4	5	5
total # of intolerant spp.	4	4	3
total # of tolerant fish	5	14	15
total # of low DO tolerant species	0	1	2
total # of forage fish tolerant to low DO	0	14	12
total # of disturbed habitat species	1	0	1
total # of fish tolerant to disturbed habitat	5	0	3
total # tolerant to turbidity	98	16	52
total # of omnivores	5	14	15
total # of insectivores	88	36	63
total # of top carnivores	84	17	47
total # with DELT	1	0	0
% of posible fish spp present	32	22	30
% non-native spp.	8	1	21
% of intolerant spp.	6	7	3
% of tolerant spp.	3	21	12
% forage fish tolerant to low DO	0	82	27
% of forage fish tolerant to disturbed habitat	9	0	7
% of forage fish tolerant to disturbed habitat AND low D	9	82	34
% tolerant to turbidity	55	24	42
% of omnivores	3	21	12
% of insectivores	50	54	50
% of carnivores	47	25	38
% with DELT	0.6	0	0

Appendix I.
Electrofishing and Netting Station Maps

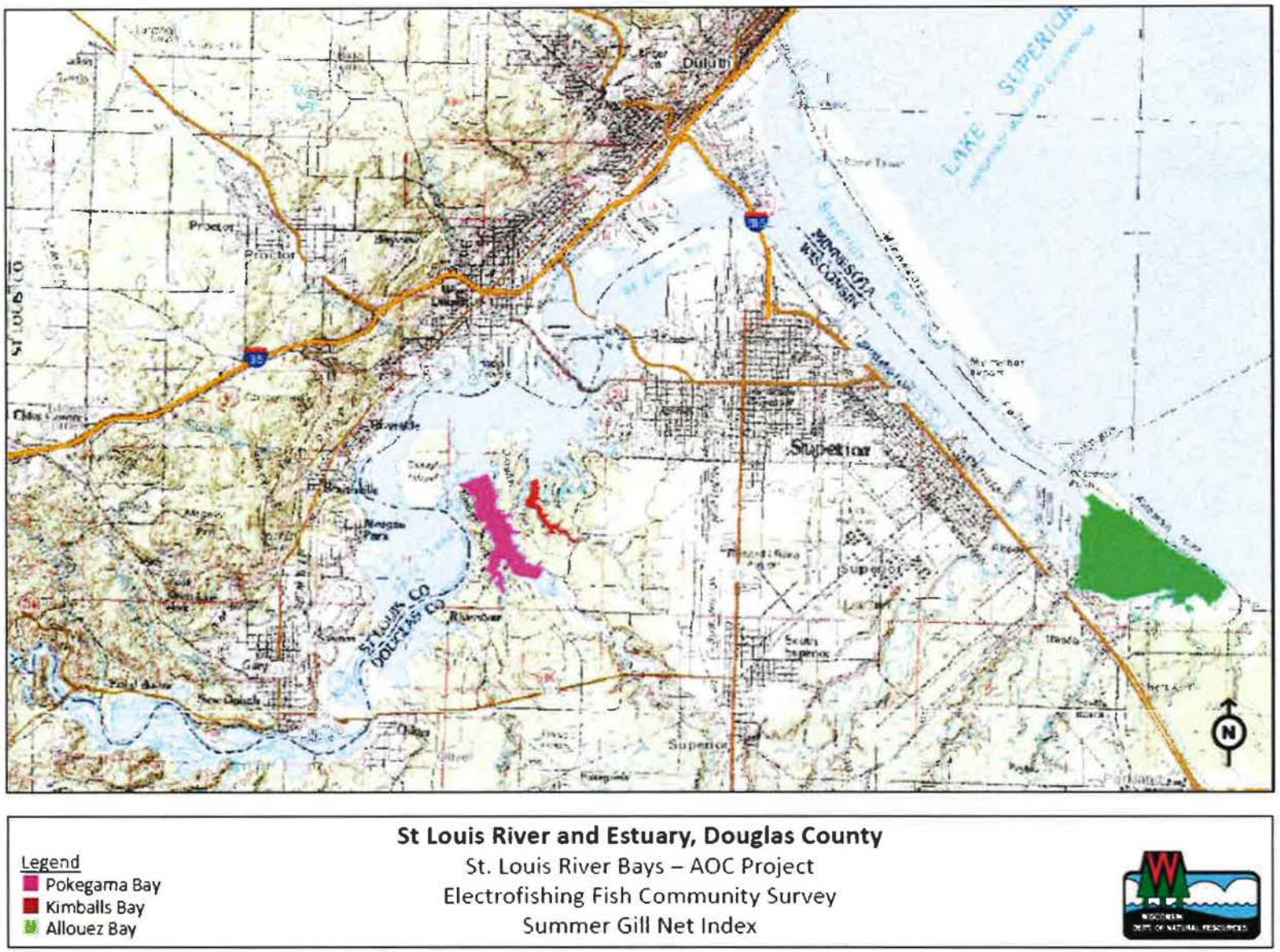


Figure 1. Map of St. Louis River and Estuary with project areas highlighted.

Figure 2. Map of gill net locations and electrofishing stations for Allouez Bay

Figure 3. Map of gill net locations and electrofishing stations for Kimball's Bay

Figure 4. Map of gill net locations and electrofishing stations for Pokegama Bay

Appendix 4

Comparative Analysis of the Nemadji River Watershed In the Lake Superior Basin (Pertains to management action 6.05)

Final Report Community GIS Services, Inc.
Comparative Analysis of The Nemadji River Watershed in the
Lake Superior Basin

Our goal of the project was to deliver a product that local, state, and federal governmental entities could use to identify small hydrological units within the Nemadji River Watershed that are exceeding forty percent open land status. For this project our definition of open land class was timber that is 15 years or less in age, agricultural land, and urban land types.

Our first task was to digitize the 0 – 15 year timber age class within the watershed. We accomplished this task by obtaining 16 years of USGS Landsat 5 and Landsat 8 satellite imagery covering the entire watershed. Covering the entire Nemadji River Watershed with Landsat scenes often took two adjacent image scenes (Row 27, Path 28 and Row 26, Path 27). For gathering 16 years of these images, Community GIS Services needed to obtain 32 images or scenes of Landsat data that were acquired or captured between the months of May and September. Scenes captured within this time period revealed peak leaf chlorophyll amounts. Community GIS Services checked for proper spatial control of the Landsat scenes by matching them with previously rectified scenes. In ArcGIS software the technician imported the native Landsat format to tif file format and clipped the imagery to the watershed bounds. The clipped Landsat images were given a band combination of 4 (Near Infrared),5 (Short-wave Infrared),3 (visual red) for Landsat 5 scenes and for Landsat 8 scenes a band combination of 5 (Near Infrared), 6 (Short Wave Infrared 1), 3 (visual red). These band combinations along with the use of high resolution aerial imagery, 1:24,000 scale digital USGS Topographic maps and wetland inventory data allowed us to distinguish between timber harvests / agricultural lands and wetlands.

Community GIS Services digitized each timber harvest occurring within the watershed by utilizing ArcGIS software with the 16 years of layered Landsat scenes as a basis and watershed boundaries and section line boundaries as secondary layers. The GIS technician would start on one side of the watershed and turn each year of the Landsat imagery on for each section and looked for changes to the landscape that indicated a timber harvest. If a harvest was found, it was digitized and attributed with the year in which it occurred. This process continued for the entire watershed on a section-by-section basis.

The next task was to digitize the urban and agricultural lands within the watershed. Since the timber harvests were already digitized, the technician could use 2013 aerial imagery from USDA and Douglas County Wisconsin to identify and digitize the boundaries of these land features on-screen using ArcGIS software.

Our third task was to digitize the small hydrological units that would be used to calculate the percentage of open land. Community GIS Services had previously

contracted hydrologist Dr. E. Sandy Verry to determine the extents of the hydrological units. This was completed by Community GIS Services printing in large format the 1:24,000 USGS digital topographic maps with USFS HUC-12 watershed boundaries of the Nemadji River Watershed and Dr. Verry cutting the HUC-12 delineations into smaller hydrological units with pencil. His criteria for cutting the watersheds were stream sinuosity, stream slope, watershed contour, and personal knowledge of the region. Community GIS Services delineated the outlines of the hydrological units on the maps and digitized onscreen using ArcGIS software. These digitized hydrological units were attributed and printed again for Dr. Verry to verify. Finally, Community GIS Services combined the harvests, agriculture, and urban land features into one GIS layer and performed an Identity function on the open land class layer and the digitized hydrological units. The identity function combined both the polygon features of the land classes and the hydrological units with the attribute records of both GIS layers. Community GIS Services was then able to calculate total percent open based on 0 – 15 year timber harvests, agriculture and urban land, and a combination of both land classes per hydrological units within the Nemadji River Watershed. Additionally, we incorporated the previous open land study data of 2003 and 2008 to measure the amount of increase or decrease of cumulative open lands within each subwatershed.

After checking and rechecking our data for accuracy and completeness Community GIS services created small format map layouts in digital map formats for replication. All data created and used for this project along with map images were copied to our web server and are available to local, state, tribal, and federal governmental entities that requested a copy.

A Summary of results:
Watershed Size: 280,787 Acres
Number of subwatersheds delineated for the study: 171

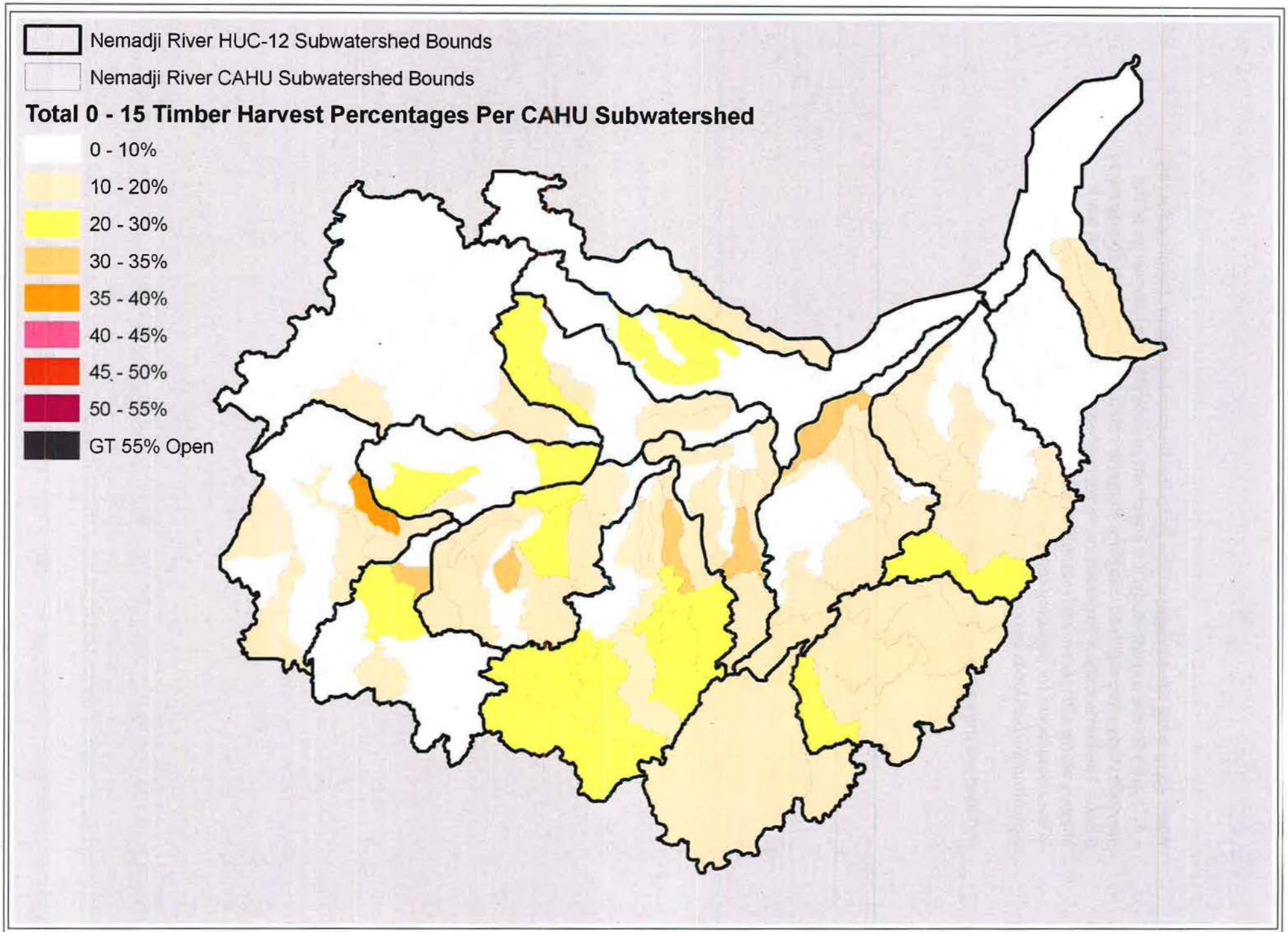
Number of subwatersheds below 40% open for current study: 125
Number of subwatersheds between 40 – 50% open for current study: 23
Number of subwatersheds between 50 – 60% open for current study: 18
Number of subwatersheds above 60% open for current study: 5

Number of subwatersheds below 40% open for previous (86' – 02') study: 136
Number of subwatersheds between 40 – 50% open for previous (86' – 02') study: 20
Number of subwatersheds between 50 – 60% open for previous (86' – 02')study: 12
Number of subwatersheds above 60% open for previous (86' – 02') study: 3

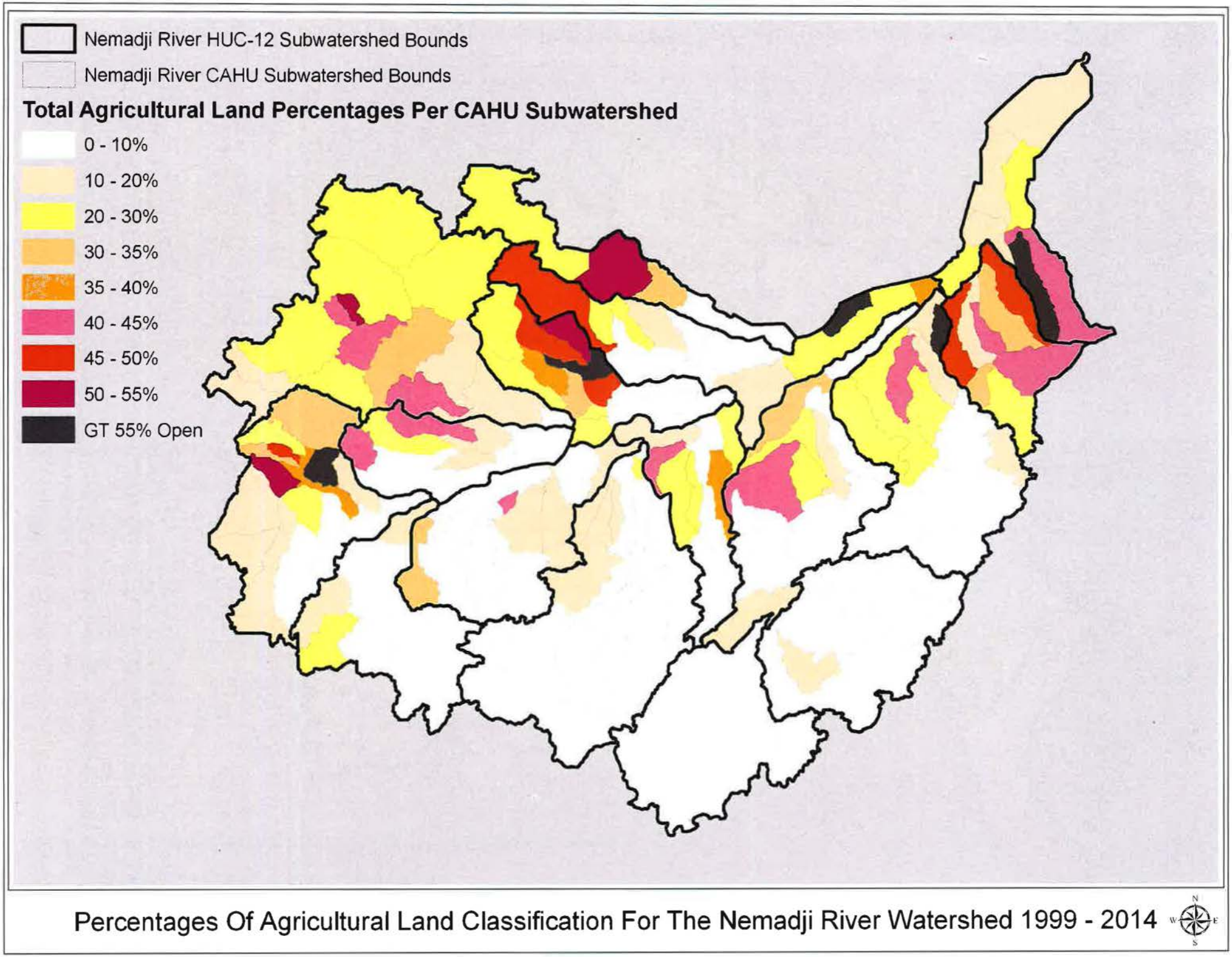
Total acres of 0 – 15 year timber age class for current study: 35,444
Total acres of agricultural/urban lands for current study: 55,825
Total acres of 0 – 15 year timber age class for previous (86' - 02') study: 27,898
Total acres of agricultural/urban lands for previous (86' - 02') study: 44,184

When comparing the acreages of the agricultural/urban lands from the previous (86' – 02') study to the current study, it is important to note that the boundaries of the previous study were digitized using the 30-meter resolution 2002 Landsat imagery as a basis. The current study had many more years of 1-meter resolution USDA NAIP imagery along with recent high resolution state and county imagery. The differences in base data layers for digitizing features could be one factor for the 26% increase in agricultural/urban lands between 2002 and 2014 studies.

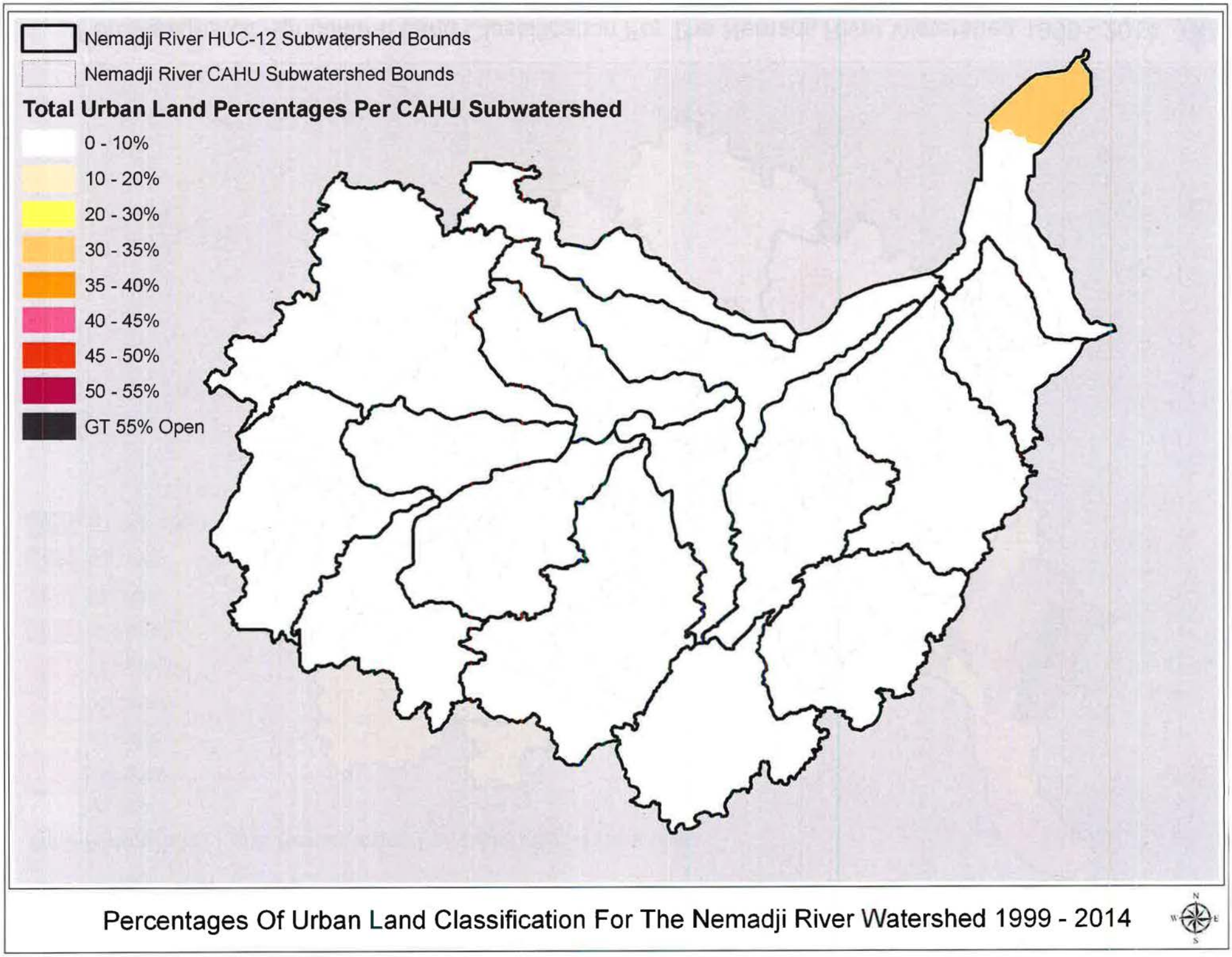
Included are the cartographic summaries of the study's analysis.



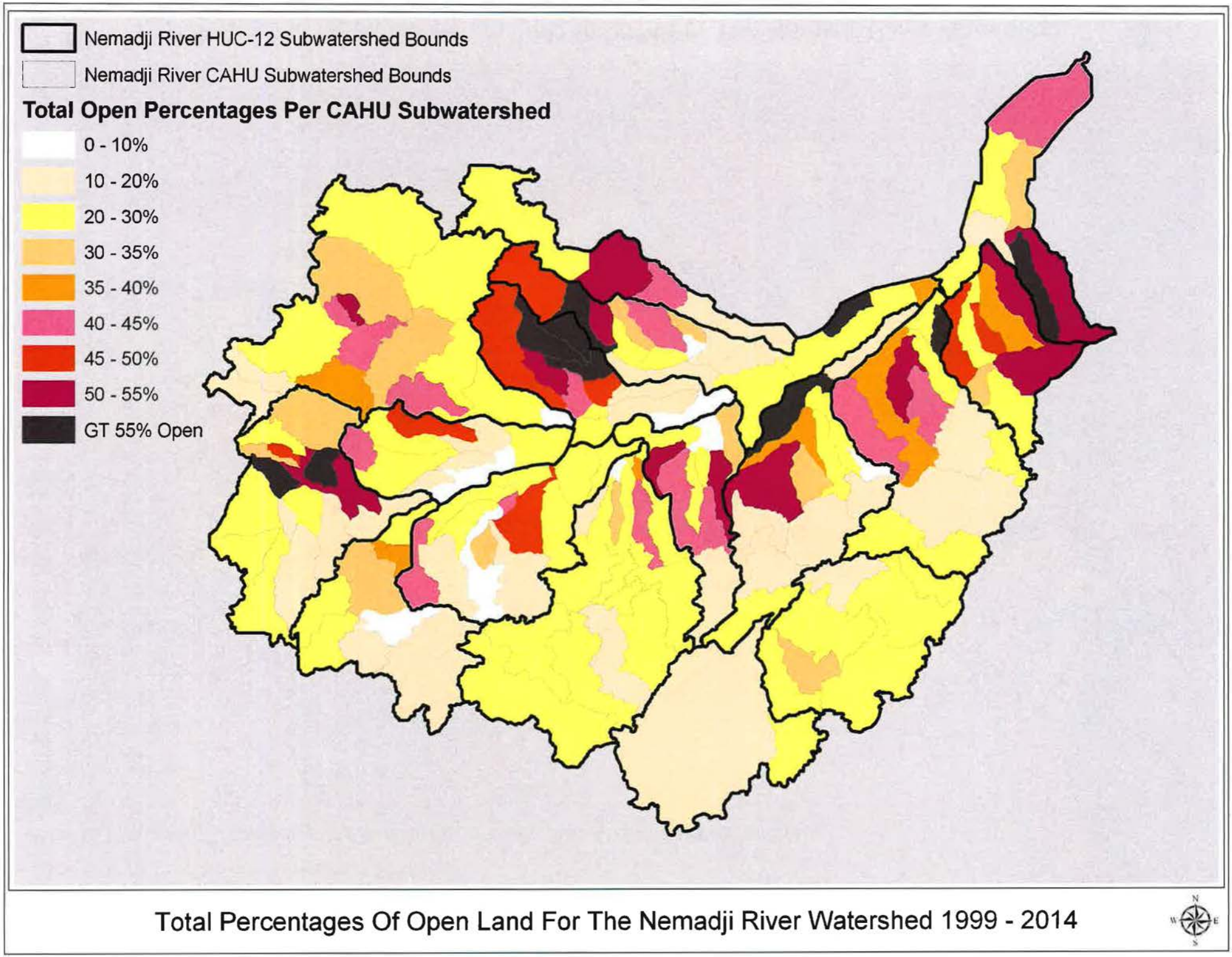
Percentages Of 0 - 15 Year Timber Age Class For The Nemadji River Watershed 1999 - 2014

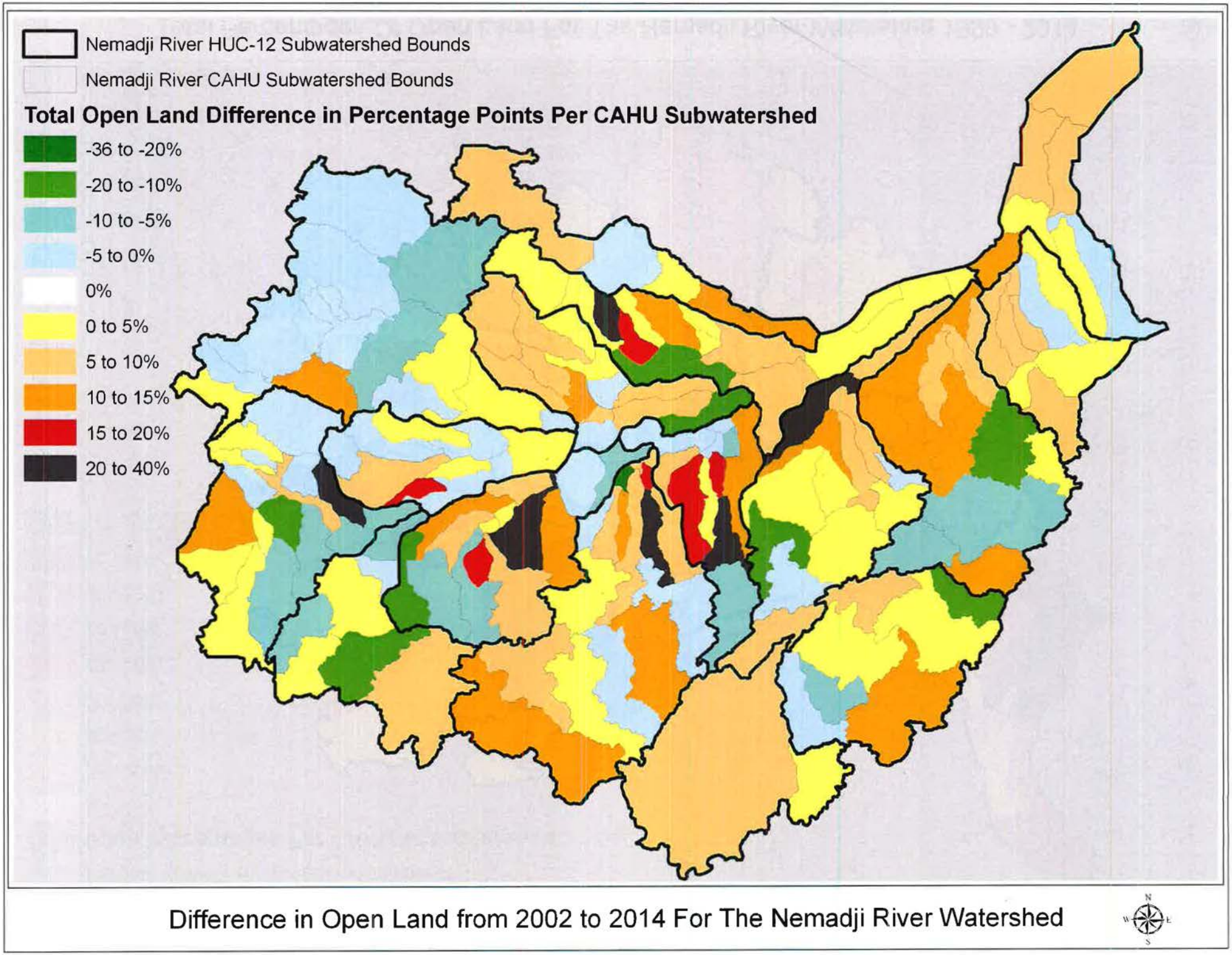


Percentages Of Agricultural Land Classification For The Nemadji River Watershed 1999 - 2014

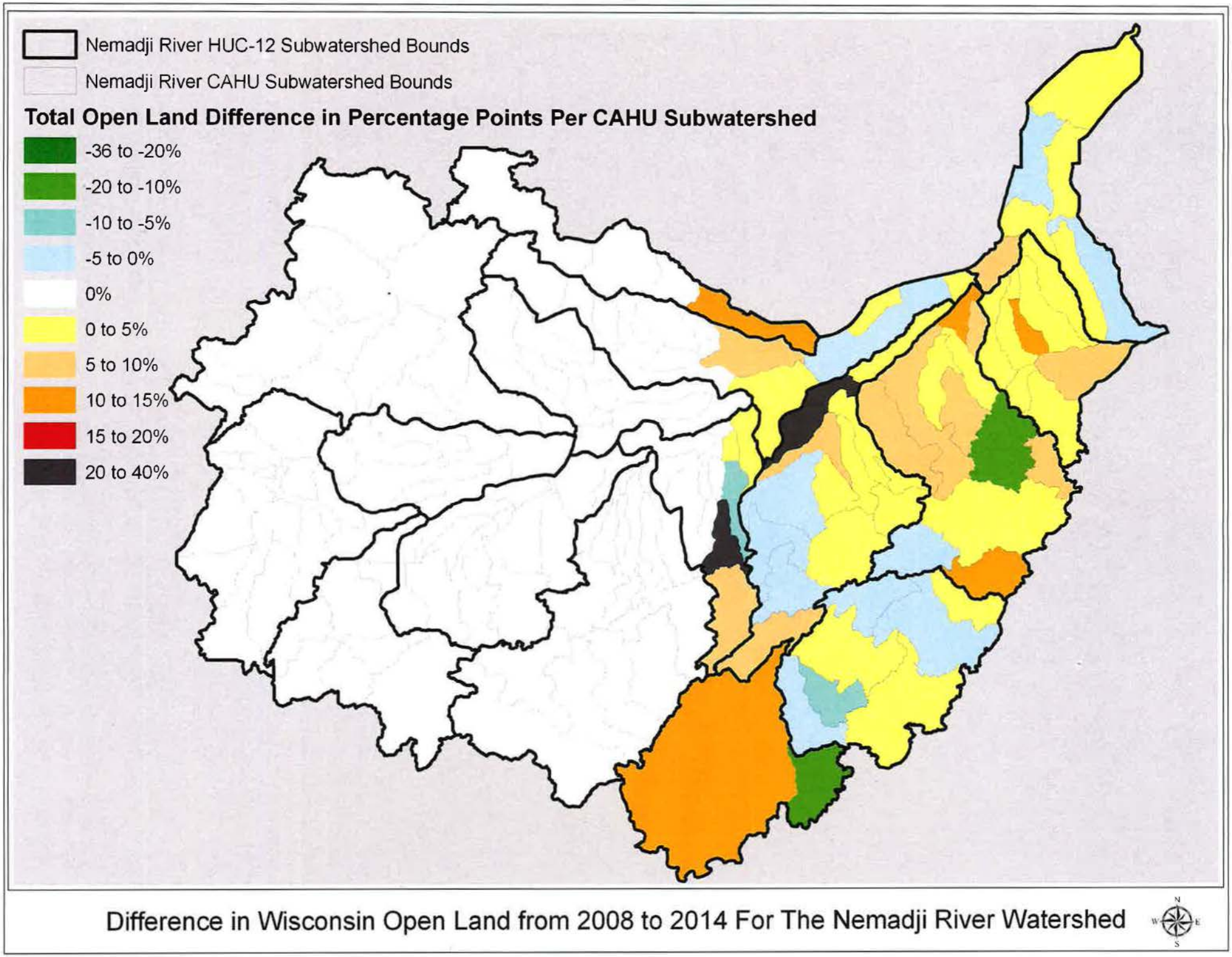


Percentages Of Urban Land Classification For The Nemadji River Watershed 1999 - 2014


Nemadji River HUC-12 Subwatershed Bounds
Nemadji River CAHU Subwatershed Bounds
Total Open Percentages Per CAHU Subwatershed
0 - 10%
10 - 20%
20 - 30%
30 - 35%
35 - 40%
40 - 45%
45 - 50%
50 - 55%
GT 55% Open
Total Percentages Of Open Land For The Nemadji River Watershed 1999 - 2014



Difference in Open Land from 2002 to 2014 For The Nemadji River Watershed


Nemadji River HUC-12 Subwatershed Bounds
Nemadji River CAHU Subwatershed Bounds
Total Open Land Difference in Percentage Points Per CAHU Subwatershed
-36 to -20%
-20 to -10%
-10 to -5%
-5 to 0%
0%
0 to 5%
5 to 10%
10 to 15%
15 to 20%
20 to 40%
Difference in Wisconsin Open Land from 2008 to 2014 For The Nemadji River Watershed
N
W
E
S

Appendix 5

Current and Historic Sediment Loading in the Nemadji River Basin
(Pertains to management action 6.05)

Current and Historic Sediment Loading in the Nemadji River Basin

August 1, 2016

PREPARED FOR

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TETRA TECH

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1.0 INTRODUCTION

The overall project goal was to simulate historical conditions for sediment loading in the Nemadji River watershed and compare current to estimates of historic conditions in an effort to understand how sediment loads have changed over time. The project makes use of an existing Hydrological Simulation Program-Fortran (HSPF, version 12.4; Bicknell et al., 2014) watershed model developed under contract with the MPCA (Tetra Tech 2016). The HSPF model was developed for MPCA's general basin planning and permit development purposes and addresses flow, sediment, dissolved oxygen, temperature, nutrient loads, and algae within the Nemadji River basin. It is a tool of opportunity for use in evaluating historic sediment loading scenarios that provides a reasonable representation of sediment loading, from both upland and channel sources, under current conditions and can be efficiently modified to address historic conditions. It is not, however, an ideal tool for sediment loading simulation because it was created at a fairly coarse spatial scale and provides a one-dimensional channel simulation that cannot resolve the details of bank stability throughout the planform of the Nemadji River and its tributaries. More detailed high-resolution channel models would be preferable for conducting analysis of sediment scour and deposition processes; however, such models require a high level of effort and detailed survey information that is not feasible under the currently available funding. Nonetheless, we believe that the HSPF model scenarios provide a reasonable first-cut estimate of the likely differences between current and historic conditions for sediment loading and transport in the Nemadji River watershed and can provide a basis for planning future, more detailed modeling studies.

The project area includes the Nemadji River's 1,130 km^2 watershed that drains to the St. Louis River Estuary on Lake Superior. The entire Nemadji watershed is included in the St. Louis River Area of Concern due to excessive sedimentation observed in the river. The lower portion of the Nemadji watershed is situated in extremely clay-rich glaciolacustrine soils (Lake Superior Red Clay Plain) that are naturally prone to erosion and sedimentation. The upper third of the basin is situated in interbedded glacial tills and beach and outwash sands and gravels. Sedimentation issues related to historic land use changes are very difficult to distinguish from natural processes in this naturally sediment-rich system. The Nemadji is a young watershed and is affected by a continuous change in base levels in Lake Superior, so equilibrium assumptions regarding channel form are likely not appropriate. An apparent increase in precipitation event intensity due to a changing climate compounds the problem further.

Changes in sediment loading since European settlement of the area are difficult to quantify. The estimated average annual suspended sediment load at the mouth of the Nemadji was 130,000 tons/yr based on sediment data collected in the 1970s and flow data through the late 1990s (NRCS 1998 and Robertson, 1996). As a check on model performance, we used the USACE FLUX program to estimate sediment loading in the Nemadji at South Superior directly from monitoring and flow gaging data for 1997-2012, obtaining an estimate of 80,107 tons/yr. This estimate is matched within 2 percent by the HSPF model (after revisions described below in Section 2.3.2): Using 2008 land cover, the HSPF model predicts an average annual suspended sediment load at South Superior of 78,751 tons/yr – both confirming model performance and indicating improvement in sediment loading conditions since the 1970s. Note that all estimates of sediment load are based on suspended sediment monitoring, which is likely to under-estimate the total sediment load because sediment moving as bedload has not been sampled.

Land use changes in the past two centuries have resulted in hydrologic alterations and accelerated erosion rates in similar rivers in Wisconsin situated in the Red Clay Plain (e.g., Fitzpatrick et al., 1999; Fitzpatrick and Knox, 2000). The Nemadji watershed was logged extensively in the late 1800s and early 1900s, and subsequently many upland wetlands and depressions were drained and converted to agriculture. Many channels were straightened, steepened, and cleared in order to raise water levels and facilitate the transport of logs downstream (Rector, 1951). These manipulations likely accelerated down-cutting and widening of streams in the basin. Trapping of beaver may also have had important effects, as beaver dams trap sediment and increase the extent of wetlands. In the 1930s, agriculture (crops, hay, pasture) is said to have occupied nearly half of the land area in the Wisconsin portion of the basin. Increased water yields during peak agriculture resulted in increased incision and entrenchment of Nemadji and its tributaries, and disconnection from the floodplain (Riedel et al., 2001). Since the mid-1900s, many acres have been converted back to forests. Today, the watershed is approximately 68% forested 11% agriculture (crop, pasture, hay), and 18% wetlands and lakes; however, the second-growth aspen/birch forest cover is not functionally equivalent to the native old growth, white pine forest cover prior to logging. Wetland area, which mitigates peak flows, has also been substantially reduced relative to pre-settlement conditions, from about 38 percent of the watershed under pre-settlement conditions to about 17 percent of the watershed under current conditions.

A better understanding of the influence of historic land use change on sediment loading will help resource managers address key areas where sedimentation is excessive by working with landowners and stakeholders to make improvements. This will also meet the actions required under the Excessive Loading of Sediment and Nutrients beneficial use impairment (RAP, 2015) for the St. Louis River Area of Concern. As a means to assess this, HSPF modeling scenarios were developed to evaluate sediment loads for the following time periods that encompass the range of upland land conditions experienced in the watershed:

- Prior to European settlement in the watershed
- During the time period with the highest percentage of agriculture and logged lands (ca. 1930)
- Under current land use conditions

Representation of current conditions uses the calibrated HSPF model previously developed by Tetra Tech (2016) for MPCA (with additional updates documented below in Section 2.3.2). The model is constructed at approximately the HUC-12 spatial scale (Figure 1-1). It uses land use information from the 2006 National Land Cover Database, the 2008 LANDFIRE coverage from the U.S. Forest Service, and the 2013 Cropland Data Layer from USDA. Channel dimensions and hydraulic behavior are based on analysis of available cross sections and regional hydraulic geometry relationships developed by Magner and Brooks (2008). Model calibration and validation demonstrate that the model provides a reasonable representation of recent sediment loads in the Nemadji River watershed, although the relatively coarse segmentation of the model limits the ability to describe conditions within individual source areas. The HSPF model provides an available platform with which to examine relative changes in sediment load over time.

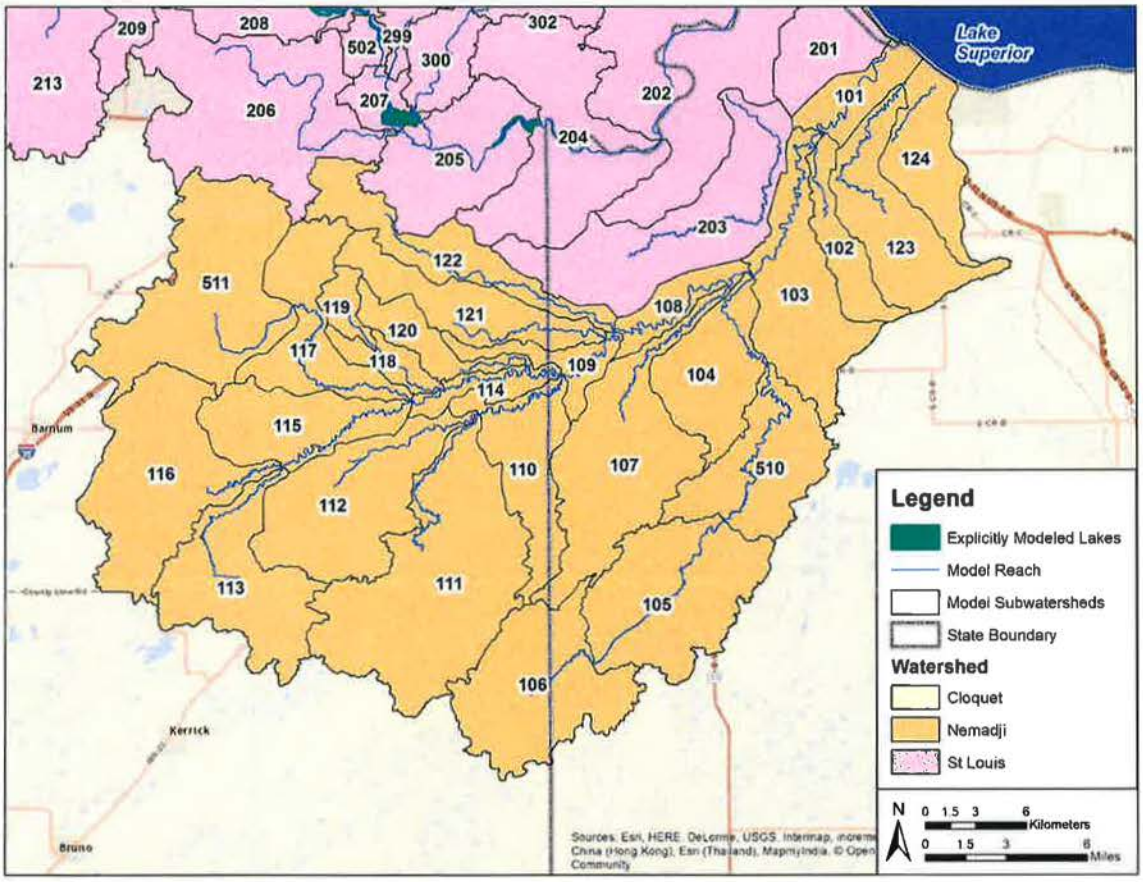

Figure 1-1. Model Subbasins in the Nemadji River HSPF Model

The existing model uses a hydrologic response unit (HRU) approach in which the landscape is simulated based on unit-area components that reflect a combination of land use, land cover, and soil properties summarized by hydrologic soil group (HSG). HSG is a measure of infiltration potential, ranging from A soils (high infiltration capacity) to D soils (very low infiltration capacity). The HRUs described in the model are listed in Table 1-1. The developed and road categories are further sub-divided into pervious and hydrologically connected impervious areas, as described in Tetra Tech (2012).

Table 1-1. Hydrologic Response Units in the Nemadji River HSPF Model

Land Use	HSG
Forest, Deciduous	AB
Forest, Deciduous	CD
Forest, Evergreen	AB
Forest, Evergreen	CD
Wetlands, Forested	CD
Wetlands, Herbaceous	CD
Grassland/Shrubland	AB
Grassland/Shrubland	CD
Pasture/Hay	AB
Pasture/Hay	CD
Row Crops	AB
Row Crops	CD
Row Crops	Drained
Developed, Open Space	All
Developed, Medium Intensity	All
Developed, High Intensity	All
Water	CD
Barren/Strip Mines	CD
Roads	All

2.0 METHODS

2.1 LAND USE/ LAND COVER

An evaluation of historical sediment dynamics was conducted by applying the existing model to land use/land cover conditions or previous time periods. To apply the model to historical time periods it is first necessary to estimate appropriate land use/land cover in the watershed. The approach and relevant information sources are provided below for each of the scenarios. An evaluation of the uncertainties associated with the available and utilized datasets is provided in the Results (Section Results3.0).

2.1.1 Current Conditions

As noted above, current conditions simulation relies on the previously developed and calibrated HSPF watershed model (Tetra Tech, 2016). Land use/land cover components of this model are described below.

Base Land Use and Land Cover: The base land use and land cover representation is as developed for the existing Nemadji River basin model for MPCA. This uses the 2008 LANDFIRE coverage (which is based on the same 2006 Landsat imagery as the 2006 NLCD) combined with 2006 NLCD and SSURGO soils.

A more detailed study of open lands in the Nemadji basin was recently completed by Community GIS Services ([2014], *Comparative Analysis of The Nemadji River Watershed in the Lake Superior Basin).* Among other things, this study used year-by-year analysis of Landsat imagery to estimate the 0-15 year timber age class, allowing identification of rates of forest harvest. The HSPF model represents a static land use and is developed at a coarser scale than the open lands analysis, so this information cannot be incorporated directly. The HSPF model does include shrub and barren land classifications into which recently timbered land (as of 2006) will fall. The open lands study suggests that the fraction of forest classified as shrubland is consistent with recent long-term average forest harvest rates in the watershed.

Surface Drainage: Surface drainage is enhanced relative to natural conditions due to ditching and the construction of roads. The natural watershed is believed to have included many internally drained depressions which were leveled and/or drained during peak agriculture. Although much of the agricultural land has reverted to forest since that time, the enhanced surface drainage remains (NRCS, 1998). The current conditions model hydrologic calibration implicitly incorporates the current enhanced surface drainage.

Wetlands: As identified in the base land use and land cover for the existing model.

Lakes: The lakes that are present in the watershed are generally small and disconnected from the main stream network. They are represented in the existing model as water land uses.

Rural Development: As identified in the base land use and land cover.

Roads/Railways: As identified in the base land use and land cover using the Tiger Census roads and local county roads coverages.

2.1.2 Peak Agriculture (ca. 1930)

Peak agriculture in the watershed was assumed to be approximately 1930 (although the agricultural census suggests that additional conversion of land to agriculture may have continued after 1930.) Satellite-based estimates of land use/land cover conditions are obviously not available for 1930. The data sources and assumptions used to describe ca. 1930 land use and land cover are described below.

Base Land Use/Land Cover: Topographic maps of the whole watershed are not available until the late 1940s. There is aerial imagery from 1939, but resources are not currently available to process this imagery. For the Wisconsin portion of the watershed, detailed information on land use in Douglas Co. as of 1933 is provided in the Wisconsin Land Economic Survey maps ("Bordner surveys"; https://uwdc.library.wisc.edu/collections/EcoNatRes/WILandInv/). The Bordner surveys have been digitized and merged to form a seamless map. The digitizing was for land use only, and does not include buildings and roads. As buildings and roads can have an important impact on hydrology they must be addressed separately, as described below. The Bordner surveys, after subtracting the road impervious area, provide the base land use/land cover for ca. 1930 conditions in the Wisconsin portion of the watershed.

The Bordner surveys do not cover the City of Superior, WI – except insofar as to show it as an urban area. Superior is at the very mouth of the Nemadji and has only a small impact on delivered sediment loads associated with the river. Therefore, we initially assumed that the land use distribution within the 1930 city limits was approximately the same as under current conditions. Population Census data are used to determine if a density-based adjustment to the impervious cover fraction in the City of Superior relative to current conditions is warranted.

There is not a spatial coverage comparable to the Bordner surveys for the Minnesota portion of the watershed (in Carlton County and a small part of Pine County). Therefore, more approximate methods must be used. Specifically, we use county level information for Carlton Co. from the 1929 and 1934 tabulations provided in the 1935 Agricultural Census (Bureau of the Census, 1936) compared to the average in the 2007-2012 Agricultural Census (USDA, 2014) to determine the relative change in cultivated, fallow, and hay/pasture land uses. We use a two-census average because these reports are based on statistical samples of farms and thus subject to uncertainty. This relative change is applied to the 2008 land cover currently in the model to estimate the area present ca. 1930. The agricultural census also contains county-level statistics on the fraction of farms that was in forest or forest-pasture that were used to make some rough estimates as to changes in forest cover.

The type of agriculture conducted around 1930 was also different from modern practices as it included moldboard plowing and much more acreage of oats, barley, and rye (with some corn as well) than are currently grown. Under current conditions there is much less production of small grains, and tillage practices involve much less soil disturbance. We use the crop acreage shown in the agricultural census to estimate likely tillage and cover conditions ca. 1930 and adjust the seasonal pattern of detached sediment on farm land accordingly. In HSPF this is done through the use of the SPECIAL ACTIONS routine to reset the amount of detached sediment on the land surface after spring plowing, seed planting and tillage, and at harvest.

Logging is not expected to have been a major factor in sediment dynamics ca. 1930. Most of the old growth forest had been long since cut and the massive fire of 1918 further degraded forest cover, such that there was likely little mature forest available for timber production in 1930. The first Forest Service Forest Inventory and Analyses for Wisconsin as well as for Minnesota were conducted in 1936 and for the

area around Lake Superior show mostly young forest with lots of aspen. Given this history, the model is not adjusted to represent differences in logging for this period relative to current conditions.

Surface Drainage: Much of the modern surface drainage network was already in place by 1930, although additional drainage of wetlands occurred after World War II, due in part to the ready availability of dynamite in that period. We use the extent of wetlands in 1930 as the primary indicator of differences in surface drainage present in 1930 as areas in wetlands are by definition not effectively drained.

Wetlands: The Bordner surveys are the primary source for the extent of wetlands in Douglas Co. ca. 1930. For Minnesota we do not have a 1930 wetlands coverage and it is believed that additional drainage occurred after this period. Lacking other information, we assume that any fraction of additional wetland loss after 1930 identified in the Douglas Co. portion of the watershed would also apply to the Minnesota portion of the watershed.

Rural Development: As noted above, the digitization of the Bordner surveys did not include buildings, driveways, and other impervious areas that are important to hydrology. Only small changes in developed area are expected. The decennial Census, however, gives counts of population on a township basis. We evaluated the change in population statistics between 1930 and 2000 by township as a basis for deciding whether changes in developed impervious area (other than transportation) are needed.

Roads/Railways: The impervious surface in roads and railways plays an important role in storm event hydrology. Unfortunately, the available digitized version of the Bordner survey does not include the roads. Tetra Tech therefore digitized the road and rail lines from the Bordner survey. We were able to obtain an image of a 1935 transportation map of Carlton Co., MN, which is used for the same purpose. For Pine Co., MN the area that is in the Nemadji watershed is sparsely developed. The best available coverage for peak agriculture roads in this small area is provided by the road lines contained on the 1941 county soil survey map.

The amount of impervious area associated with road lines depends on the lane width. The travel lanes on paved roads, gravel roads, and unimproved dirt roads can all be considered as essentially impervious for storm response modeling. However, lane width ca. 1930 was generally less than under today's engineering guidelines. An FHWA (1994) survey discusses the presence of many older rural roads that have only an 8 or 9 foot lane width. Roads present in 1930 were generally two lane, and interstate highways had not yet been built. The following assumptions are made for road impervious area:

- Improved numbered route: 2 lanes, total 20' paved width.
- Rural road: 2 lanes, total 16' paved width
- Unimproved dirt/gravel road: 1 lane, 8' paved width
- Railroad corridor: 12' effective impervious width (gravel, compacted earth)

2.1.3 Pre-Settlement / Intact Forest (ca. 1850)

Base Land Use and Land Cover: The pre-settlement land use is simpler to describe, but harder to observe. In both Wisconsin (Finley, 1976) and Minnesota (Marschner, 1974) the notes and descriptions of lines and corners in the original land plats have been used to reconstruct the pre-settlement vegetation. Both are available in digital form. Tabulations obtained from these maps were checked for consistency against the focused analysis of original vegetation in the Nemadji of Koch et al. (1977).

Much of the watershed prior to settlement was covered with mature pine forest, although fire was also a natural part of the landscape and, along with disease and blowdowns, resulting in a patchwork of ages

and types of stands in the pre-settlement forest (NRCS, 1998). This differed in both quantity and quality from current second growth forest. It was not feasible to incorporate all the potential effects of mature forest, such as impacts on the seasonal evapotranspiration cycle or higher amounts of soil piping in undisturbed forest land; however, the shift to evergreen forest for pre-settlement conditions automatically incorporates a greater degree of shading (for snowmelt), higher rates of rainfall interception, and greater cover relative to erosion.

Surface Drainage: Prior to settlement there were no ditches or roads, which reduced the hydrologic connectivity of the uplands to streams. This is approximated in two ways. First, we use the wetland studies described in the next paragraph to approximate areas that were either true wetlands or internally drained depressions with hydric soil characteristics prior to settlement. Second, we modified the model to include longer average overland flow path lengths to better reflect a roadless and unditched situation.

Wetlands: The original vegetation maps (Finley, 1976; Marschner and Heinselman, 1974) provide a rough representation of the extent of pre-settlement wetlands in the Nemadji watershed through comparison to the potentially restorable wetland coverages (National Wetlands Inventory (http://www.fws.gov/wetlands/), Minnesota Restorable Wetlands coverage (http://mnwetlandrestlore.org), and work on restorable wetlands in Douglas Co. (Stark and Robertson, 2013)). Wetlands present in 1930 or shown on the National Wetlands Inventory (see Section 3.1). In addition to drainage of upland wetlands, it is speculated that reduction in riparian wetland area along the middle and lower reaches of the Nemadji may have occurred due to overbank sedimentation and entrenchment of channels.

Rural Development: None.

Roads/Railways: None

2.2 WEATHER

All model simulations for both current and historic land use employ the same weather series, covering the period 1/1/1993 – 12/31/2012. Climate ca. 1930 and ca. 1850 may well have been different from the weather of 1993 - 2012; however, there are not detailed observations available and use of the same weather series for all time periods enables comparison of differences due only to changes in the landscape and channel form.

The model operates on an hourly time step, but hourly precipitation data are not available for the Nemadji watershed. Instead, records from summary-of-the day stations are used, with temporal disaggregation against the hourly pattern at Duluth International Airport. Only four precipitation stations with sufficient periods of record were available for the Nemadji watershed: WI478349 (Superior, WI), WI476413 (Pattison State Park, WI), MN213863 (Holyoke, MN), and MN211630 (Cloquet, MN, used only for the northeast edge of the watershed). Unfortunately, three of these stations had incomplete records and needed to be filled from nearby stations using the normal ratio method: Records for WI478349 end on 12/31/2005 and later dates were filled from WI476413, records for WI476413 commence on 4/30/1998 and earlier dates were filled from WI478349), and records of MN213863 end on 12/31/2006 and later dates were filled from WI476413. There is thus considerable uncertainty in the precipitation record, which propagates into the hydrologic simulation. Air temperature was also taken from local stations, while the third major driver of the energy balance, potential evapotranspiration, was calculated using the Penman Pan energy balance method. The Penman Pan calculations combine local air temperature with inputs for

cloud cover, dew point, solar radiation, and wind taken from Duluth International Airport, and are thus also subject to uncertainty.

It is worth noting that gridded meteorological data products have become more widely available since the original development of the Nemadji model, including PRISM daily precipitation and hourly energy balance components from NLDAS. Use of these gridded weather inputs would likely improve the performance of the Nemadji model, particularly as there can be strong gradients in weather over short distances due to lake effects.

2.3 CHANNEL PROCESSES

2.3.1 Channel Hydraulics

Land use changes over the past 150 years have resulted in hydrologic alterations and accelerated erosion rates in similar rivers in Wisconsin situated in the Red Clay Plain (e.g., Fitzpatrick et al., 1999; Fitzpatrick and Knox 2000). The Nemadji watershed was logged extensively in the late 1800s and early 1900s. Many channels were straightened, steepened, and cleared in order to raise water levels and facilitate the transport of logs downstream (Rector, 1951). These manipulations likely caused down-cutting and widening of streams in the basin, as is evident from abandoned terraces. Increased water yields during peak agriculture (largely associated with loss of wetlands) are believed to have resulted in incision and entrenchment of the Nemadji and its tributaries, and disconnection from the floodplain (Riedel et al., 2001).

Studies of the Nemadji and similar watersheds such as Knife River and Fish Creek that are dominated by lacustrine clay at the western end of Lake Superior conclude that the soils are generally resistant to erosion under natural land cover (NRCS, 1998; Stone et al., 2010), but that activities within the watershed that expose soils, concentrate flows, or disturb banks can increase fluvial erosion and destabilize channels (Riedel et al., 2005; Riedel et al., 2002).

The existing HSPF model is developed at a relatively coarse scale and does not provide a detailed hydraulic simulation because it is a one-dimensional approximation. Channel/bank scour and deposition processes are simulated as a function of flow velocity (for sand) and average boundary shear stress (for silt and clay). An important source of sediment load in the Nemadji is mass wasting from bluffs where the river impinges on valley walls. The existing HSPF model does not explicitly represent bluff mass wasting, but approximates this process (to the extent that information and monitoring data are available) by increasing the maximum potential scour rate in reaches where these processes occur. Simulation performance could likely be improved through development of a finer scale model that explicitly represents areas where bluff mass wasting contributes sediment loads to the channel.

Both flow velocity and boundary shear depend on the channel cross-sectional area as a function of volume. HSPF uses "Functional Tables" (FTables) to describe the relationships between channel dimensions, volume, and discharge. The channel dimensions in the current model are not based on detailed cross-section surveys, but are developed in one of two ways: There are eight model reaches where the Minnesota Department of Natural Resources (MNDNR) or the U.S. Geological Survey (USGS) has developed rating curves with cross sections (the cross sections generally go only to bank full, but have been extended with LiDAR). These enable a direct estimate of volume-flow-cross sectional area-hydraulic radius and velocity relationships, albeit truly appropriate only to the immediate locale of the rating curve. Most of these are at or near road crossing. For other reaches, we used the regional

geometry regressions that Magner and Brooks (2008) developed for bankfull cross-sectional area and bankfull flow, together with measured slope and some assumptions about entrenchment ratio and roughness to calculate hydraulics.

The regional regression equations are available in Magner and Brooks (2008) and accompanying files provided by Tim Larson of MPCA and describe bankfull cross-sectional area A_{bank} (ft^2) and flow Q_{bank} (cfs) as a function of drainage area DA (mi^2).

The following inputs are obtained from GIS:

DA	drainage area	mi^2
L	reach length	ft
W_m	stream width	ft
m_F	floodplain slope (inverse – expressed as run over rise)	
s	reach slope	

We also assume the following based in part on the standard method for FTables in BASINS Technical Note 2 (USEPA, 2007):

$W_F = W_{bank} = W_m$ (i.e., the bankfull width is the same as the observed width and the floodplain side width is assumed equal to the channel width)

$m_C = 1.5$ (channel side slope is assumed 1:1.5 due to somewhat incised nature of many streams in this area)

We then calculate:

A_{bank} (bankfull cross-sectional area in ft^2) = $5.5209 \times DA^{0.7744}$ (Magner 15-sites equation, $R^2 = 0.9744$)

Q_{bank} (bankfull flow in cfs) = $41.913 \times DA^{0.7946}$ (Magner regression, $R^2 = 0.9001$)

Y_c (bankfull depth, ft) = A_{bank}/W_m

$Y_m = Y_c/1.25$ (standard method assumption)

We can use Q_{bank} to back-solve for the channel Manning's coefficient (n) (roughness or friction parameter applied to the flow):

P_{bank} (bankfull wetted perimeter) = $W_m - 2\, m_c\, Y_c + 2\, Y_m\, (m_c^2 + 1)^{0.5} = b + 2\, Y_m\, (m_c^2 + 1)^{0.5}$,

$n = A_{bank}/Q_{bank} \times 1.486 \times (A_{bank}/P_{bank})^{2/3} \times s^{0.5}$

A separate Manning's coefficient is assigned to overbank flow (0.06 in the absence of other information.) We then calculate discharge using Manning's equation, assuming no friction loss between the channel and overbank segments, as is done in WinXSPro (Hardy et al., 2005).

HSPF simulates channel sediment transport, scour, and deposition as a function of the channel hydraulics. Three generalized size classes are represented (sand, silt, and clay). Potential transport of non-cohesive sediment (represented by the sand fraction) is simulated as a power function of the flow velocity (as an average across the cross-section width) and sand scours or deposits depending on the difference between potential and actual mass transport. The behavior of cohesive sediments in HSPF is a function of cross-sectional average shear stress (τ; mass per area). The shear stress at any given flow

rate is simulated as the product of slope, the unit mass of water, and the hydraulic radius of the channel, which is defined as the ratio of the cross-sectional area of the channel to the wetted perimeter, and thus depends on the hydraulic geometry described above. Silt and clay both have a critical shear stress below which deposition may occur (τ_{CD}) and a critical shear stress above which scour may occur (τ_{CS}). When $\tau > \tau_{CS}$ the scour rate of the relevant class of cohesive bed material (if available) is simulated as $M \times (\tau/\tau_{CS} - 1)$, where M is an erodibility coefficient (mass per area per hour).

Because HSPF uses a one-dimensional representation of stream channels, scour and deposition from channel banks and the channel bed are not explicitly distinguished. For the Nemadji the largest source of sediment loads is associated with bank and bluff failures, especially where the stream channel impinges on valley-edge bluffs. Detailed surveys of the location and recession rates of bluffs are not available for the Nemadji, so only an approximate representation of these contributions is available at this time. Specifically, contributions from banks and bluffs in reaches where such contributions are believed to be important (based largely on anecdotal and qualitative information) are represented in the model primarily by assigning increased values of the erodibility coefficient (M) to the reach, and both M and τ_{CS} were adjusted on a reach-by-reach basis during the model calibration process to provide a better fit to observed suspended sediment concentrations. This representation could be refined and improved through the collection of survey data, use of a finer-scale spatial resolution, and through application of more sophisticated channel sediment scour and deposition models.

2.3.2 Model Revisions

The upland simulation for current conditions is unchanged from that reported in Tetra Tech (2016). During the course of this work we did, however, identify an error in the implementation of the Magner regional geometry equations for the Nemadji. This prompted us to re-estimate the model FTables that relied on this method, which in turn causes minor changes to the hydrograph shape and larger changes to the simulation of sediment scour and deposition. These changes required recalibration of the model to observed total suspended solids concentrations by adjusting the channel sediment parameters.

The recalibration effort was successful and yields results that are similar to those reported in Tetra Tech (2016). For the downstream station, Nemadji River near South Superior, WI, the recalibrated model has an average error on paired simulated and observed TSS concentrations of 0.83% and an average error on paired simulated and observed TSS daily loads (where "observed" load is calculated from concentration and flow) of -12.17%, both within the relative error performance target of ±30% that is recommended as indicative of a good quality sediment calibration by Donigian (2000) and Duda et al. (2012). Note that the apparent error on load is highly leveraged by one outlier on 4/16/2012 when an observed concentration of 3,500 mg/L TSS was reported.

Observed and simulated TSS time series are compared for the South Superior monitoring station are compared in Figure 2-1 (note the logarithmic scale). The model generally replicates the trend in observed data, although the lowest observed concentrations are over-predicted due to assumptions of a minimum background concentration during low flow conditions. These assumptions reflect non-flow related processes such as disturbances by human and animal activity and have little impact on annual load estimates. A small number of high flow observations are substantially under-estimated. This is commonly observed in water quality simulation models and reflects a number of factors: (1) the observations are point-in-time measurements, whereas the model predicts averages over time, (2) the observations are also point-in-space measurements that may not be representative of the average concentration across the entire cross-sectional area, and (3) individual observations may be affected by

stochastic events, such as sudden bank failure, that are not predictable within the model. Figure 2-2 is a power plot that compares the relationship of TSS load to flow at this station and demonstrates general consistency in the relationship to flow between observed and simulated values.

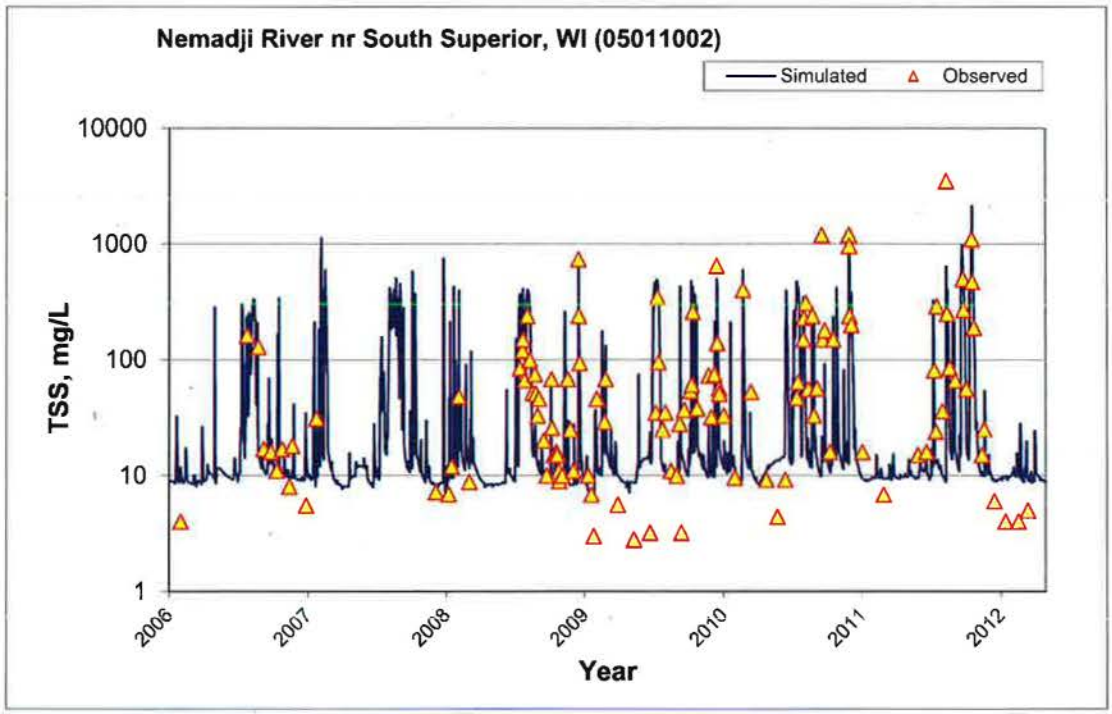

Figure 2-1. TSS Concentration Times Series, Nemadji River near South Superior, WI (05011002)

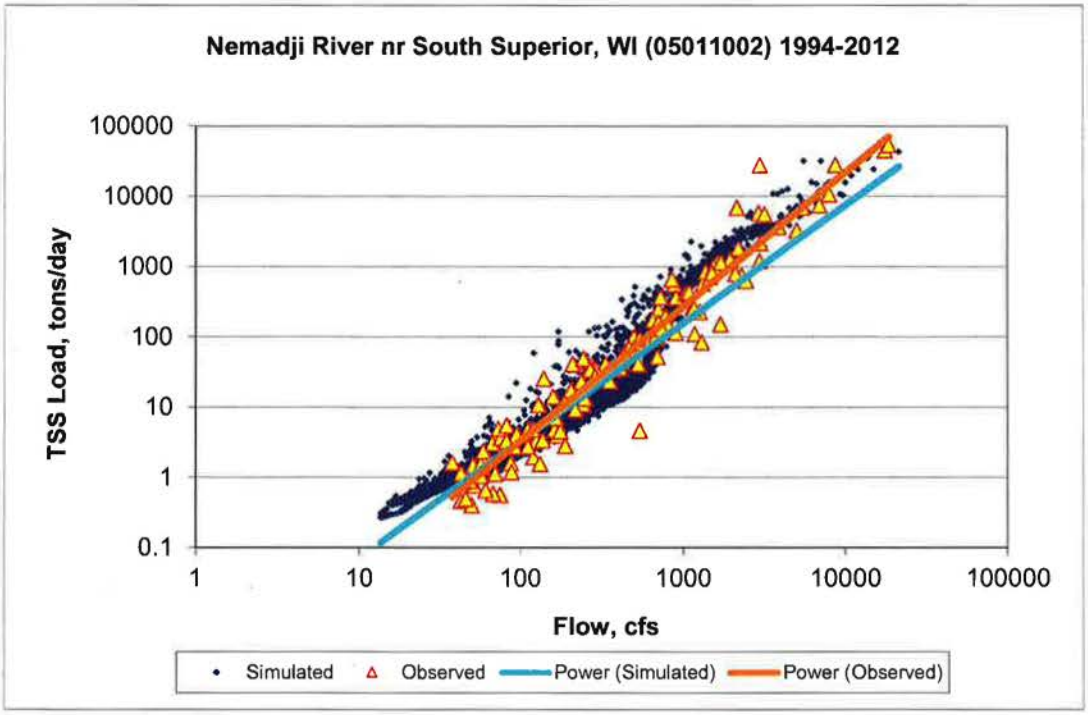

Figure 2-2. TSS Load Power Plot, Nemadji River near South Superior, WI

As an additional check on model performance we compared model predictions of suspended sediment load to estimates derived directly from the monitoring data at the South Superior monitoring station. Suspended sediment load is not observed directly and can only be estimated with uncertainty, using statistical methods, due to the strong correlation between flow and load. The "observed" monthly loads are estimated using the USACE FLUX32 program (a Windows-based update of the FLUX program developed by Walker, 1996; available at https://www.pca.state.mn.us/water/watershed-pollutant-load-monitoring-network#flux32-8f1620f5), and are themselves subject to significant uncertainty. FLUX provides a variety of methods for estimating load, but the estimates with the lowest relative uncertainty are obtained using FLUX method 6 without stratification against flow or season. Method 6 is a bias-corrected regression method based on establishing a relationship between observed flow and concentration and using this relationship to extrapolate to a complete loading time series. For the period 1997 – 2012, FLUX estimates the average suspended sediment load passing the South Superior Station as 80,107 tons/yr, with a coefficient of variation of 0.13 (indicating 95% confidence limits of from 59,696 to 100,518 tons/yr). Over the same time period, the revised HSPF calibration estimates average loading of 76,680 tons/yr, which is only 2 percent lower than the FLUX estimate and near the center of the 95% confidence limit boundaries. Alternative estimation methods in FLUX yield similar results, suggesting that the HSPF model provides a good match to suspended sediment loads on an average annual basis.

2.3.3 Historic Channel Conditions

Anthropogenic changes in land use and land cover are likely to have induced changes in hydrology that induced changes in channel morphology, which in turn would affect the transport, scour, and deposition of sediment. Humans may also have induced geomorphic changes indirectly through trapping and

reduction of beaver populations, as beaver dams slow the flow of water and encourage sedimentation, but can also be a cause of episodic scour events during dam failure (Butler and Malanson, 2005). Dramatic changes in channel conditions over time have been documented through the examination of relict channels in detailed studies of Fish Creek (Fitzpatrick et a., 1999; Fitzpatrick and Knox, 2000), which borders the Nemadji to the south. While there are ample grounds for speculation about how channels in the Nemadji basin may have changed in response to human activities there is a lack of hard data to constrain the simulation for this basin. The HSPF model can provide a reasonable representation of how hydrology has changed, which in turn drives erosive stresses on channels, but we do not have a strong basis for assigning pre-settlement channel morphology. We discussed these issues with Dr. Joe Magner and Dr. Mark Riedel, and the consensus seems to be that the Nemadji has never been in geomorphic equilibrium, in part due to changing base levels in Lake Superior and the recent geologic time since the end of glaciation, and that the basin is attempting to continue expansion. The Nemadji likely always generated large sediment loads where the channel impinged on valley walls and there have likely been progressive headcuts in the system even prior to settlement due to natural conditions such as ongoing base level change, wildfires, and so on. While we think channel condition may have been somewhat worse around 1930 (due to a history of logging, damming, straightening, and removal of large woody debris accompanied by clearing and drainage of the uplands) and somewhat better pre-settlement, that is mostly speculative and we do not have a good basis for describing morphology for these periods.

As a result of these uncertainties, this analysis begins by simulating pre-settlement conditions with present morphological/channel geometry relationships, but with the simulation applying different hydraulic stresses associated with changes in land use. This enables a direct examination of the probable effects of changes in land use and cover independent of channel morphology. For this run the only change to channel form is to back out (partially) the effects of road crossings. This is done by assuming that the hydraulic geometry relationships developed by Magner and Brooks (2008) apply to all stream segments and removing the altered hydraulic geometries obtained from the gage rating tables/cross sections (most of which are located at road bridges/culverts).

After this initial pre-settlement run we present a sensitivity analysis to speculate about the additional impacts on sediment loading associated with possible differences in pre-settlement channel form. This includes sensitivity to potentially reduced bankfull cross-sectional area, potential reductions in the maximum channel erosion (due to better root and riparian cover development, plus lower groundwater pore pressure), channels that were somewhat less entrenched, and channels with greater sinuosity prior to de-snagging and use of splash dams to float logs. Increasing sinuosity increases channel length, resulting in a net reduction in the energy grade and thalweg slope. While it is likely that channel roughness may also have been different under pre-settlement conditions (e.g., greater roughness associated with large woody debris) this cannot be investigated as an independent variable in this study because roughness is implicitly defined as a function of channel geometry, slope, and bankfull flow through use of the Magner hydraulic geometry relationships described in Section 2.3.1.

3.0 RESULTS

3.1 HISTORIC LAND USE

Base Land Use/Land Cover: As described in Section 2.1.pre-settlement land use was estimated based on Finley (1976) and Marschner (1974). Land use ca. 1930 was based largely on the Bordner surveys for rural Douglas Co. The Bordner surveys do not cover the City of Superior or the Minnesota portion of the watershed.

For the City of Superior, the U.S. Census for 1930 and 2000 (reported in Bureau of the Census, 1932b, and U.S. Census Bureau, 2012b) show a small net decline in population (within the same boundaries), from 30,113 in 1930 to 27,368 in 2000. Given the lack of growth, land use within the City of Superior (exclusive of state and county roads) is assumed to be unchanged between 1930 and the present.

For the Minnesota portions of the watershed, the change in agricultural land uses within the watershed is approximated by county-level statistics from the agricultural Census for Carlton Co. Total plowed land ca. 1930 was 1.694 times that present ca. 2008, while open pasture land ca. 1930 was about 1.8 times that present ca. 2008. Accordingly, current crop acreage was increased by this ratio on a model subbasin by subbasin basis to create the 1930 scenario for the Minnesota portion of the watershed, with a corresponding reduction in deciduous forest. This approximation likely introduces some error but has the advantage of associating the additional agricultural land proportional to current agricultural use, thus focusing the gain on the locations likely most suitable for cropping. The agricultural Census also indicates that a large portion of the forest area present in 1930 was used for woodland pasture, a practice that has declined substantially (from about 63,000 acres to under 8,000 acres in Carlton Co.) The effects of pasturing in woodlands on hydrology and sediment yield have not been incorporated into the model.

Agricultural practices for the ca. 1930 scenario are believed to have been more intensive than those in use today. The agricultural Census for Douglas Co., WI, and Carlton Co., MN (Bureau of the Census, 1936) shows that the dominant crops were oats, barley, and potatoes, with smaller amounts of corn. Old-style tillage for oats and barley would involve spring moldboard plowing followed by a cultivator on planting. These crops have slightly different timing, with later harvest for potatoes and corn, and we have no spatial coverage of crop types in 1930. Based on local characteristics for oats and barley, we assume spring plowing occurs on April 24, followed by planting on May 1 and harvesting around August 20 (typical oat harvest date would be August 15, but a slightly later date is assumed due to the mix of other crops). The model represents the effects of tillage on sediment availability by increasing the detached sediment store to 2.5 tons/ac consistent with older tillage practices. (In most years the model is not very sensitive to the exact value of this assumption because sediment load from flatter fields with fair or better drainage is primarily limited by overland sediment transport capacity of runoff.)

Surface Drainage and Wetlands: The Bordner surveys are the primary source for the extent of wetlands in Douglas Co. ca. 1930. We also consulted the Douglas Co. Restorable Wetlands spatial analysis of Stark and Robertson (2013) and discussed this coverage with one of the authors who agreed that the Bordner survey provides a good source for 1930 and matches well with their results (personal communication from Andy Robertson, St. Mary's University, 1/28/2016). Comparison of current conditions to 1930 conditions reveals almost no change in forested wetlands, while herbaceous wetland acreage in 1930 is less than that under current conditions. Therefore, contrary to expectation, there does

not appear to have been a significant additional loss of wetlands after 1930 and the extent of wetlands in the Minnesota portion of the watershed is not adjusted for the ca. 1930 scenario.

For pre-settlement conditions, all wetlands identified in 1930 and under current land use, plus those in the National Wetlands Inventory (http://www.fws.gov/wetlands/), were combined with the pre-settlement land use coverages to create the wetlands layer. Areas identified on the Minnesota Restorable Wetlands coverage (http://mnwetlandrestlore.org) are generally included within these boundaries. The Douglas Co. Restorable Wetlands work of Stark and Robertson (2013) appear to be of limited use in establishing the extent of pre-settlement wetlands. The authors noted that there were problems in their approach for the Nemadji clay plains because the soils coverages do not clearly distinguish between hydric and non-hydric soils and their analysis for this area is primarily an evaluation of which soil areas are most likely to contain hydric soils. They noted that there has been substantial land leveling to improve agricultural conditions that complicates the topographic analysis, and that the effort does not yield a reliable estimate of the extent of pre-development wetlands (personal communication from Kevin Stark and Andy Robertson, St. Mary's University, 1/28/2016). Based on that discussion we relied on the original vegetation coverage for pre-settlement wetland extent (beyond currently existing wetlands). Stark and Robertson noted that a refined estimate might be obtained from a more detailed analysis of the original land survey notes, but that was beyond the scope of this project.

The major difference in wetland extent over time is between pre-settlement conditions and current conditions for forested wetlands, which declined from over 100,000 acres to about 19,000 acres. Apparently, much of the loss of wetlands had occurred prior to 1930. In contrast to forested wetlands, area in herbaceous wetlands appears to show an increase over time. This likely represents regrowth on a fraction of forested wetlands that were cut but not ditched for agriculture.

We do anticipate that there may have been some increases in drainage efficiency and ditch density from 1930 to present. This is roughly approximated in the model (which is lumped at the subbasin scale) by assuming an increase in the overland flow path length (which defines average travel distance to a simulated stream reach) by 50% to 750' for the ca. 1930 and pre-settlement scenarios. In addition, the small amount of crop land estimated to have tile drainage under current conditions was converted to crop land without artificial sub-surface drainage.

Rural Development: As noted above, the digitization of the Bordner surveys did not include buildings, driveways, and other impervious areas that are important to hydrology. We examined changes in population by census enumeration district (primarily township) between 1930 and present with mixed results (housing data were not available by enumeration district, just at the county level, for 1930). Within the rural townships (Summit and Superior townships, exclusive of the City of Superior) that intersect the watershed in Douglas Co., WI population increased from 2,033 to 3,229; however, agriculture has also declined so the amount of building area, including barns and outbuildings, has not necessarily increased. In enumeration districts intersecting the Minnesota portion of the watershed (Blackhoof, Clear Creek, Holyoke, and Wrenshall townships in Carlton Co. and Nickerson Township in Pine Co.) the total population increased from 2,058 to 2,508, with net decreases in two of the five townships. As the enumeration district boundaries do not line up very well with watershed boundaries and considering the uncertainty in the ratio of total building area to population we decided there was not sufficient evidence to justify changing the acreage of developed area between 1930 and current conditions.

Roads/Railways: The extent of transportation land uses ca. 1930 was analyzed using the approach described in Section 2.1. The total area in these land uses ca. 1930 appears to have been slightly greater than current conditions due to the subsequent abandonment of many minor roads. Note that road

construction and maintenance prior to 1930 was more variable than current practices and may have produced greater sediment loading rates than modern roads, although this is not included in the simulations.

The net results of the land use analysis are summarized in Table 3-1 and Figure 3-1 through Figure 3-3. A much larger percentage of the watershed was in crops and pasture ca. 1930 compared to current conditions, although the percentage of the landscape in agricultural land uses (excluding woodland pasture) was still only about 20% based on available data on a whole watershed basis. (Note that the agricultural census shows that cropland area may have been significantly greater in 1934 than in 1929, which is the basis used here). Relative to pre-settlement conditions there are two major changes: a shift from evergreen forest to second-growth deciduous forest and a reduction in the area of forested wetlands. The assignments of land uses to hydrologic soil groups, as well as the average slope for land use class, were re-calculated based on the spatial distribution of land use types for each time period.

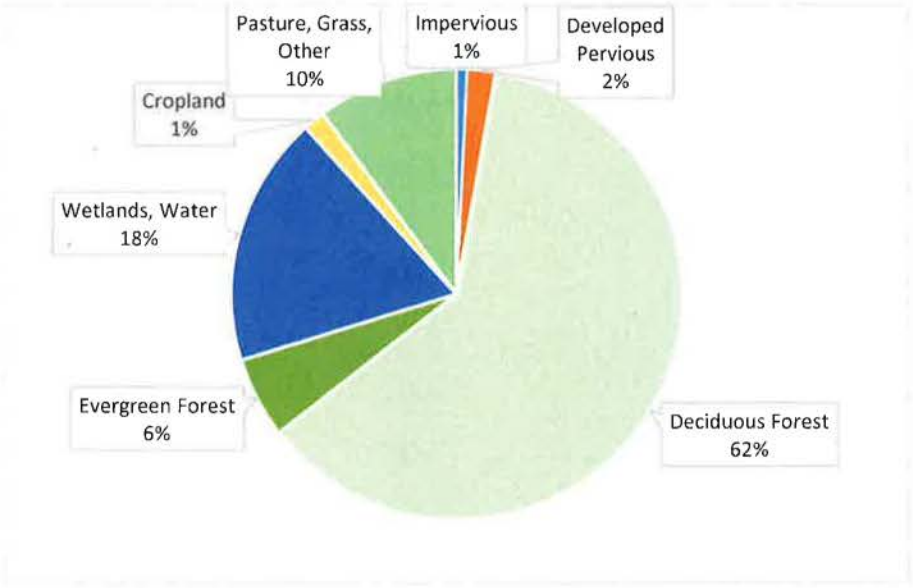

Figure 3-1. Land Use Distribution, 2006

Table 3-1. Historic Land Use Summary (acres)

Land Use/Land Cover (plus HSG)	2006	1930s	Pre-settlement
Dev OS Impervious	392	255	0
Dev MD Impervious	109	74	0
Dev HD Impervious	26	17	0
Road Impervious	1,879	1,936	0
Deciduous Forest (A,B)	47,981	40,323	23,719
Deciduous Forest (C,D)	138,165	136,380	34,051
Evergreen Forest (A,B)	6,933	6,591	33,930
Evergreen Forest (C,D)	10,489	8,777	91,141
Wetlands, Forested	18,856	18,559	107,201
Wetlands, Herbaceous	32,632	21,713	8,665
Grass/Shrub (A,B)	478	345	7
Grass/Shrub (C,D)	2,216	1,224	1,901
Pasture/Hay (A,B)	8,897	15,255	0
Pasture/Hay (C,D)	19,084	19,946	0
Row Crops (A,B)	1,279	4,195	0
Row Crops (C,D)	3,224	17,978	0
Row Crops w/ tile drains	324	0	0
Developed Open Space Pervious	5,925	3,821	0
Developed Medium Density Pervious	132	87	0
Dev High Density Pervious	11	7	0
Water (excluding simulated reaches)	2,643	2,452	1,390
Barren	330	2,070	0
Total	***302,005***	***302,005***	***302,005***

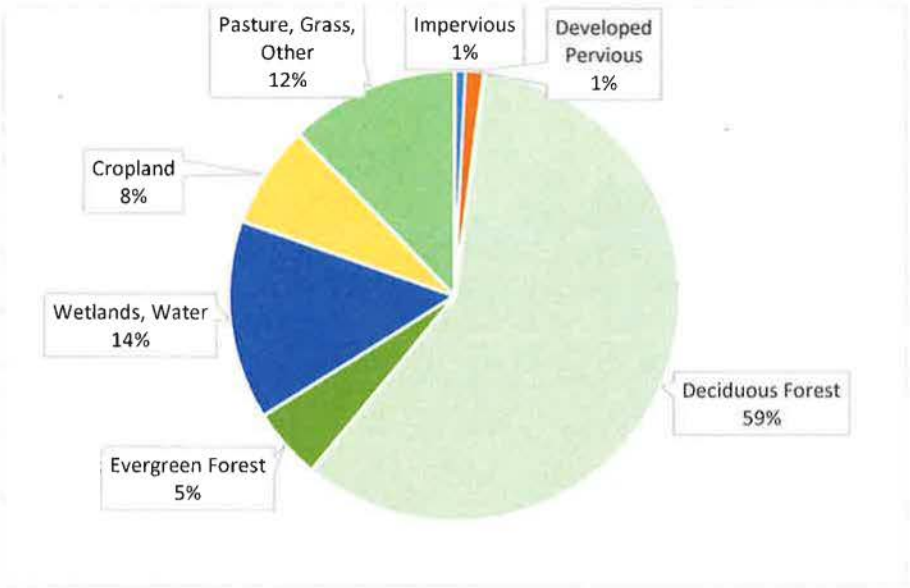

Figure 3-2. Land Use Distribution, ca. 1930

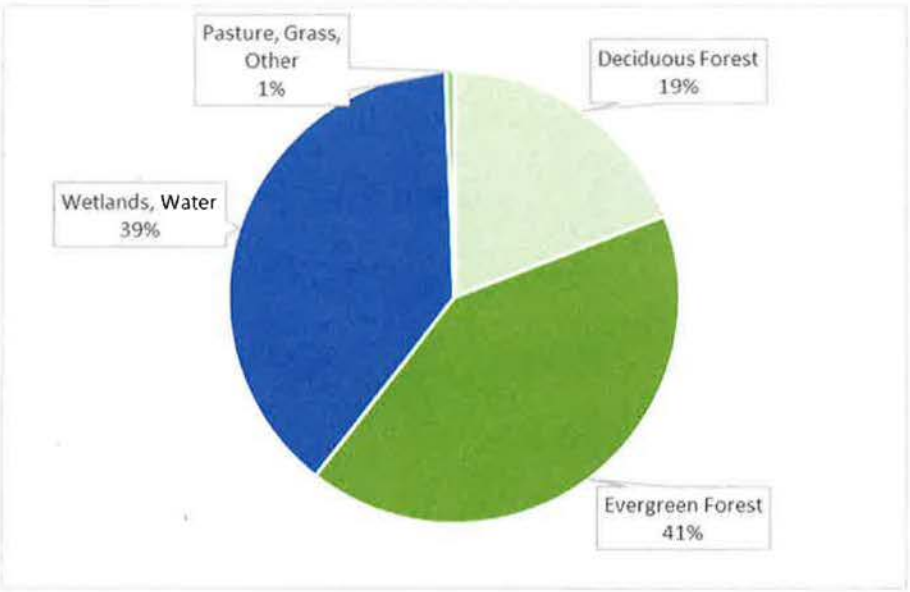

Figure 3-3. Land Use Distribution, Pre-Settlement

Pre-settlement and 2006 land use/land cover extents are mapped in Figure 3-4. A map is not provided for conditions ca. 1930 as these are inferred from a variety of spatial and non-spatial data sources as described above.

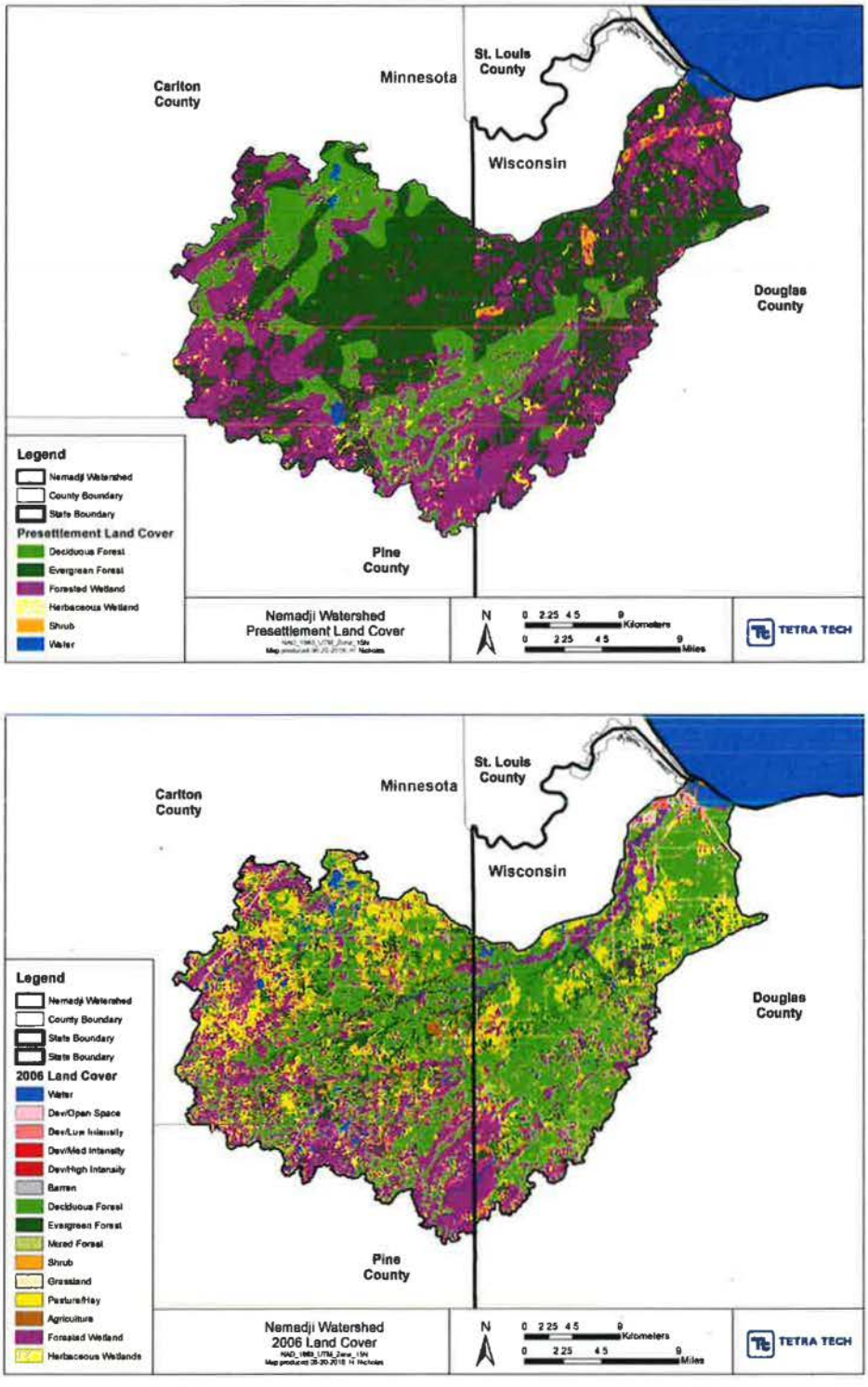

Figure 3-4. Pre-Settlement and 2006 Land Cover for the Nemadji River Watershed

3.2 HYDROLOGY

Changes in land use lead to changes in simulated hydrology, some expected and some not anticipated. In general, greater open space and greater impervious area after development would be expected to increase surface runoff and decrease evapotranspiration, which the model indeed shows. The pre-settlement average water yield across the watershed was 10.9 in/yr, while that for current conditions is 11.1 in/yr over the simulation period. For 1930 conditions, the total water yield is 11.3 in/yr. The differences are relatively small on an annual basis because of the importance of snowmelt volume. During the summer growing season the direct surface runoff, which drives upland sediment erosion, is noticeably higher for current conditions (Figure 3-5).

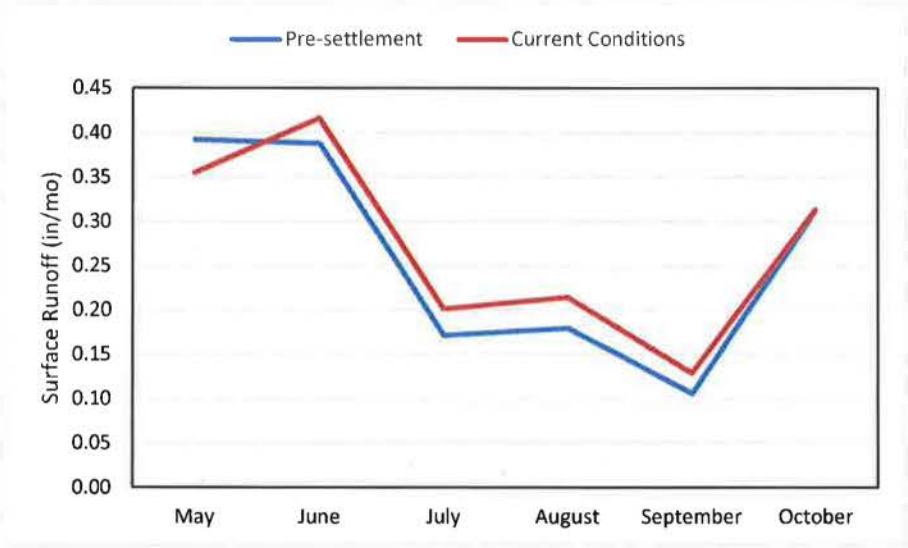

Figure 3-5. Comparison of Growing Season Direct Surface Runoff for Current and Pre-Settlement Conditions

The largest land use differences between pre-settlement and post-settlement conditions is a shift from mature evergreen forest to second-growth deciduous forest, along with a loss of forested wetlands and increase in cleared areas. The shift from evergreen to deciduous (or no) tree cover has a significant effect on the dynamics of snow melt as an evergreen canopy provides shade (and insulation) year round while a deciduous canopy does not. The denser canopy results in less solar energy reaching the snowpack, as well as less longwave energy exchange with the atmosphere, causing spring snow melt to be delayed. (There are many other differences in snowmelt dynamics between evergreen and other cover that are not discussed here, but the later spring snow melt is a key factor.) Under the right weather conditions this can allow more snowpack to persist until warm spring rains cause a sudden melt (see example in Figure 3-6). Higher soil moisture in spring due to later snowmelt can also increase the response to storms, although conifer stands in northern Minnesota are expected to have greater net evapotranspiration losses than deciduous forest on an annual average basis (Verry, 1986).

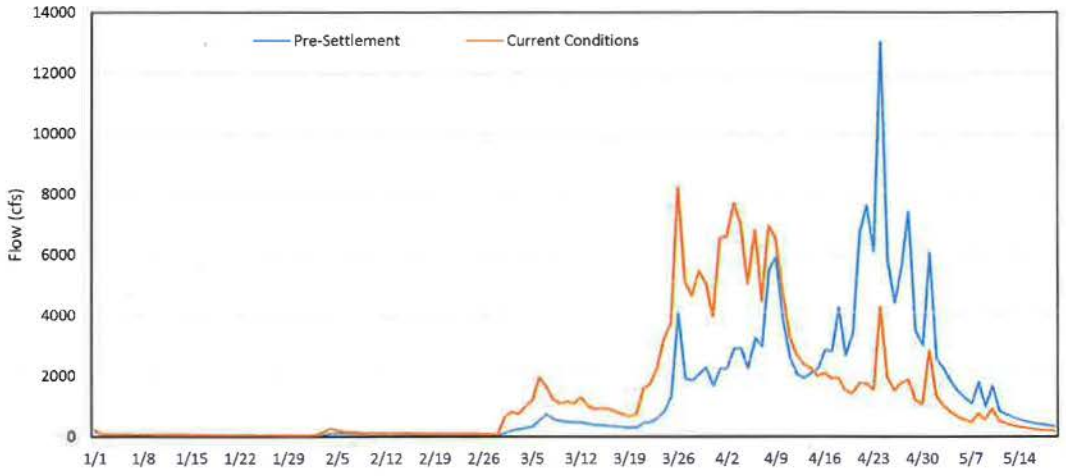

Figure 3-6. Example of Delayed Snowmelt under Pre-Settlement Evergreen Cover (Flow at Watershed Outlet, 1993 Weather)

3.3 UPLAND SEDIMENT LOADS

Upland sediment yield rates (tons/ac/yr) from the baseline model are summarized in Table 3-2. The highest per-acre loads are for cropland, followed by barren and shrub land uses, which primarily represent recent forest harvest and regrowth of timbered areas. The lowest sediment yield rates (excluding water) are for forest land uses (both evergreen and deciduous forest are averaged here), while the highest loading rates are for crops. These changes suggest that the total upland sediment load should be lower for pre-settlement conditions and likely higher around 1930, compared to current conditions, due to the decline in agricultural acreage in the watershed.

Table 3-2. Average Sediment Loading Rates by Land Use, Baseline Model

Land Use	Avg (tons/ac/yr)	Area	% area
Forest	0.031	204,573	67.5%
Wetland	0.045	51,488	17.0%
Shrub	0.339	2,699	0.9%
Pasture	0.133	28,030	9.2%
Crop	0.665	4,856	1.6%
Developed (pervious plus impervious)	0.208	6,595	2.2%
Water	0.000	2,645	0.9%
Barren	0.554	330	0.1%
Roads	0.135	1,879	0.6%

Consistent with these expectations, and despite other factors, such as changes in hydrology, the watershed model predicts dramatic changes in upland sediment load. Under current conditions, the average upland loading rate across the whole watershed is 22,120 tons/yr (based on weather inputs for 1993 – 2012). For pre-settlement conditions, the estimated upland loading is 12,263 tons/yr or 45% less than current conditions, based on the same weather time series. For conditions ca. 1930 the estimated upland loading to streams is 41,929 tons/yr or 90% greater than present conditions. While these changes are dramatic, upland loading represents only about one quarter of the sediment load delivered by the Nemadji River under current conditions, with the remainder coming from channel degradation processes.

3.4 CHANNEL, BANK, BLUFF, AND TOTAL SEDIMENT LOADS

In contrast to upland sediment loads, the model predicts relatively little change in sediment loads derived from the channel, banks, and bluffs over time (Table 3-2). Indeed, the net loading from channel/bank/bluff sources is estimated to be slightly higher under current conditions than during the ca. 1930 period, and there is little predicted reduction in channel sources for the pre-settlement period under the initial pre-development scenario. This reflects the assumption in the initial pre-settlement scenario that channel morphometry was not significantly different pre-settlement; alternative assumptions are investigated in Section 3.5. Under the assumptions of no significant change in channel morphometry the total sediment load delivered to the St. Louis River Estuary under conditions ca. 1930 is predicted to be 21% higher than under current conditions (using the same weather forcing), while the pre-settlement delivered load is predicted to be 15% less than the load under current conditions.

Table 3-3. Total Sediment Loads for Primary Scenarios

Time Period	Upland Load Delivered to Stream (tons/yr)	Net Channel, Bank, and Bluff Sources (tons/yr)	Total Load (tons/yr)
Current Conditions	22,120	66,276	88,396
ca. 1930	41,929	65,197	107,126
Pre-settlement (with current hydraulics)	12,263	63,066	75,329

These estimates are subject to considerable uncertainty. Of particular importance may be the contribution from bluff loads, which are roughly approximated in the model, as described in Section 2.3.1. Bluff load is expected to be a major component of the total sediment load in most tributaries to western Lake Superior. For instance, 67 percent of the sediment load in North Fish Creek is attributed to bluffs (Fitzpatrick et al., 1999), while Nieber et al. (2008) conclude that approximately 90% of the sediment load in the Knife River watershed originates from bank erosion and bluff slumping. NRCS (1998), based on a limited monitoring record, estimated that bluffs contributed 89% of the fine sediment source load and 98% of the delivered load from the Nemadji River. This study suggests that about 75 percent of the sediment load in the Nemadji River derives from bank erosion and bluff sources under current conditions, but how the loads associated with bluff slumping may have differed under historical conditions is not clearly known at this time. Riedel et al. (2005) found that mass wasting appeared to increase exponentially with rate of bankfull discharge normalized to watershed area in this basin. Riedel et al. also show that the rate of bankfull discharge is greater where cover has changed from coniferous to deciduous forest and in watersheds with reduced amounts of wetland area. These findings suggest that mass wasting contributions to the Nemadji were likely lower under pre-settlement conditions dominated by coniferous forests and with larger wetland areas.

Model predictions of change in channel sediment balance by reach over the 20-year simulation period are shown in Figure 3-7 through Figure 3-9. Because HSPF has a one-dimensional representation of stream reaches, both bed scour (incision) and bank scour/bluff contributions (widening) are represented as an equivalent net change in bed elevation by the model. The differences in simulated outcomes by reach are small, and most reaches in the lacustrine core of the Nemadji are predicted to be net sources of sediment under both current and historical conditions, consistent with the hypothesis that the Nemadji is a naturally unstable system that was experiencing channel degradation even under pre-settlement conditions.

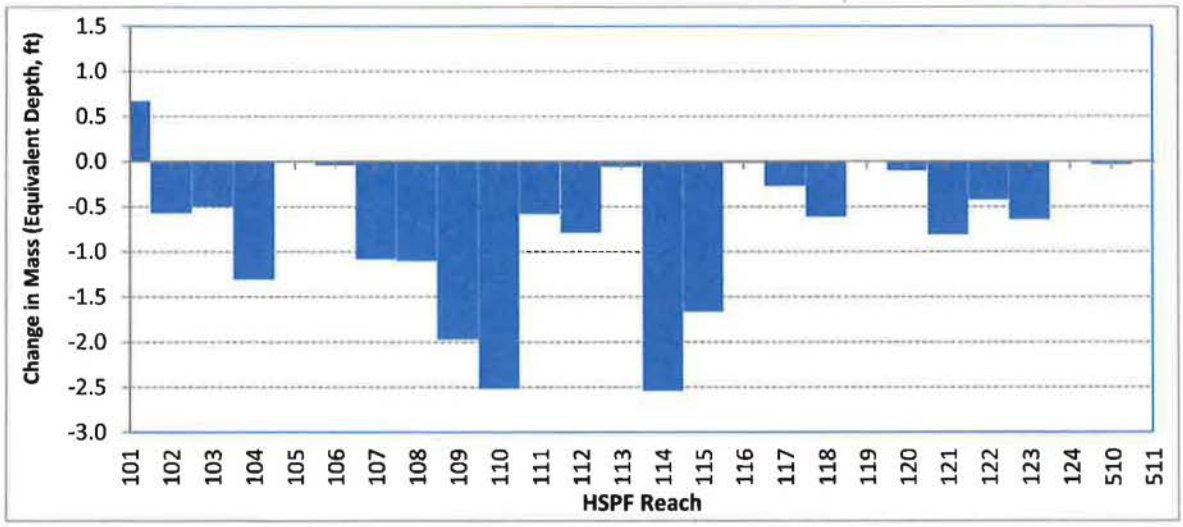

Figure 3-7. Predicted Change in Channel Sediment Balance over 20 years, Current Conditions

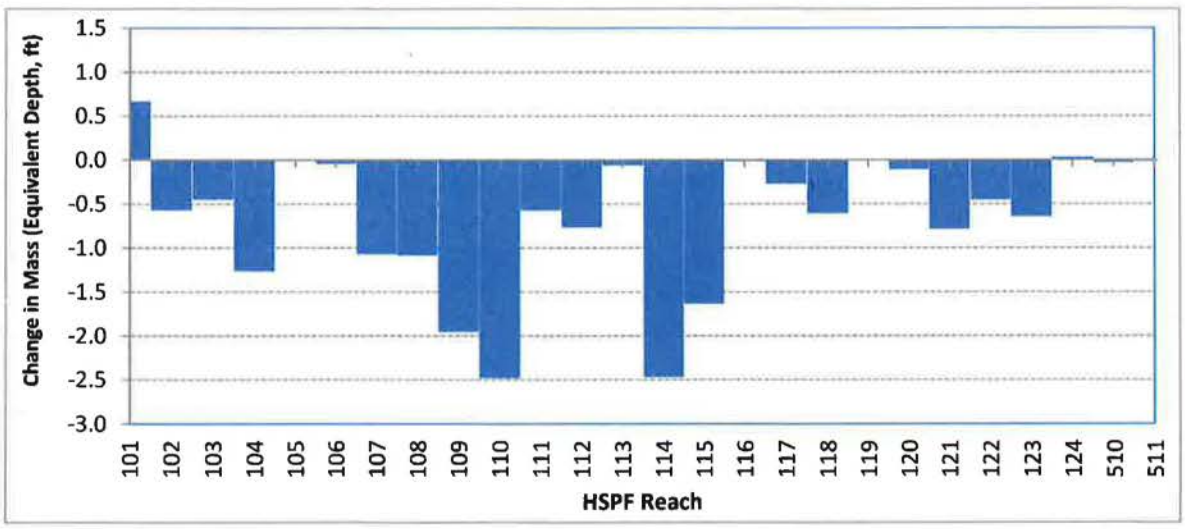

Figure 3-8. Predicted Change in Channel Sediment Balance over 20 years, ca. 1930 Conditions

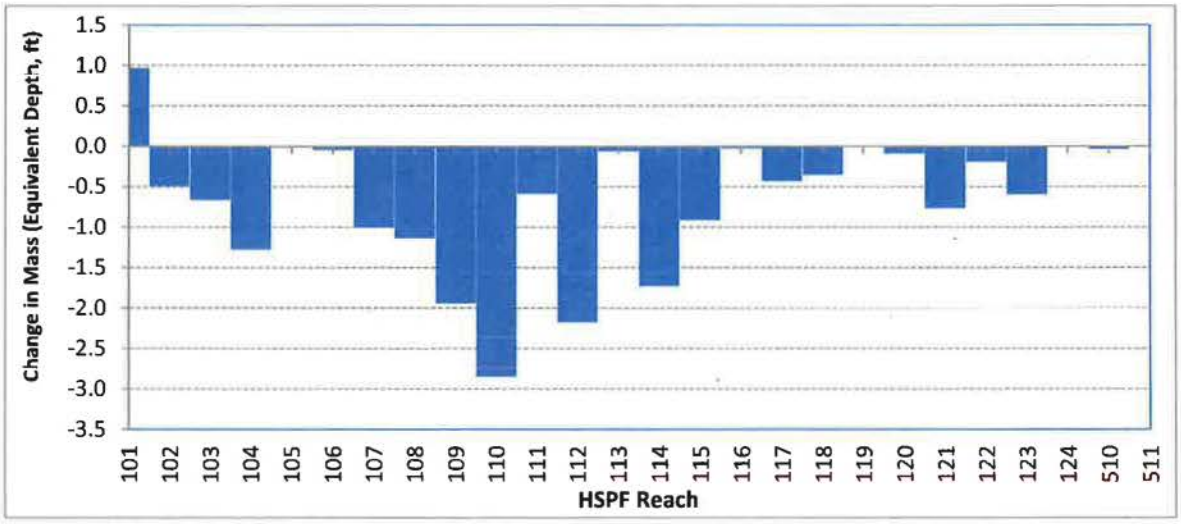

Figure 3-9. Predicted Change in Channel Sediment Balance over 20 years, Pre-Settlement

3.5 SENSITIVITY ANALYSIS FOR PRE-SETTLEMENT CONDITIONS

The results provided in Section 3.4 for pre-settlement conditions assume no change in channel geometry. As noted in Section 2.3.3, it is possible, but not certain, that somewhat different channel geometry conditions applied prior to human activity during the initial logging of the watershed. To test the sensitivity of results to assumptions regarding channel form a second, speculative version of the pre-settlement run was constructed. The major change for this run was an assumption of increased channel sinuosity, resulting in smaller effective slope and greater reach length. In many parts of the Nemadji, the river belt width is constrained by valley walls, so this increase in sinuosity, if it applied, would likely not be great. The scenario assumes a 20% increase in sinuosity. In addition, channels are assumed to have been somewhat less incised through specification of a channel bank width to depth ratio of 2, rather than 1.5. Both assumptions are speculative, but thought to be physically reasonable based on best professional judgment. Finally, the Manning's roughness coefficient for the floodplain was increased from 0.06 to 0.12 to reflect heavily wooded conditions, consistent with values cited in Arcement and Schneider (1989). The revised channel conditions were then used to regenerate the HSPF FTables for the simulation. Channel slopes (which influence the calculation of shear stress) and lengths were also modified in the HSPF input file. The upland simulation is unchanged from the pre-settlement run.

Under these conditions, the total net sediment loading generated from bank and bed scour is predicted to average 48,139 tons/yr, or about 24 percent less than the amount generated under the pre-settlement scenario without hydraulic modifications (see Table 3-4, which repeats Table 3-3 with the addition of the alternate pre-settlement scenario). Even so, the sediment load generated from the channel is still predicted to be much larger than the upland load (12,263 tons/yr). Under these modified pre-settlement conditions the total sediment load delivered to the St. Louis River Estuary was 60,402 tons/yr, 32% less than the load under current conditions, and 44 % less than when the watershed was most degraded (ca 1930)

Table 3-4. Total Sediment Loads for Primary and Alternate Scenarios

Time Period	Upland Load Delivered to Stream (tons/yr)	Net Channel, Bank, and Bluff Sources (tons/yr)	Total Load (tons/yr)
Current Conditions	22,120	66,276	88,396
ca. 1930	41,929	65,197	107,126
Pre-settlement (with current hydraulics)	12,263	63,066	75,329
Pre-settlement modified hydraulics	12,263	48,139	60,402

Changes in channel sediment balance by reach under this alternative scenario are shown in Figure 3-10. Comparing to the pre-settlement run without modifications to channel geometry (Figure 3-9), the pattern remains similar, but the amounts of predicted degradation are less.

As with the results presented earlier, all these results are reasonable, but uncertain, and depend on the accuracy of the existing coarse-resolution model and the assumptions made to describe historical conditions. The true magnitude of change, for which direct evidence is not available, could differ significantly from these projections; however, the qualitative differences between scenarios are believed to be reasonable.

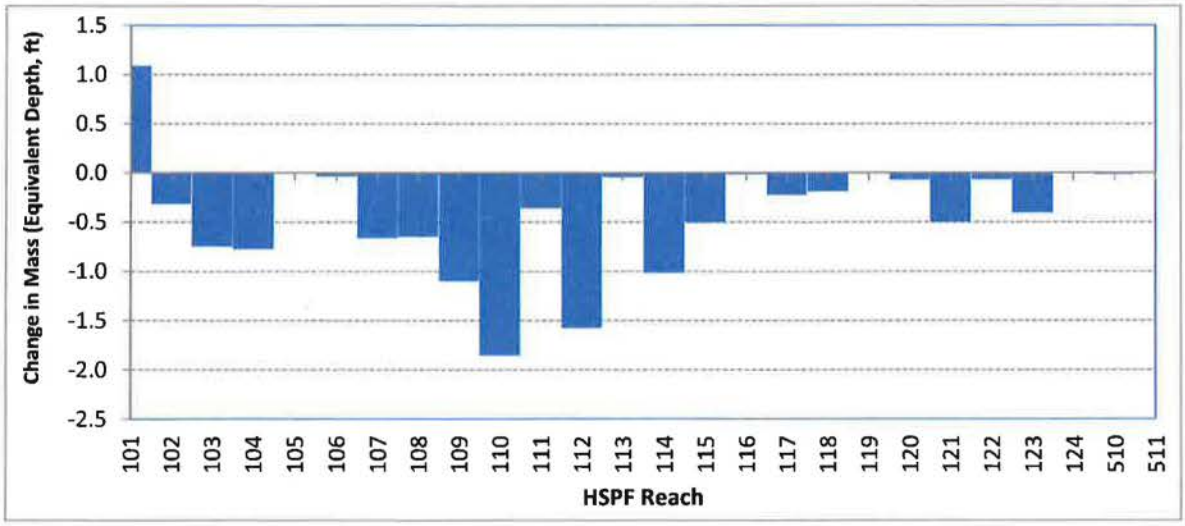

Figure 3-10. Predicted Change in Channel Sediment Balance over 20 Years, Pre-settlement Conditions with Modified Reach Hydraulics

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4.0 SUMMARY

An existing HSPF watershed model was used to examine likely changes in sediment load in the Nemadji River for current conditions relative to pre-settlement conditions and conditions ca. 1930, soon after harvest of the virgin forest had had ceased and when agriculture was more extensive than at present. HSPF is a well-established and widely used tool for dynamic simulation of hydrology and upland sediment loading. The model also provides predictions of scour and deposition of sediment from and to the channel bed and banks as a function of channel geometry and simulated flows. This aspect of the model is perhaps weaker, as the channel is represented as a one-dimensional approximation. Also, in absence of adequate data on changes in channel morphology since pre-settlement times, a sensitivity analysis was conducted to identify the likely range of possible pre-settlement channel conditions. Further, the model is developed at a relatively coarse spatial scale without the benefit of detailed field measurements of channel dimensions, relying instead on regional hydraulic geometry relationships developed for the Nemadji by Magner and Brooks (2008), and thus cannot resolve the fine-scale details of reach erosion processes. Nonetheless, the model provides a credible fit to observed TSS concentrations in the Nemadji and is believed to provide a useful basis for historical comparisons.

Figure 4-1 summarizes the results of the simulation of sediment load delivered from the Nemadji River to the St. Louis River Estuary, based on consistent 20-year weather forcing. The upland sediment loading is predicted to have increased substantially from pre-settlement conditions to the 1930s, and to have subsequently declined; however, the predicted net load generated from bank, bluff, and channel processes shows only small changes when current hydraulic geometry relationships are assumed to apply. The sensitivity analysis of pre-settlement conditions with greater channel sinuosity (and several other changes) does predict a substantial reduction in bank, bluff, and channel-associated sediment load; however, those results are again speculative as direct information is not available on the pre-settlement channel form.

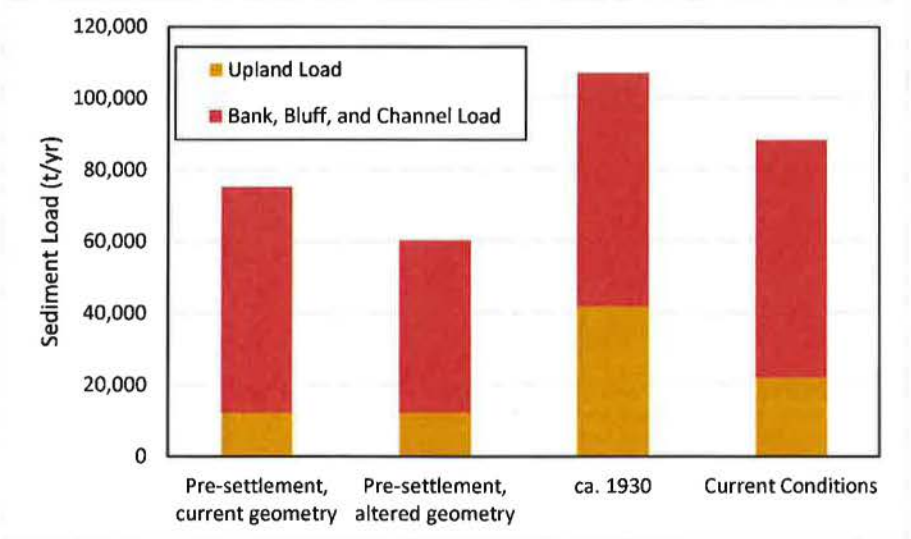

Figure 4-1. Summary of Current and Historic Simulations of Annual Average Sediment Load Delivered from the Nemadji River to the St. Louis River Estuary

It should be noted that conditions ca. 1930 were selected to represent peak agriculture, in part because of the availability of the Bordner surveys at about this time. A comparison of harvested cropland acres by county from the agricultural census (Figure 4-2) suggests that peak agricultural activity may actually have occurred between 1934 and 1945; possibly accompanied by even greater upland sediment loads, although the increased acreage may have been offset to some extent by better tillage practices.

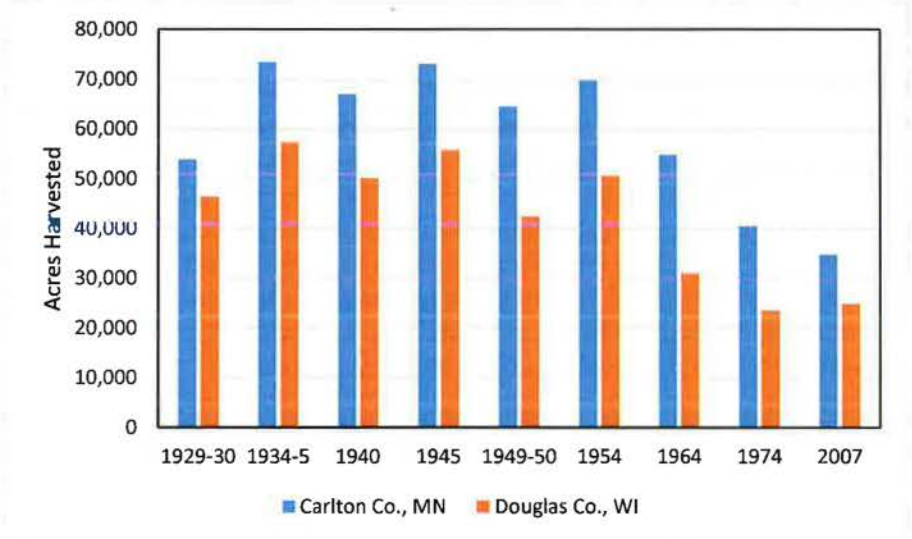

Figure 4-2. County-Level Trends in Harvested Cropland over Time

Finally, the analysis is based on a consistent set of weather data (1993 – 2012 observations) and does not account for any changes in climate over time.

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Appendix 6

Sediment Characteristics of Northwestern Wisconsin's Nemadji River, 1973-2016

(Pertains to management action 6.05)

USGS
science for a changing world

Prepared in Cooperation with the Wisconsin Department of Natural Resources

Sediment Characteristics of Northwestern Wisconsin's Nemadji River, 1973-2016

By Faith A. Fitzpatrick

Open-File Report

13 January 2020

U.S. Department of the Interior
DAVID BERNHARDT, Secretary

U.S. Geological Survey
Jim Reilly, Director

U.S. Geological Survey, Reston, Virginia: 2020

Cover photograph of the Nemadji River near County Highway C, on July 12, 2016, by Molly Wick, formerly of the Wisconsin Department of Natural Resources

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Technical field assistance was provided by Josef Schuler, Lucas Weegman, and Scott Hagar of the USGS Upper Midwest Water Science Center (UMidWSC) for the collection and processing of suspended sediment, bedload, and bed material samples. Troy Rutter, also of the UMidWSC compiled the data needed for the modified Einstein procedure for calculation of total sediment loads.

The modified Einstein procedure results were reviewed by Christopher Ellison (formerly with the U.S. Geological Survey, Wyoming Water Science Center). Colleague reviews were provided by Joel Groten (U.S. Geological Survey, UMidWSC) and Molly Wood (U.S. Geological Survey, Idaho Water Science Center).

Contents

Figures

Tables

Conversion Factors

U.S. customary units to International System of Units

Multiply	By	To obtain
	Length	
foot (ft)	0.3048	meter (m)
	Area	
square foot (ft^2)	929.0	square centimeter (cm^2)
square foot (ft^2)	0.09290	square meter (m^2)
	Volume	
cubic foot (ft^3)	28.32	cubic decimeter (dm^3)
cubic foot (ft^3)	0.02832	cubic meter (m^3)
	Flow rate	
foot per second (ft/s)	0.3048	meter per second (m/s)
cubic foot per second (ft^3/s)	0.02832	cubic meter per second (m^3/s)
	Mass	
ton, short (2,000 lb)	0.9072	metric ton (t)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F = (1.8 \times °C) + 32.$$

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows:

$$°C = (°F - 32) / 1.8.$$

Abbreviations

USGS	U.S. Geological Survey
WDNR	Wisconsin Department of Natural Resources
UMidWSC	Upper Midwest Water Science Center
SSC	Suspended sediment concentration
TSS	Total suspended solids

Sediment Characteristics of Northwestern Wisconsin's Nemadji River, 1973-2016

By Faith A. Fitzpatrick

Abstract

The Nemadji River is part of the U.S. Environmental Protection Agency's St. Louis River Area of Concern (AOC). One of the goals of the AOC cleanup is to reduce sediment loads from the Nemadji River into the estuary of the St. Louis River. The goal of this study was to determine if there was a reduction in sediment loads over the last 45 years due to land use improvements. A change in suspended sediment sampling and analytical methods over the period of interest necessitated a comparison the two types of data before any conclusions could be drawn. Suspended sediment samples from the Nemadji River near South Superior (USGS identification number 04024430) were collected by the U.S. Geological Survey (USGS) from 1973 through 1986 using isokinetic samplers with width- and depth-integrated procedures and suspended sediment concentration (EWI SSC) laboratory analyses. From 2006 through 2016 the Wisconsin Department of Natural Resources and the Minnesota Pollution Control Agency collected grab samples and conducted total suspended solids (grab TSS) laboratory analyses. Previous studies in the region had shown that TSS concentration may be much less than SSC, and for this study

regression equations were developed to provide an adjustment factor to the grab TSS data before they could be compared to the EWI SSC data.

In 2015-16, the USGS, in cooperation with the Wisconsin Department of Natural Resources, conducted a comparison study of the USGS and State methods for collection and laboratory analyses of suspended sediment concentration and loads. From this comparison, regression equations were generated between the EWI SSC and grab TSS data. In addition to suspended sediment, bedload and bed material samples also were collected. The modified Einstein procedure was used to estimate total sediment discharge of the 2015-16 period. Thus, the relations among suspended sediment load, bedload, and total sediment load could also be examined.

As part of the 2015-16 study, historical streamflow data were examined over the two periods of suspended sediment data collection. Mean annual flows during 2006-16 were about 84 percent less than during 1973-86. In contrast, two extreme floods in 2011 and 2012 were over 2.5 times larger than any peak flow in the 1973-86 period. The 2009-16 annual total sediment loads ranged from a low of 18,000 tons per year for a relatively dry year in 2015 to almost 180,000 tons per year in 2012 that included a peak of record. Bedload ranged from 20 percent of the total sediment load during low mean annual flow years and dropped to 5 to 6 percent of the total load during high flow years.

Using adjusted TSS concentrations (TSSadj), the TSSadj curve for 2006 through 2015 had a similar slope but a lower intercept than its 1973-86 SSC-based counterpart. Although not statistically significant, the negative offset resulted in a potential reduction of about 15 percent of the annual suspended sediment loads for an example data set from 2009-16. These results suggest that land use management has started to influence suspended sediment loads. Altogether,

these various data sets collected over different periods and using different methods helped to describe the Nemadji River's sediment characteristics as well as provide a calibration tool for future sediment data collections.

Introduction

The Nemadji River (fig. 1) is part of the U.S. Environmental Protection Agency's (EPA) St. Louis River Area of Concern (AOC). One of the Beneficial Use Impairments (BUI) is excessive loading of sediment and nutrients (St. Louis River Alliance, 2015). A major goal of the AOC cleanup is to reduce sediment loads in the Nemadji River, which is one of the largest contributors of sediment to Lake Superior (Robertson, 1996). The Nemadji Basin Plan (Natural Resources Conservation Service, 1998) estimated the average annual suspended sediment discharge at the mouth of the Nemadji to be about 130,000 tons per year (tons/year).

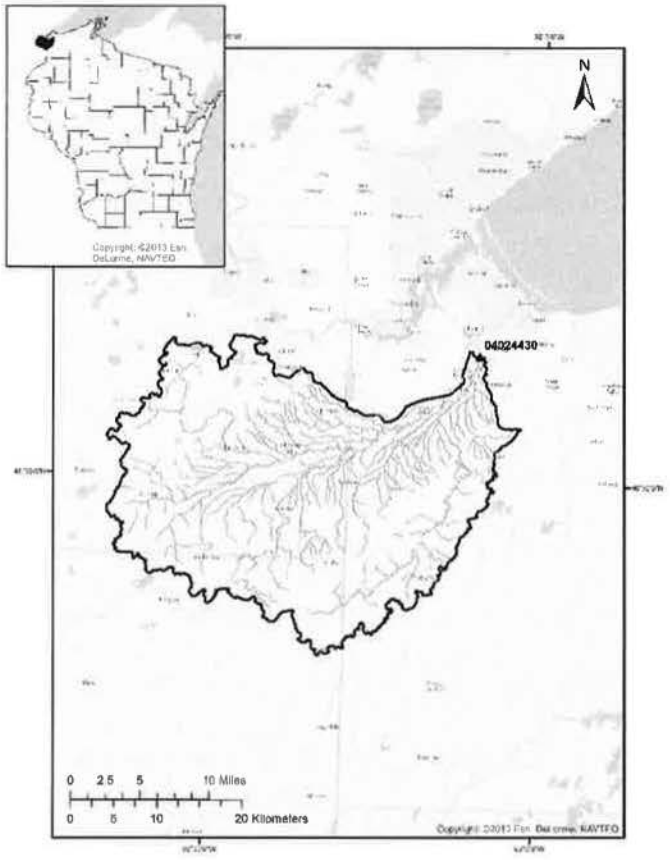

Figure 1. Location of the Nemadji River streamgage (U.S. Geological Survey identification number 04024430).

A U.S. Geological Survey (USGS) streamgage has been operating on the Nemadji River near South Superior, Wisconsin (USGS identification number 04024430) since 1970 (fig. 1). Over the last 45 years, sediment samples were collected and analyzed with two different sets of standard sampling and laboratory methods used by the USGS and state agencies. The USGS used an isokinetic sampler with width- and depth- integrated procedures (EWI) (Edwards and Glysson, 1999; Gray and Landers, 2014) and suspended-sediment concentration (SSC) laboratory methods and sampled from 1973-86. The Wisconsin Department of Natural Resources

(WDNR) and Minnesota Pollution Control Agency (MPCA) used the grab sampling and total suspended solids (TSS) laboratory methods, starting in about 2006. State agencies adopted the grab TSS procedure over the EWI SSC procedure because it is faster and less expensive and it was thought to be adequate for assessing sediment-related impairments as well as other water quality issues (Ellison and others, 2014). Comparison studies of grab TSS and EWI SSC have shown that grab TSS samples are biased negatively compared to EWI SSC samples, especially when the samples had more than 20 percent sand (Gray and others, 2000; Ellison and others, 2014). The negative bias could be caused by the sampling, laboratory procedure, or both.

For the Nemadji River, the gaps in the sediment data collection record combined with the different types of data for the two time periods required the development of a calibration between the two data types. Additionally, it commonly difficult to separate upstream changes in suspended sediment supply from year-to-year hydrologic variability and thus a method comparing sediment concentration to water discharge curves used by Warrick (2014) was adopted for helping to distinguish whether there were changes in the suspended sediment supply potentially associated with land management practices.

In 2015-16, the USGS, in cooperation with the WDNR, conducted a methods and laboratory comparison study for suspended sediment collected at the Nemadji River streamgage. In addition to suspended sediment, bedload and bed material samples also were collected. These data were used to help describe present sediment conditions as well as provide a calibration relation to be able to compare historical suspended sediment data sets. The results from the comparison will be used by the WDNR to identify if sediment loading is increasing, decreasing, or staying the same. This study was part of a larger assessment of the Excessive Loading of Sediment and Nutrients BUI in the Nemadji watershed, funded by the EPA in 2015, which also

included evaluation of macroinvertebrate and fish communities in the Lower Nemadji River and modeling historic sediment loads (Minnesota Pollution Control Agency and Wisconsin Department of Natural Resources, 2015).

Purpose and Scope

This report describes three main study objectives related to sediment data collected at the USGS Nemadji River streamgage near Superior, Wisconsin. The first objective was to develop a calibration curve between EWI SSC and grab TSS data. The second objective was to compare the EWI SSC suspended sediment rating curves from 1973-86 data with adjusted TSS-based curves from 2006-15 data and determine if there was a potential change in suspended sediment loads. The third objective was to describe 2015-16 total sediment loads, which were determined directly by measuring suspended sediment loads and bedload and indirectly by estimating total sediment discharge using the modified Einstein procedure.

The USGS collected 12 sets of suspended, bedload, and bed material samples at the Nemadji River streamgage for 11 sampling events from July 2015 through July 2016. For each event, two types of suspended sediment samples were collected including a grab sample from the middle of the river and a composite EWI depth-integrated sample from a representative cross section of the river. Both types of suspended sediment samples were submitted for both SSC and TSS laboratory analyses. The resulting four types of concentration data were used to develop log 10 based regression relations between EWI SSC and grab TSS. Bedload and bed material samples also were collected during the sampling events and submitted to the laboratory for particle size analyses. The modern (2006-15) suspended sediment concentrations relations were used to compare historical (1973-86) sediment curves and discharges. Lastly, a modern 6-yr TSS

dataset collected by the MPCA was available for calculation of suspended and total sediment discharges.

Physical Setting

The Nemadji River drains 430 square miles and empties into Lake Superior in Superior, Wisconsin (fig. 1). The watershed straddles eastern Minnesota and northwestern Wisconsin. The USGS streamgage is 2 miles south of Superior, Wisconsin and the river has a drainage area of 420 square miles at the streamgage. The streamgage has operated continuously from 1973 to present.

The steep, mainly forested Nemadji River basin is known for problems with flashy runoff and large sediment discharges (Robertson, 1996; Natural Resources Conservation Service, 1998). The average slope of the river is 2 ft per mile but the thick clayey glacio-lacustrine deposits that cover much of the watershed promote rapid runoff during rainfall events (Natural Resources Conservation Service, 1998; Reidel and others, 2001; 2005). Mass wasting and channel incision along deeply incised stream valleys contribute a large portion of the load (Natural Resources Conservation Service, 1998; Reidel and others, 2001; 2005).

Methods

Methods first included gathering published historical and ongoing sediment concentration, water discharge, and sediment load data collected by the USGS, WDNR, and the MPCA at the Nemadji River streamgage. Additional comparative measurements of suspended sediment, bedload, and bed material were collected by the USGS from July 2015 through July 2016 and analyzed by the USGS Kentucky Sediment Laboratory and Wisconsin State Laboratory of Hygiene (WSLH). Instantaneous total sediment discharges for 2015-16 were calculated from

summing the measured suspended sediment discharge and bedload discharge. Estimations of total sediment discharge were calculated with the modified Einstein procedure. Sediment data processing followed four main steps – tabulation, evaluation, editing, and verification, following guidelines in Porterfield (1972), Guy (1969) and the USGS Office of Surface Water Memorandum 91.15. Lastly, analysis of covariance (ANCOVA) was used to for comparing sediment concentration-water discharge rating curves.

Historical Suspended Sediment Data Mining and Sediment Rating Curves

Historical sediment data at the Nemadji River streamgage included SSC collected and analyzed by the USGS from 1973 through 1986 and TSS collected and analyzed by the WDNR and MPCA from 2006 through 2015. Data were gathered from the USGS National Water Information System (NWIS) (U.S. Geological Survey, 2017) and the WDNR Surface Water Integrated Monitoring System (SWIMS) and included SSC, instantaneous water discharge, and suspended sediment loads. The TSS data were collected by either the WDNR or MPCA on a monthly plus events basis for a total of about 30 to 40 samples per year.

The continuous-record streamgage on the Nemadji River has operated by the USGS since 1973 with streamflow data consisting of instantaneous, mean daily, mean annual, and annual instantaneous peak flows. These data were retrieved, along with the historical sediment data, from the NWIS (U.S. Geological Survey, 2017).

TSS mean daily and annual sediment load computations for 2009-16 were made using USGS methods and the Graphical Constituent Loading Analysis System (GCLAS) (Porterfield, 1972; Koltun and others, 2006). The monthly plus events sampling frequency of 30 to 40 samples per year at a variety of water discharges were used to calculate mean daily suspended sediment load. The data sets were sparser than the daily sampling frequency recommended for

GCLAS and the suspended sediment loads are considered estimates. TSS data from 2006-08 were too few to calculate mean daily or annual discharges using this method.

2015-16 Sediment Data Collection, Laboratory Analyses, and Discharge Calculations

From July 2015 through July 2016, 12 sets of sediment samples were collected by USGS at the Nemadji River streamgage along with ongoing water discharge measurements during 11 sampling events from July 2015 through September 2016 (table 1, fig. 2). Water discharge measurements are routinely collected as part of existing funding from WDNR and the USGS Cooperative Water Program to run the realtime continuous-record streamgage (http://waterdata.usgs.gov/usa/nwis/uv?site_no=04024430). A range of water discharges from 300 to over 12,000 cubic feet per second (ft^3/s) were sampled for sediment. The sampling was spread among seasons, except for the winter months of December through March when the river was frozen. The July 2016 flood had an exceedance probability of less than 0.002 (Fitzpatrick et al., 2017).

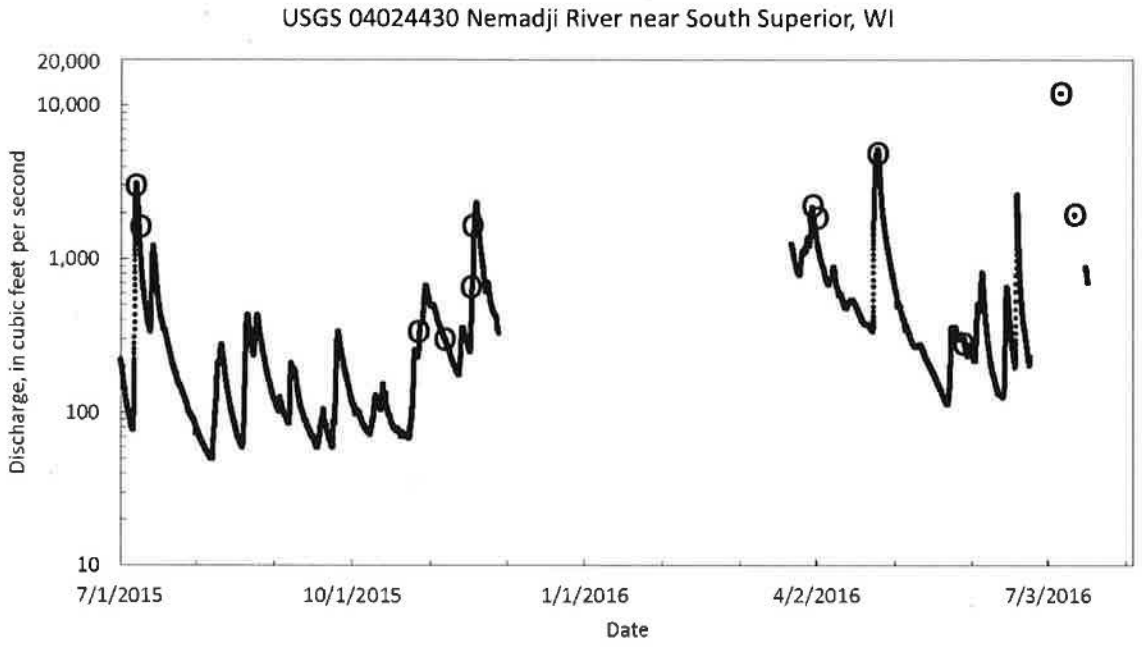

Figure 2. Hydrograph of instantaneous discharge for the Nemadji River streamgage near South Superior, Wisconsin during the study period from July 2015 to July 2016 (U.S. Geological Survey identification number 04024430). Sediment sampling dates shown in circle symbols. Instantaneous discharge data are not available for winter months and for a short time during the July 2016 flood. Data from the National Water Information System.

Table 1. Date and discharge associated with suspended sediment and bedload samples collected by the U.S. Geological Survey at the Nemadji River near Superior, Wis. (identification number 04024430) from July 2015 through July 2016.

Date	Time	Discharge (cubic feet per second)	Comments
7/7/2015	10:29	2,960	None
7/8/2015	10:23	1,540	None
10/28/2015	09:15	385	None
11/6/2015	9:25	300	None
11/17/2015	10:59	654	None
11/18/2015	10:43	1,870	None
3/31/2016	10:16	2,090	None
3/31/2016	15:58	1,950	None
4/25/2016	12:09	4,790	None
5/31/2016	12:08	250	None
7/12/2016	20:29	12,800	Suspended sediment grab sample only
7/15/2016	10:07	1,920	No bedload sample

Suspended Sediment

Cross-sectional composite samples of suspended sediment were collected with a depth-integrating model D-74 sampler using standard USGS methods, including EWI and equal transit rate (ETR) (Edwards and Glysson, 1999; Gray and Landers, 2014). Stream water was collected at 15-20 verticals across the channel cross section with a distance between verticals of about 5 ft. The resulting composite sample, abbreviated in the report as EWI SSC, includes the horizontal and vertical variability in flowing water. These samples were collected followed similar techniques used by the USGS to collect suspended sediment data at the streamgage in 1973-86. The exact depth integrating sampler used in 1973 is not known.

A grab sample of suspended sediment was collected during cross-sectional sampling using a weighted open-bottle sampler, replicating sampling methods used by WDNR and MPCA for the TSS sampling. The grab sample, abbreviated as grab TSS, was collected from approximately 0.3 to 1 m below the water surface in the centroid of flow. The WDNR was able to get a grab sample on 7/12/2016 close to the peak of flooding when the EWI sample was not able to be transported to the site as quickly as needed.

The EWI and the grab sample were each split into two samples using a churn splitter. One set of samples was analyzed for SSC and particle-size determinations at the Kentucky Water Science Center Sediment Laboratory using standard filtration methods (Guy, 1969) and under a quality assurance plan (Shreve and Downs, 2005). The exact particle size determination used depended on the concentration of the sediment and the minimum amount needed for analyses. The second set of samples were analyzed for TSS at the Wisconsin State Laboratory of Hygiene using standard method for the examination of water and waste-water method 2540D (American

Water Works Association, 2012). In hindsight, the churn splitter added another possible source of error to the sediment concentration results, since biases have been documented, both positive and negative, associated with the particular settling velocity of the particles and the amount of sand (Barr, 2018).

The instantaneous water discharge at the time of sampling was used with the results from the concentration analyses to compute an instantaneous suspended sediment load using the equation:

$$Q_s = Q_w \times C_s \times K$$

where Q_s is the suspended sediment load, in tons (English short tons) per day (tons/day), Q_w is the instantaneous streamflow (water discharge), in cubic feet per second (ft^3/s), C_s is suspended sediment concentration (SSC or TSS), in milligrams per liter (mg/L), and K is a coefficient (0.0027) to convert the units of measurement of water discharge and SSC or TSS into loads of tons/day and assumes a specific gravity of 2.65 (Porterfield, 1972). Sometimes the water discharge for the EWI samples was different than the grab sample if the flow conditions were changing between the two types of sampling. The EWI samples generally took about 45 minutes to collect, whereas the grab sample represents more or less a minute of time.

Bedload and Bed Material

Bedload samples were collected with a BL-84 sampler using the single equal-width-increment (SEWI) method (Edwards and Glysson, 1999). Bedload samples were collected at the same verticals as the suspended sediment samples. A sample bag mesh size of 0.125 mm was employed for all events. The more standard 0.250-mm mesh bag was used for the first event for the two out four cross sectional passes, otherwise two traverses of the channel cross section were completed. The sampler was held on the bottom for usually 30 seconds at each vertical. The

composited bedload samples were dried at 105 deg C, weighed, and sieved for sand and gravel sizes at the preparatory laboratory at the Wisconsin Water Science Center to obtain bedload mass and sand and larger particle sizes.

For computing bedload discharge, the "total cross-section method" was used (Edwards and Glysson, 1999). This method requires that (1) the sample times at each vertical are equal, (2) the verticals were evenly spaced across the cross section, and (3) the first sample was collected at half the sample width from the starting bank or edge with active bedload transport. The bedload discharge was calculated as:

$$Q_b = K \times (W/t) \times M$$

where Q_b = bedload discharge, in tons per day; K is a conversion factor of 0.381 for the type of sampler (the BL-84 has a 3-inch wide opening); W = total width of the channel from where the bedload samples were collected, in feet; t = total time the sampler was on the bed, in seconds; and M = total mass of sample collected from all verticals sampled in the cross section, in grams.

The measured bedload discharge (Q_b) and suspended-sediment discharge (Q_s) were summed to get measured total sediment discharge (Q_{Mts}):

$$Q_{Mts} = Q_b + Q_s$$

This simple addition of the two loads assumes 100 percent efficiency of the BL-84 bedload samples and that the bedload sampler is sampling the approximately 3-inch unsampled zone near the bed not reached by the suspended sediment sampler (Edwards and Glysson, 1999).

Bed material samples were collected at each of the verticals using methods described in Edwards and Glysson (1999). Particle size determinations were done using standard sieve methods at the Kentucky Water Science Center Sediment Laboratory using standard sieving methods (Guy, 1969).

Modified Einstein Procedure

The USGS program MODEIN for the modified Einstein procedure was used to estimate a total sediment load (Einstein, 1950; Colby and Hembree, 1955; Colby and Hubbell, 1961; Stephens, 1985). This procedure is appropriate for alluvial channels that have mixed sand and gravel beds finer than 16 mm. Additional field data needed for the procedure included water discharge, average water depth, top width of channel, water temperature, particle size of suspended sediment, particle size of bed material, and suspended sediment concentration. Bedload discharge (Q_b) was estimated by subtracting the calculated suspended sediment discharge (Q_s) from the calculated total sediment discharge (Q_{Cts}):

$$Q_b = Q_{Cts} - Q_s$$

The Einstein procedure for calculated estimates of total sediment load and bedload were compared to load estimates based on the sum of the measured suspended sediment and bedload.

Suspended Sediment Rating Curves

Sediment rating curves for SSC and TSS data were constructed by use of standard procedures in Glysson (1987). The graphical based curves represent the fit of the relation between water discharge (Q_w) and sediment concentration (C_s) and were used for both SSC and TSS. Ordinary least squares regression analyses were applied in Excel to the base-10 logarithm transformed concentration and discharge data following methods used in Gray and others (2000) and Warrick (2014). The rating curves used in this study are of the power-law form

$$C_s = a \times Q_w^{\,b}$$

where a and b are fitted values for the intercept and slope, respectively, of the least squares regression line.

The regression technique was also used for comparison of base-10 logarithm pairs of SSC and TSS data collected in 2015-16. The regression was used to adjust the historical TSS data before it could be compared with the historical SSC data.

Historical data sets of log-transformed SSC and TSS (adjusted by the paired data) data were compared for statistically significant differences using analysis of covariance (ANCOVA) (Clausen and Spooner, 1993). This technique was used in the R Data Analysis and Graphics Program (R Core Team, 2016) to determine if there was a statistical difference in the log-transformed suspended sediment rating curves from 1973-86 compared to the 2006-2015 data. The ANCOVA ($\alpha = 0.05$) determines if there is a significant difference between the slopes and the intercepts of the regressions for the two time periods.

Hydrologic Conditions 1973-2015

For the Nemadji River, mean annual flow from 2006-15 during the TSS sampling was on average 84 percent of mean annual flow from 1973-86 during SSC sampling (fig. 3). During the SSC sampling mean annual flows were highly variable. The highest mean annul flow was measured in 1986. In contrast, the lowest mean annual flow on record was in 2007 during the TSS sampling, with the 10-year moving centered average in mean annual flow increasing from about 2010 to present.

Instaneous peak flows are also helpful for distinguishing years with large floods, even when annual mean flows may be lower than usual (fig. 4). Peak flows during the TSS data collection were higher compared to the SSC data collection. Two large floods in 2011 and 2012 were more than double any previous flood from 1980 onward. The July 2016 flood of 15,600 ft^3/s also had a probability of less than 0.2 percent (Fitzpatrick and others, 2017).

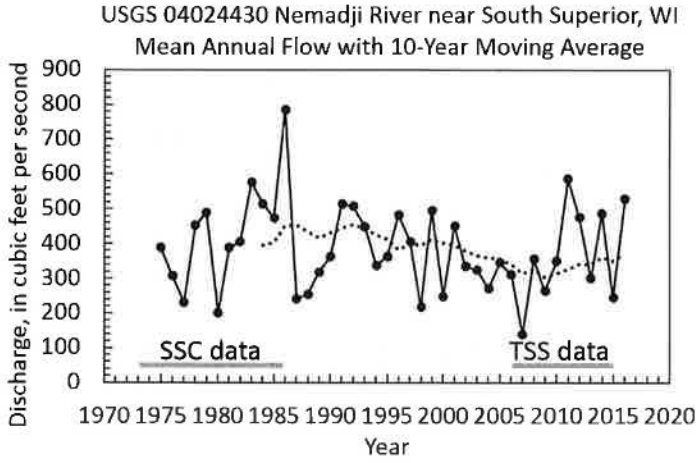

Figure 3. Mean annual flow for Nemadji River streamgage near South Superior, Wisconsin, 1973-2016 (U.S. Geological Survey identification number 04024430). The dotted line represents the 10-year moving average. Suspended-sediment concentration (SSC) data were collected 1973-86 and total suspended solids (TSS) were collected 2006-2015.

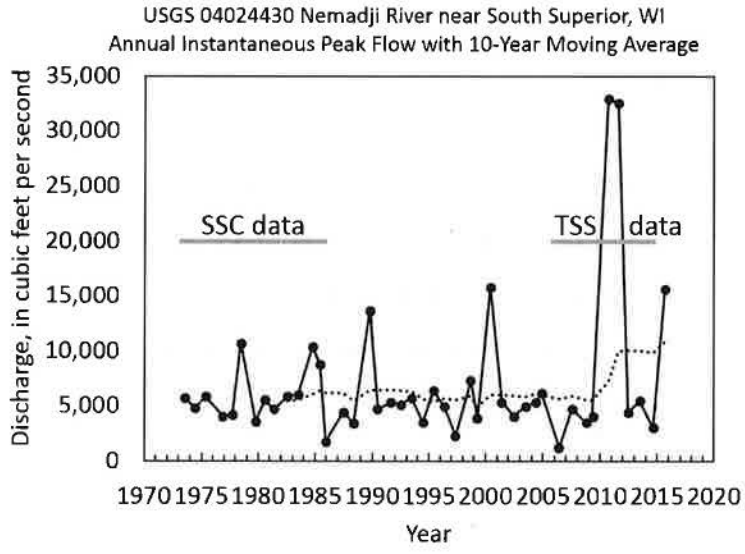

Figure 4. Instantaneous peak flows for the Nemadji River streamgage near South Superior, Wisconsin during the study period from 1973-2016 (U.S. Geological Survey identification number 04024430). The dotted line represents the 10-year moving average. Suspended-sediment concentration (SSC) data were collected 1973-86 and total suspended solids (TSS) were collected 2006-2015.

Flood-frequency characteristics, calculated using the USGS PEAKFQ program (Flynn and others, 2006; Interagency Committee on Water Data, 1982) for three consecutive 30-year periods of streamgage record, indicate a potential decrease in the size of low magnitude frequent floods (95 percent probability of occurring in any given year) and an increase in high magnitude floods (1 percent probability of occurring in any given year) during the data collection period for TSS (fig. 5). The decrease in the size of small frequent floods for 1980-1999 and 1985-2014 periods compared to previous periods is complementary to the decrease in mean annual flows over a similar time period (fig. 3), reflective of the region-wide decade-long drought. The increase in the size of floods with a 1 percent probability for the same period is in part affected by the large floods in 2011 and 2012 (Czuba et al., 2012). The non-stationarity in mean annual flows and instantaneous peak flows over the same period with differing collection methods for suspended sediment preclude any direct interpretations from increasing or decreasing annual sediment loads without considering flow variability.

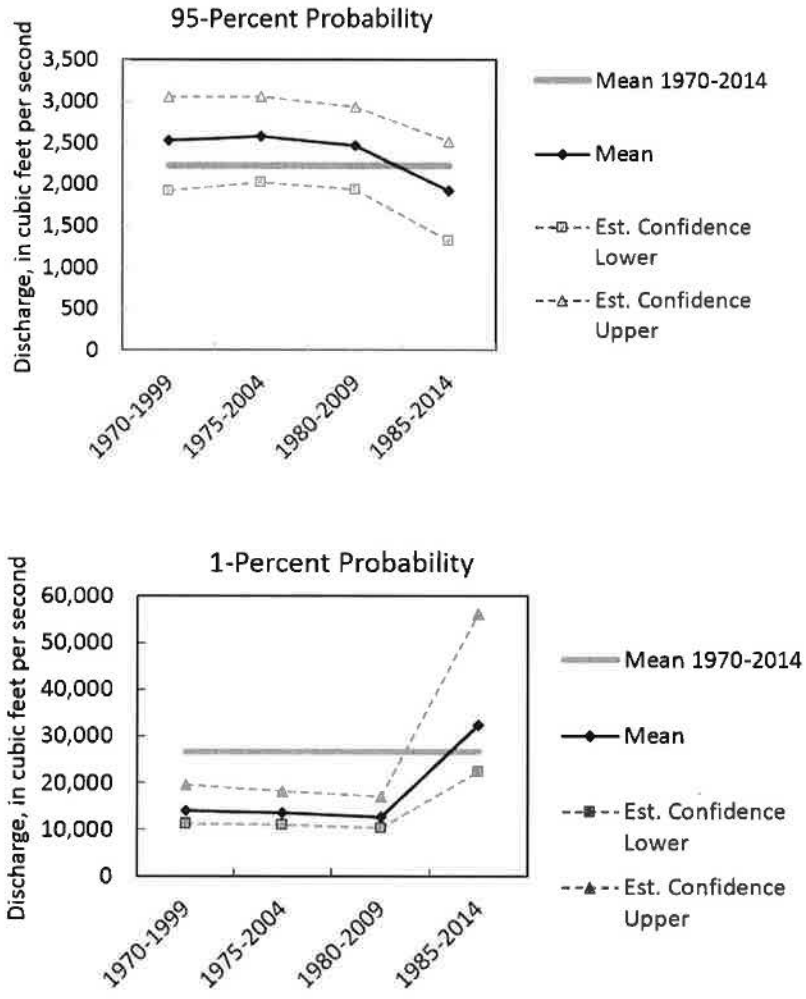

Figure 5. Trends in 30-year moving averages of flood frequency characteristics for Nemadji River near South Superior, WI USGS ID 04024430 for frequent small floods (95-percent probability) and infrequent large floods (1-percent probability). Peak flow data from the National Water Information System. Flood frequency statistics calculated using the PEAKFQ program (Flynn and others, 2006) and confidence limits set at 95 percent.

Sediment Characteristics 2015-16

The 2015-16 sediment sampling at the Nemadji River streamgage was mainly done to develop a calibration curve that could be used for comparing the historical suspended sediment data sets collected with different methods. In addition, bedload and bed material sampling was done to better characterize the proportion of bedload to suspended sediment discharge and ultimately the total amount of sediment discharged to the St. Louis River estuary and AOC.

Suspended Sediment

Suspended sediment samples from 2015-16 were collected during a wide range of flow conditions, from a low flow of 250 cfs to flood flows of 12,800 cfs (table 2). Concentrations based on EWI SSC covered three orders of magnitude from less than 25 to greater than 2,000 mg/L. Concentrations of TSS were lower than SSC in all except four pairs. Concentrations of TSS were on average 84 percent of SSC concentrations. Gray and others (2000) also concluded that TSS was almost always lower than SSC for a 1993-94 data set from three tributaries to Lake Michigan. Moreover, Ellison and others (2014) found SSC concentrations to be on average two times larger than TSS concentrations for Minnesota streams.

Table 2. Suspended sediment concentration (SSC) and total suspended solids (TSS) data collected in 2015-16 at the Nemadji River near South Superior, Wisconsin using two field sampling methods. A composite sample from an equal width increment equal depth interval was collected as well as a grab sample from the centroid of flow.

[EWI, Equal-width-increment; SSC, suspended sediment concentration; TSS, total suspended solids; ns, no sample]

Date and Time	Sampling method, 10 = EWI and 60 = grab	Discharge, instantaneous, cubic feet per second	SSC, milligrams per liter	TSS, water, unfiltered, milligrams per liter	TSS/SSC	Percent greater than 0.063 mm in SSC sample
7/07/2015 18:33	10	2590	297	271	0.91	7
07/07/2015 18:54	60	2560	269	344	1.28	ns
07/08/2015 15:45	10	1350	135	150	1.11	3
07/08/2015 16:20	60	1330	128	161	1.26	ns
10/28/2015 11:10	60	385	34	27	0.79	ns
10/28/2015 13:30	10	388	61	13	* 0.21	15
11/06/2015 11:40	60	298	25	23	0.92	16
11/06/2015 12:54	10	298	33	27	0.82	ns
11/17/2015 14:22	10	698	139	11	* 0.08	14
11/17/2015 14:50	60	704	121	111	0.92	ns
11/18/2015 12:12	60	1980	482	298	0.62	ns
11/18/2015 13:26	10	1990	419	282	0.67	6
03/31/2016 14:21	60	2010	286	262	0.92	ns
03/31/2016 14:41	10	1990	298	270	0.91	8
04/25/2016 15:15	60	4750	2000	1790	0.90	ns
04/25/2016 17:02	10	4820	1550	1320	0.85	6
04/25/2016 17:03	10	4820	1450	1240	0.86	4
04/26/2016 13:25	10	4830	461	372	0.81	12
05/31/2016 15:31	60	248	32	33	1.03	ns
05/31/2016 15:58	10	247	33	29	0.88	2
07/12/2016 17:10	60	15300	1970	1840	0.93	ns
07/13/2016 16:10	10	5930	510	458	0.90	4

*Outliers

The proportion of sand in the SSC samples ranged from 2 to 16 percent, with most of the sand in very fine to medium sand categories (0.063 to 0.500 mm). The proportion of sand greater than 10 percent generally happened during low water discharges, except for a sample on April

26, 2016 that had relatively high discharge and low suspended sediment concentration (table 2). This sample was taken on the receding limb of a spring rainfall event.

The paired samples of SSC and TSS collected from July 2015 through July 2016 were plotted in four ways to illustrate how the sampling and laboratory methods affected the relations between TSS and SSC (fig. 6). The comparison of EWI SSC with grab TSS (fig. 6*A*) has the most variability for multiple potential reasons, including differences between getting a full cross section representation compared to a grab sample and analyzing the entire sample compared to an aliquot in the lab. There may also be a sample difference because of the time offset between the EWI sample, which takes about 45 minutes to collect, compared to the instantaneous grab. For EWI TSS and EWI SSC, concentrations of TSS were particularly low compared to SSC from two pairs (fig. 6*B*). Differences between EWI SSC and grab SSC were present but not as noticeable as the first two comparisons (fig. 6*C*), and the grab only paired samples had close to a 1:1 relation (fig. 6*D*). The regression equation for the relation between EWI SSC and grab TSS (fig 6*A*) was used to adjust the historical grab TSS data to be comparable to the historical EWI SSC data.

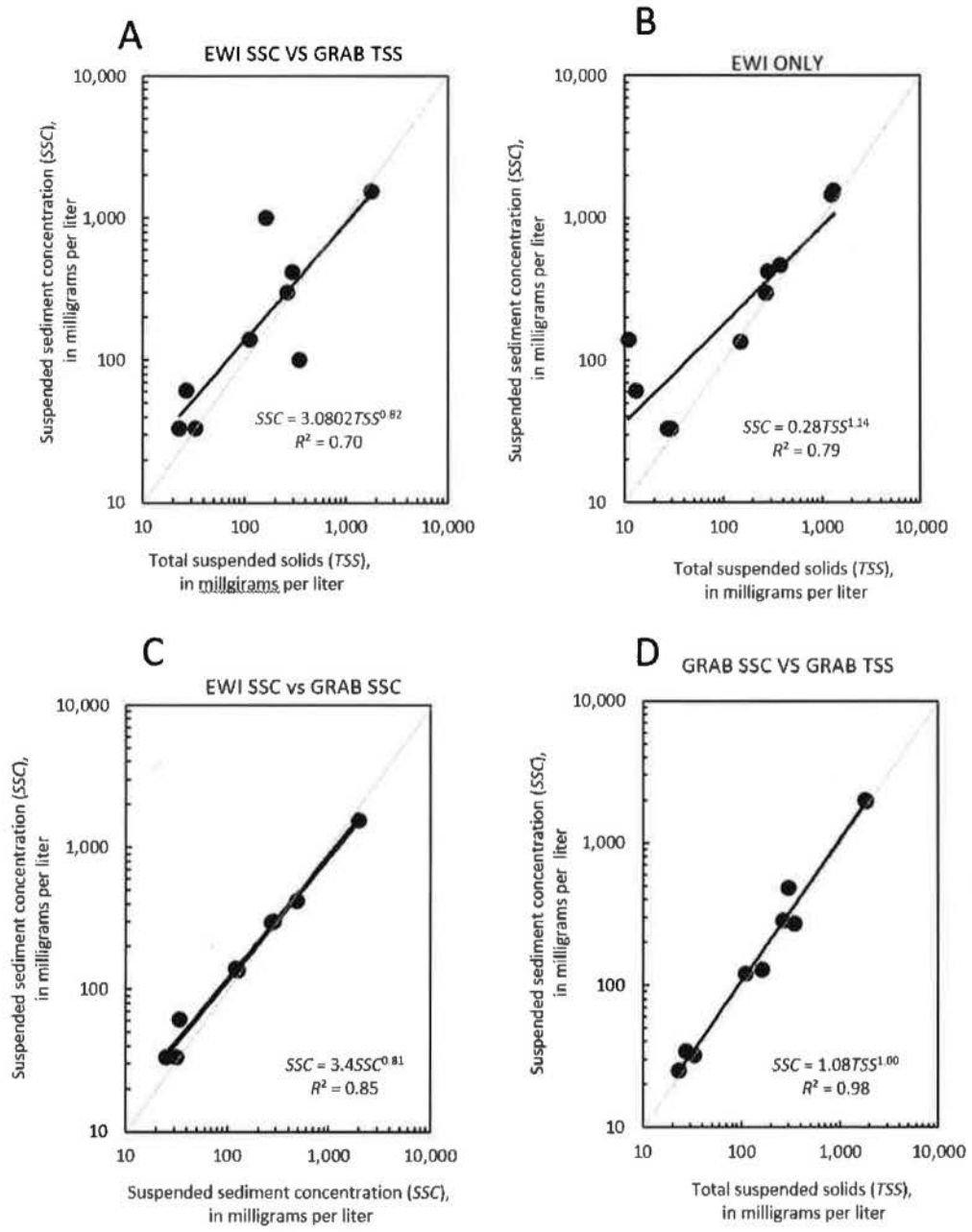

Figure 6. Comparison of paired suspended-sediment concentration (*SSC*) and total suspended solids (*TSS*) data from 2015-16 for the Nemadji River near South Superior, WI USGS ID 04024430. Black lines are power functions fit through log-10 normalized data. A) equal-width-increment (EWI) SSC and grab TSS B) EWI SSC and EWI TSS, C) EWI SSC and grab SSC, and D) grab SSC grab and grab TSS. The line for a 1:1 relation is shown in gray.

The proportion of TSS to SSC was between about 80 and 90 percent for most pairs (fig. 7). Two outliers had particularly low proportions but also had low suspended sediment concentrations of which sand made up 14 and 15 percent (table 2). This suggests that some of the sand portion was likely lost from the TSS sample, either from splitting using the churn sampler or the aliquot sampling during the TSS laboratory analyses. The grab only plot (fig. 6*D*) had an almost 1:1 relation between SSC and TSS and very little variability, indicating that the EWI sampling, more so than the laboratory analytical methods, resulted in higher concentrations for SSC than TSS, especially for concentrations below about 600 mg/L.

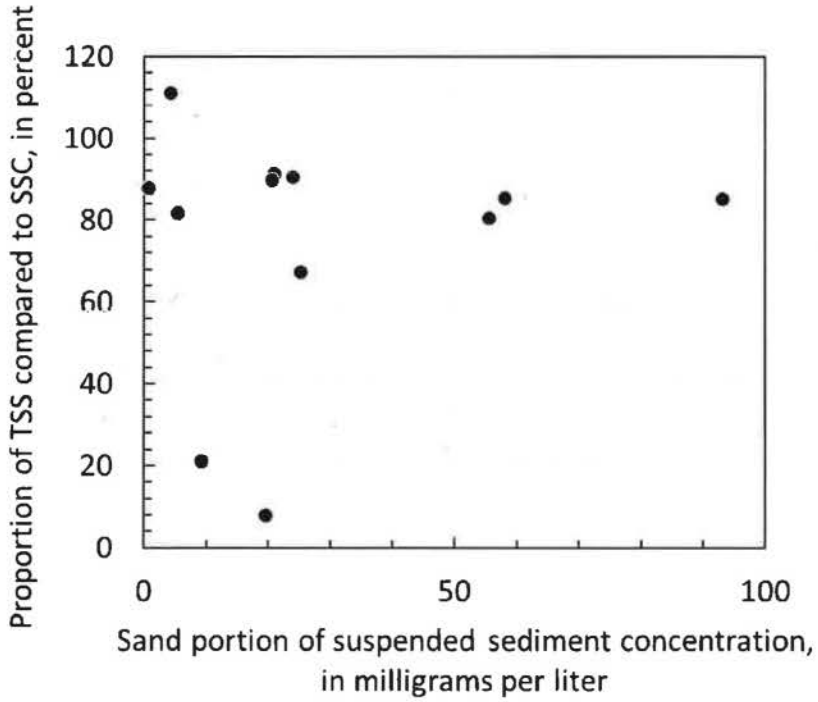

Figure 7. Proportion of total suspended solids (TSS) compared to suspended-sediment concentration (*SSC*) compared with sand concentration in the SSC samples for Nemadji River near South Superior, WI USGS ID 04024430, 2015-16.

Bedload and Bed Material

During the 2015-16 sampling sediment sampling activities, 9 of the 12 suspended sediment sampling events had collection of bedload and bed material samples (table 3). Over the nine events sampled the measured bedload ranged from 5 to 62 tons/day, and less than 1 to 47 percent of the total load. The highest proportions of bedload (and lowest proportions of suspended sediment load) were during low water discharges (fig. 8).

The particle size distribution of the measured bedload was mainly sand (fig. 9). The sampled bed material and calculated bedload component from the modified Einstein procedure also was mainly composed of sand (table 3). The greater than 2 mm fraction was less than 10 percent in all but 1 sample. The mesh size used for the bedload sampler was 0.125 mm except the first two passes on July 7 when the more traditional 0.250 mm mesh was used. The mesh size was changed to 0.125 mm because sediment could be seen evacuating from the bag during retrieval. Whereas silt- and clay-sized particles tend to have a uniform vertical distribution through the water column, the sand sized particles may be more prevalent in the lower part of the water column, especially for the 0.125 to 0.25 mm sizes (Colby, 1963). Most of the bedload was composed of three sand sizes of greater than 0.5 to 1.0 mm, greater than 0.25 to 0.5 mm, and greater than 0.125 to 0.25 mm (fig. 9).

Table 3. Results from 2015-16 sediment load measurements and modified Einstein calculations for Nemadji River near South Superior, WI USGS ID 04024430.

Date of sediment sampling	7/7/15	7/8/16	10/28/15	11/6/15	11/17/15	11/18/15	3/31/16	4/25/16	5/31/16
Measurements									
Channel wetted width (ft^2)	110.0	98.8	70.6	75.4	79.4	88.6	68.0	203.0	71.4
Average water depth (ft)	10.0	7.6	3.4	3.3	4.7	8.1	7.2	9.3	3.1
Channel wetted area (ft^2)	1,100	747	240	245	373	720	489	1,890	222
Average velocity (ft/s)	2.7	2.1	1.6	1.2	1.8	2.6	4.0	2.5	1.1
Average discharge for suspended sediment (ft^3/s)	2,960	1,540	385	300	654	1,870	1,950	4,790	250
Average discharge for bedload (ft^3/s)	2,750	1,490	385	300	689	1,970	2,030	4,755	248
Suspended sediment concentration (mg/L)	297	135	61	33	139	419	298	1,550	33
Suspended sediment load (t/d)	2,374	561	63	27	245	2,116	1,569	20,046	22
Bedload (t/d)	62	25	42	24	58	28	32	41	5
Measured total sediment load (t/d)	2,436	586	105	50	303	2,143	1,601	20,087	28
Percent of measured suspended sediment to total sediment load	97	96	60	53	81	99	98	100	80
Modified Einstein calculated suspended sediment load (t/d)									
0.0020 - 0.0625 mm size range	2,159	529	50	21	202	1,942	1,400	19,008	20
0.0625 - 0.1250 mm size range	77	6	4	2	14	62	76	396	0
0.1250 - 0.2500 mm size range	53	6	3	1	12	41	30	198	0
0.2500 - 0.5000 mm size range	28	6	1	1	5	21	15	198	-
0.5000 - 1.0000 mm size range	5	-	1	0	2	-	-	-	-
1.0000 - 2.0000 mm size range	-	-	-	-	-	-	-	-	-
2.0000 - 4.0000 mm size range	-	-	-			-	-	-	-
Calculated suspended sediment load (t/d)	2,322	545	59	24	234	2,066	1,522	19,800	21
Modified Einstein calculated total sediment load (t/d)									
0.0020 - 0.0625 mm size range	2,230	546	55	26	216	2,057	1,532	19,258	22
0.0625 - 0.1250 mm size range	93	6	5	3	18	90	146	411	0
0.1250 - 0.2500 mm size range	101	10	5	3	18	103	169	234	0
0.2500 - 0.5000 mm size range	140	38	5	4	18	149	304	440	0
0.5000 - 1.0000 mm size range	84	7	5	1	13	55	127	111	-
1.0000 - 2.0000 mm size range	5	0	-	-	-	2	34	4	-
2.0000 - 4.0000 mm size range	0	-	-			-	4	-	-
Calculated total sediment load (t/d)	2,653	607	75	36	282	2,456	2,316	20,459	23
Comparison of calculated and measured results									
Difference betwteen calculated and measurement for total sediment load (t/d)	217	21	-30	-14	-21	313	715	371	-5
Percent difference between calculated and measured	9	4	-29	-29	-7	15	45	2	-18

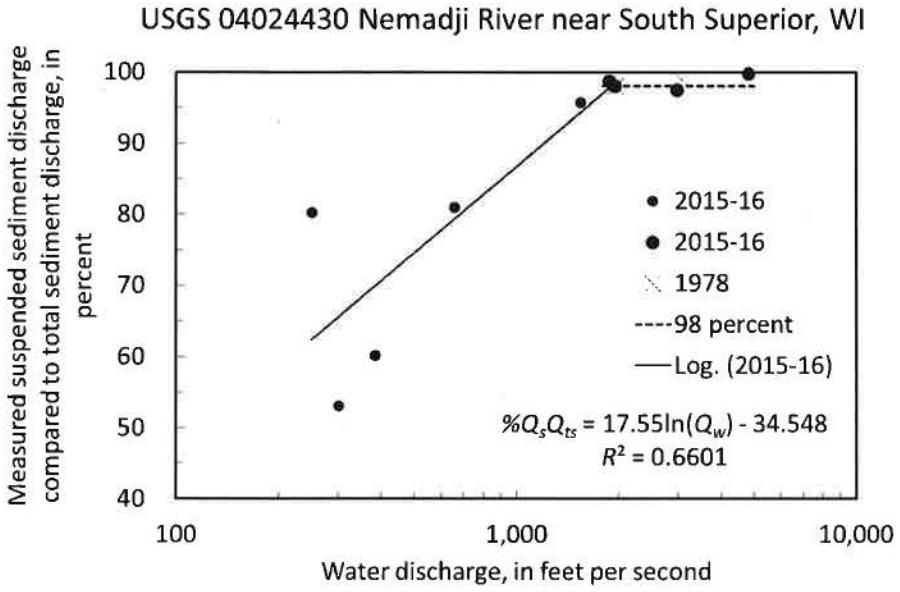

Figure 8. Comparison of the proportion of measured suspended sediment discharge to total sediment discharge with water discharge for nine events sampled in 2015-16 and two events in 1978 with measured bedload at the Nemadji River streamgage near South Superior, Wisconsin, 1973-2016 (U.S. Geological Survey identification number 04024430). The fitted linear regression (solid line) extends to a water discharge of about 1,800 cubic feet per second. Above 1,800 cubic feet per second the percentage of suspended sediment discharge was assumed to be 98 percent (dashed line). Data from 1978 from Rose (1980).

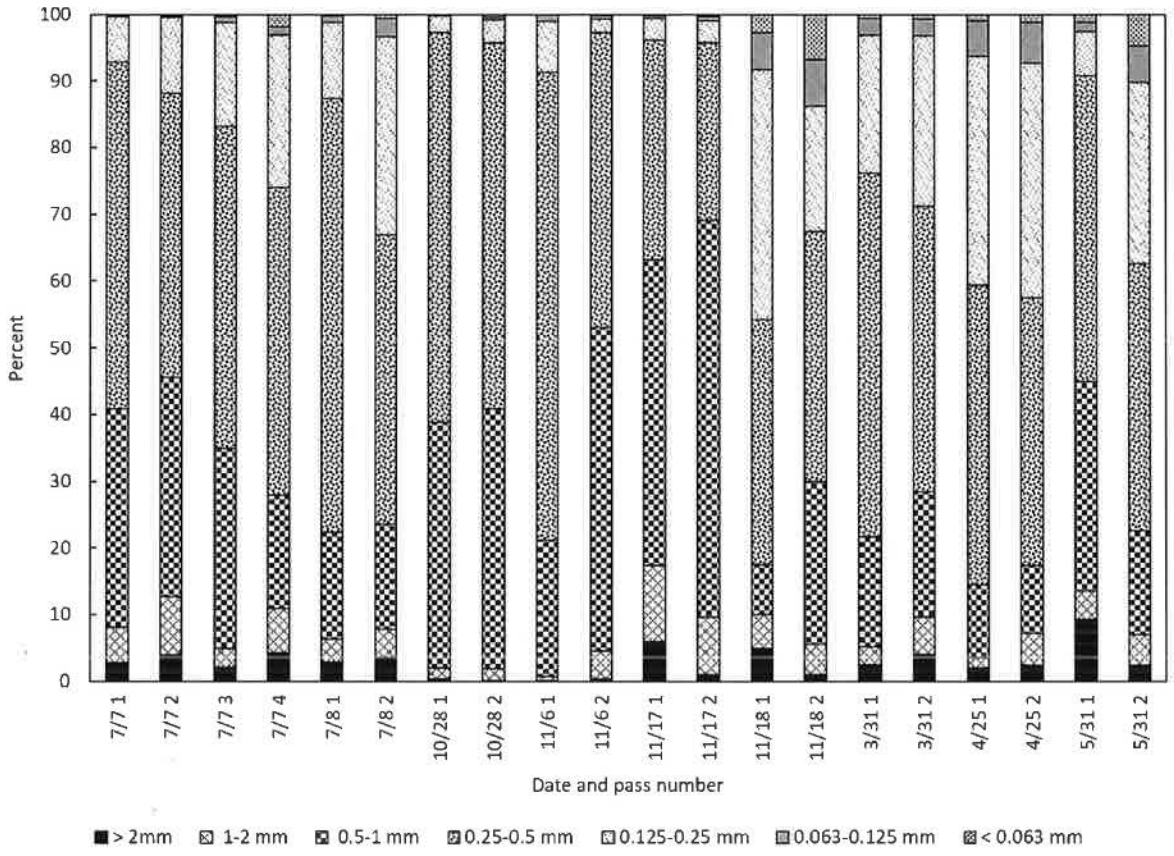

Figure 9. Particle size distribution of bedload from multiple passes from 2015-16 sampling events at the Nemadji River streamgage near South Superior, Wisconsin, 1973-2016 (U.S. Geological Survey identification number 04024430).

Total Sediment Loads

Total sediment loads for the 2015-16 event samples were computed from summing the measured suspended sediment and bedload and compared to loads calculated by the modified Einstein procedure (table 3). Total sediment loads calculated from the modified Einstein procedure varied from being less than the measured for streamflows below about 1,500 ft^3/s to more than the measured for streamflows above 1,500 ft^3/s (table 3, fig. 10). Data for two events sampled in 1978 by Rose (1980) were similar. One potential reason for the modified Einstein

procedure calculating more load during high flows than the measured may be from how well the 0.125- to 0.25-mm sand portion was distributed in the water column relative to its possible higher concentrations closer to the bed.

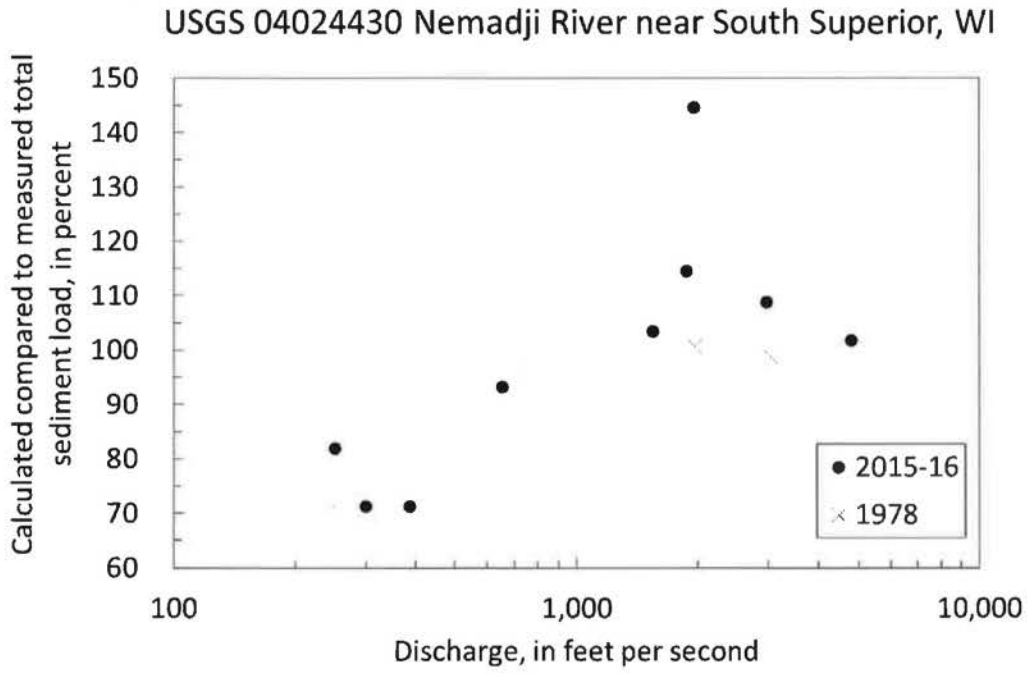

Figure 10. Comparison of percentage of calculated and measured total sediment discharge with water discharge for 2015-16 and 1978 at the Nemadji River streamgage near South Superior, Wisconsin, 1973-2016 (U.S. Geological Survey identification number 04024430). The 1978 data are from Rose (1980).

Estimates of Annual Suspended and Total Sediment Loads 2009-16

The 2009-16 period covered a range of hydrologic conditions and annual suspended and total sediment loads reflected the wide range water discharges (table 4). Estimates were lowest TSS-based annual suspended sediment load of just over 18,000 tons in 2015 in contrast with the almost order of magnitude higher 180,000 tons in 2012 (table 4) which included the flood of record (Czuba and others, 2012).

Table 4. Annual suspended and total sediment loads for 2009-16 based on TSS, *AdjTSS*, and the $\%Q_sQ_{ts}$ relation developed with 2015-16 data. Mean daily values computed with GCLAS.

[TSS, total suspended solids; adjTSS, adjusted total suspended solids; % percent; Qs, suspended sediment discharge; Qts, total sediment discharge; cfs, cubic feet per second]

Year	Water discharge (cfs-days)	TSS annual suspended sediment load (tons)	*AdjTSS* annual suspended sediment load (tons)	$\%Q_sQ_{ts}$-based annual total sediment load (tons)	Suspended sediment load as a percentage of total sediment load
2009	96,168	19,886	26,715	32,566	82
2010	127,869	32,942	42,509	48,484	88
2011	213,770	142,015	153,084	163,018	94
2012	173,792	177,484	180,990	190,233	95
2013	109,599	27,579	36,366	40,379	90
2014	177,284	158,380	166,137	175,048	95
2015	89,125	18,682	24,422	30,354	80
2016	192,926	130,515	134,853	143,308	94

The TSS-based mean daily suspended sediment concentration was updated with the power-function established between the 2015-16 EWI SSC and grab TSS data (fig. 6*A*):

$$AdjTSS = 3.0802 \times TSS^{0.82}$$

Likewise, the mean daily suspended sediment loads calculated from GCLAS were updated using *AdjTSS* concentrations. The resulting annual suspended sediment loads based on *AdjTSS* concentrations are larger than their TSS counterparts (table 4; fig. 11).

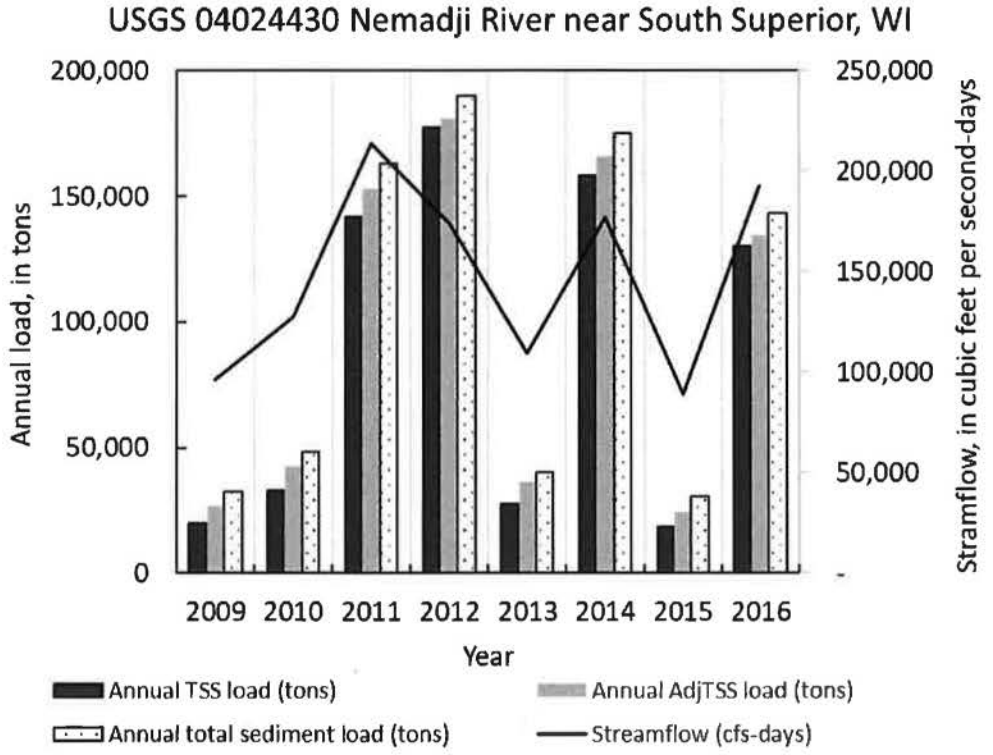

Figure 11. Annual streamflow, suspended sediment load and total sediment load for Nemadji River streamgage near South Superior, Wisconsin, 2009-2016 (U.S. Geological Survey (USGS) identification number 04024430). Suspended sediment load calculated for total suspended solids (TSS) and adjusted total suspended solids (*AdjTSS*). Total sediment load calculated from *AdjTSS* mean daily loads and relation of the percentage of suspended sediment with streamflow.

Rough estimates of annual total sediment load for 2009-16 were calculated by applying the 2015-16 regression relation for suspended sediment loads as a percentage of total sediment loads ($\%Q_sQ_{ts}$) compared to water discharge (Q_w) (fig. 8) to the mean daily data:

$$\%Q_sQ_{ts} = 17.55\ln(Q_w) - 34.548$$

This log-normal regression relation was used for the 2009-16 mean daily water discharges of less than or equal to 1,800 ft3/s. For mean daily water discharges over 1,800 ft3/s the suspended sediment load was assumed to be 98 percent of the total sediment load. Using this rough estimate

indicated that during dry years the annual bedload is probably about 20 percent of the total sediment load whereas in years with large floods the bedload is 5-6 percent (table 4).

Comparison of Suspended Sediment Rating Curves 1973-86 and 2006-15

Trend analyses requires continuous data sets. Having historical data grouped into two data sets with different collection and analytical methods and a gap of 20 years in between required an alternative approach. Another technique can be used that involves suspended sediment concentration and water discharge rating curves. This technique has been used in other studies to determine if there has been a difference in sediment loading in a watershed from land use changes. The assumption for this method is that the sediment relations are supply limited and not transport limited (Warrick, 2014). Using the side-by-side sampling procedures and paired sample linear regression analyses, a calibration coefficient was established between the EWI SSC and the grab TSS data using 2015-16 data (previous section). The sediment rating curve for the adjusted TSS was compared to SSC data for the final determination if there was a change in sediment discharge in the Nemadji River from 1973-86 to 2006-16.

The WDNR and MPCA both collected TSS samples at the Nemadji River gaging station from 2006-2015 through coordinated efforts. Both agencies used the weighted bottle grab technique. A check on suspended sediment rating curves with data grouped by state agency indicates the relation between suspended sediment concentration and water discharge for the two state agencies are virtually the same, indicating no difference in their sampling or analytical methods (fig. 12). The MPCA data cover a higher range of water discharges and also have about double the number of samples. The similarity of the data sets confirm that it is appropriate to combine the data sets into one for comparison with the historical 1973-86 SSC data.

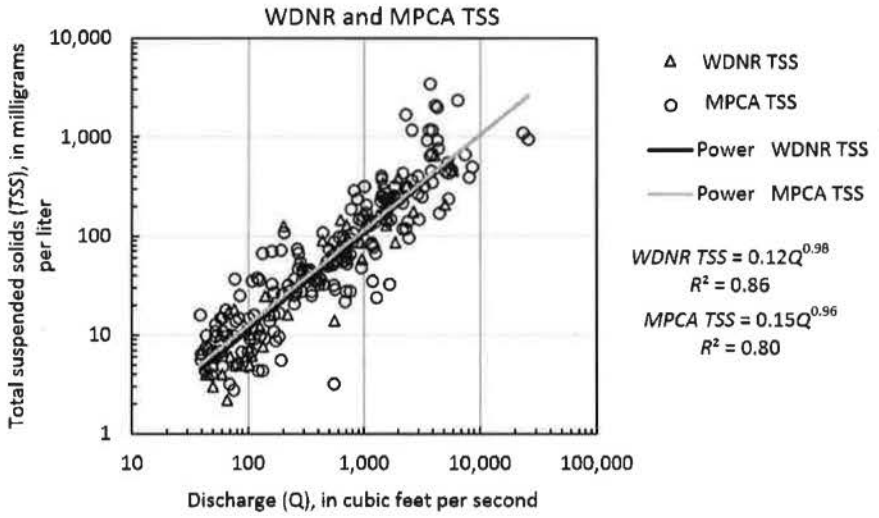

Figure 12. Total suspended solids (TSS) concentrations collected by the Wisconsin Department of Natural Resources (WI) and the Minnesota Pollution Control Agency (MN) from 2006-2015 for Nemadji River near South Superior, WI USGS ID 04024430. Lines are power functions fit through log-10 normalized data from each state agency.

Determination of the presence of a difference in the 1973-86 EWI SSC and 2006-16 grab TSS data required adjusting the TSS data to SSC equivalent data before any comparison technique could be performed. Sediment concentration and water discharge rating curves for 1973-86 SSC and 2006-15 TSS data illustrate how the rating between suspended-sediment concentration and discharge appear lower (less suspended sediment) for the later period covered by grab TSS sampling compared to the earlier period covered by the EWI SSC sampling (fig. 13*A*). There were more samples for SSC (330) than TSS (230), but the two data sets covered a similar range of water discharges and sediment concentrations. Water discharges ranged from about 50 to almost 10,000 cubic feet per second. Sediment concentrations ranged from less than 10 mg/L to over 1,000 mg/L. Power law functions for sediment rating curves fit to each data set in relation to water discharge indicate that the TSS samples had lower concentrations than SSC

samples, especially at lower water discharges (fig. 13*A*). This relation is like the relation between EWI SSC and EWI TSS concentrations in the 2015-16 paired comparison sampling (fig. 6*C*).

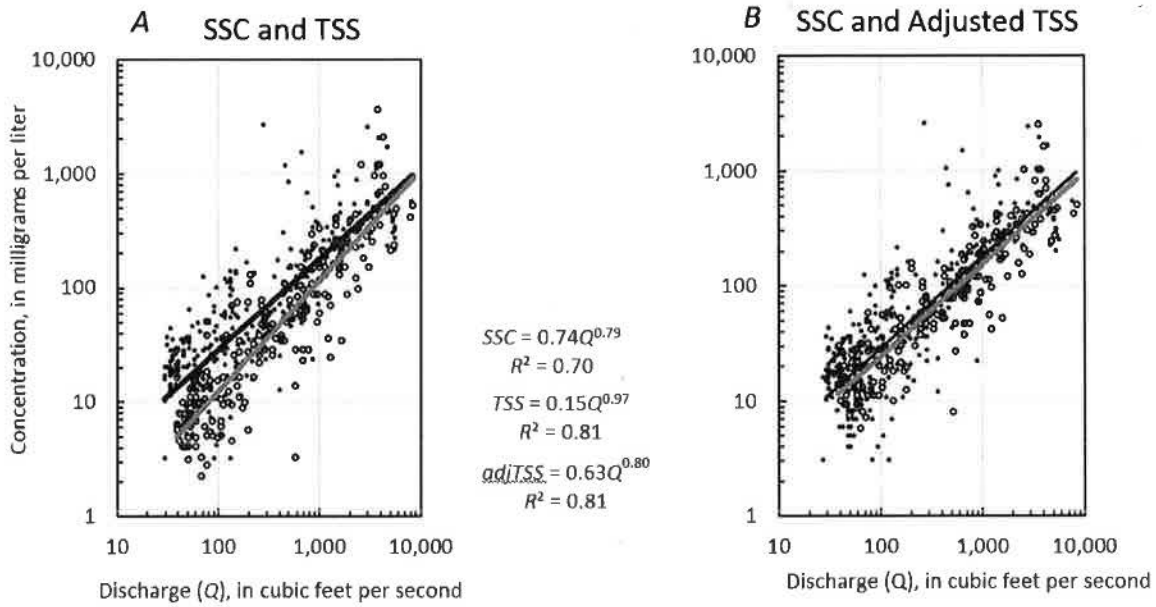

Figure 13. Suspended sediment rating curves for base-10 logarithms of suspended-sediment concentration (*SSC*) from 1973-86 and (*A*) total suspended solids (*TSS*) from 2006-15, and (B) adjusted total suspended solids (*Adj1TSS*) from 2006-15 for Nemadji River near South Superior, WI USGS ID 04024430.

After the 2006-16 TSS concentrations were adjusted (*AdjTSS*), the offset of the rating curves for the two time periods disappeared (fig. 13*B*). The 2006-16 adjusted TSS (*Adj1TSS*) rating curve had similar slope but a small negative offset of the intercept compared to the 1973-86 SSC rating curve. Results from the ANCOVA analyses indicate that the negative offset between the two regressions is not statistically significant (table 5).

Table 5. Analysis of covariance results for suspended sediment rating curves from 1973-86 and 2006-16, using adjusted total suspended solids data for Nemadji River near South Superior, WI USGS ID 04024430.

Coefficients	Estimate	Standard error	t value	Probability (greater than absolute value of t)
Intercept	-0.131	0.060	-2.177	0.030*
Log10(Q)	0.795	0.026	30.951	<2e-16***
Group	-0.111	0.102	-1.086	0.278
Log10(Q)	0.015	0.040	0.382	0.702
Residual standard error	0.3038			
F-statistic	585.2 on 3 and 571 degrees of freedom			
Degrees of freedom	3 and 571			
p-value	<2.2e-16			
Multiple R-squared	0.755			
Adjusted R-squared	0.753			

Although statistical significance remains elusive, annual total sediment loads calculated in GCLAS were compared to the two sediment rating curves for an example time period of 2009-16. Using the same set of mean daily water discharges over the 8-year period, the negative offset between the two curves resulted in the 2006-15 annual total sediment discharges being 86 percent of the 1973-85 sediment discharges. This decrease of 14 percent is not statistically significant, yet it does however give an indication that perhaps sediment discharges are heading toward decreasing. The 2006-15 sediment rating curve derived discharges were higher compared to the GCLAS calculated in six out of eight years, with the highest year in 2013 which had up to almost 170 percent of the GCLAS derived discharge (table 6). The GCLAS method takes into account seasonal and runoff hydrograph (hysteresis) variations that are not possible for with the sediment rating curve method.

Table 6. Comparison of 2009-16 annual total sediment discharges for 2009-2016 using multiple methods.

[TSS, total suspended solids; adjTSS, adjusted total suspended solids; % percent; Qs, suspended sediment discharge; Qts, total sediment discharge; cfs, cubic feet per second]

Year	Water discharge (cfs-days)	Annual total sediment discharge from GCLAS (tons)	Annual total sediment discharge using 2006-15 sediment rating curve (tons)	Annual total sediment discharge using 1973-85 sediment rating curve (tons)	Annual total sediment discharge from the 2006-15 rating curve as a percentage of discharge from the 1973-85 rating curve	Annual total sediment discharge from 2006-15 rating curve as a percentage of discharge from GCLAS
2009	96,168	32,566	32,378	37,741	86	116
2010	127,869	48,484	57,925	67,489	86	139
2011	213,770	163,018	196,812	229,022	86	140
2012	173,792	190,233	230,069	267,613	86	141
2013	109,599	40,379	58,176	67,773	86	168
2014	177,284	175,048	133,692	155,681	86	89
2015	89,125	30,354	22,848	26,644	86	88
2016	192,926	143,308	135,430	157,686	86	110

Summary and Conclusions

A variety of sediment characteristics were examined at the USGS Nemadji River streamgage in northwestern Wisconsin (USGS 04024430), which has flow and sediment data starting in 1973 and continuing through the present. Even though the available sediment data was collected sporadically with different methods and analytical techniques by the USGS and state agencies, some innovative techniques were used to describe differences in suspended sediment collection methods as well as compare historical data sets.

Before any comparisons of historical sediment data sets were done, the hydrologic context was examined in terms of mean annual discharges and annual peak floods. A range of

mean annual discharges were covered in an earlier historical data set collected by the USGS in 1973-86 as well as a later data set collected by the Wisconsin DNR and MPCA in 2006-15. However mean annual flows in 2006-15 were about 84 percent of 1973-86 mean annual flows and extreme floods in 2011 and 2012 punctuated the latter period.

From 2009-16, estimated annual total sediment discharges ranges from a low of about 18,000 tons per day in 2015 to a high of almost 180,000 tons in 2012. The percentage of bedload depended on water discharge, with about 20 percent in years with relatively low annual water discharge compared to only 5-6 percent in years with extreme floods.

The sediment rating curve approach was employed to determine if there was a change in annual suspended sediment loads from 1973-86 and 2006-15. An ANCOVA analyses indicated that the suspended sediment rating curves for the two periods were not statistically significantly different after the TSS data were adjusted to be equivalent to SSC data. However, a very rough, although not statistically significant offset in the curves resulted in a reduction of possibly 15 percent when the two curves were applied to 2009-16 annual sediment discharges. This value is not statistically sound, it should be used with caution.

The hydrologic context with what is seeming to have more year-to-year variability will likely become more important than the overall value of annual loading at face value. It is unknown if the extreme floods will become more or less frequent over the coming years. The 10-fold increase in the size of sediment discharges during extreme floods compared to more average flood condition suggest that restoration done at the mouth of the Nemadji River needs to be resilient to large floods and sporadic, highly variable sediment deposition, even though overall the amount of suspended sediment per unit of water discharge may have been reduced.

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Glossary

The following definitions, except where otherwise noted, are from Colby (1963), Edwards and Glysson (1999), and are repeated from Rose (1980) and Czuba and others (2012).

Annual exceedance probability The probability that a given event magnitude will be exceeded or equaled in any given year. The annual exceedance probability is directly related to the recurrence interval. For example, the chance that the 100-year peak flow will be exceeded or

equaled in any given year is 1 percent. A flood probability of 0.01 has a recurrence interval of 100 years. The recurrence interval corresponding to a particular flood probability is equal to 1 divided by the flood probability (Holmes and Dinicola, 2010).

Bedload discharge The sediment that moves by rolling, sliding, and bouncing along the streambed.

Bed material Material that constitutes the streambed.

Bed-material discharge Part of the total sediment discharge having particle sizes in the same range as the bed material. In an alluvial stream, bed-material discharge is related to the hydraulic properties of the flow.

Continuous-record streamgage A site where stage, discharge, and other hydrologic data are collected with sufficient frequency to define daily mean values and variations within a day (U.S. Geological Survey, 2011).

Flood peak The highest value of the stage or discharge attained by a flood; thus, peak stage or peak discharge. "Flood crest" has nearly the same meaning, but because it connotes the top of the flood wave, flood crest is properly used only in referring to stage—thus, "crest stage," but not "crest discharge."

Recurrence interval (return period) The average interval of time within which the given flood will be equaled or exceeded once. The recurrence interval is directly related to the flood probability. The recurrence interval corresponding to a particular flood probability is equal to 1 divided by the flood probability. For example, a 100-year recurrence interval has a flood probability of 0.01.

Sediment concentration Ration of dry weight of sediment to the total weight of the water-sediment mixture.

Streamflow The discharge of water in a natural channel. Although the term "discharge" can be applied to the flow of a canal, the word "streamflow" uniquely describes the discharge in a surface stream course.

Streamgage A site on a stream, canal, lake, or reservoir where systematic observations of stage, discharge, or other hydrologic data are obtained (U.S. Geological Survey, 2011).

Suspended-sediment discharge Sediment that is supported by upward components of turbulent currents.

Total sediment discharge All sediment moving downstream, bedload discharge plus suspended-sediment discharge.

Wash-load discharge Part of the total sediment load that is comprised of the particle sizes finer than those in the bed material. Unlike bed-material discharge, there is not a functional relation between wash-load discharge and the hydraulic properties of the flow. Wash load is normally delivered to the stream by overland flow or bank sloughing and is transported at the rate that is made available to the stream.

Water discharge The discharge of water in a natural channel or canal.

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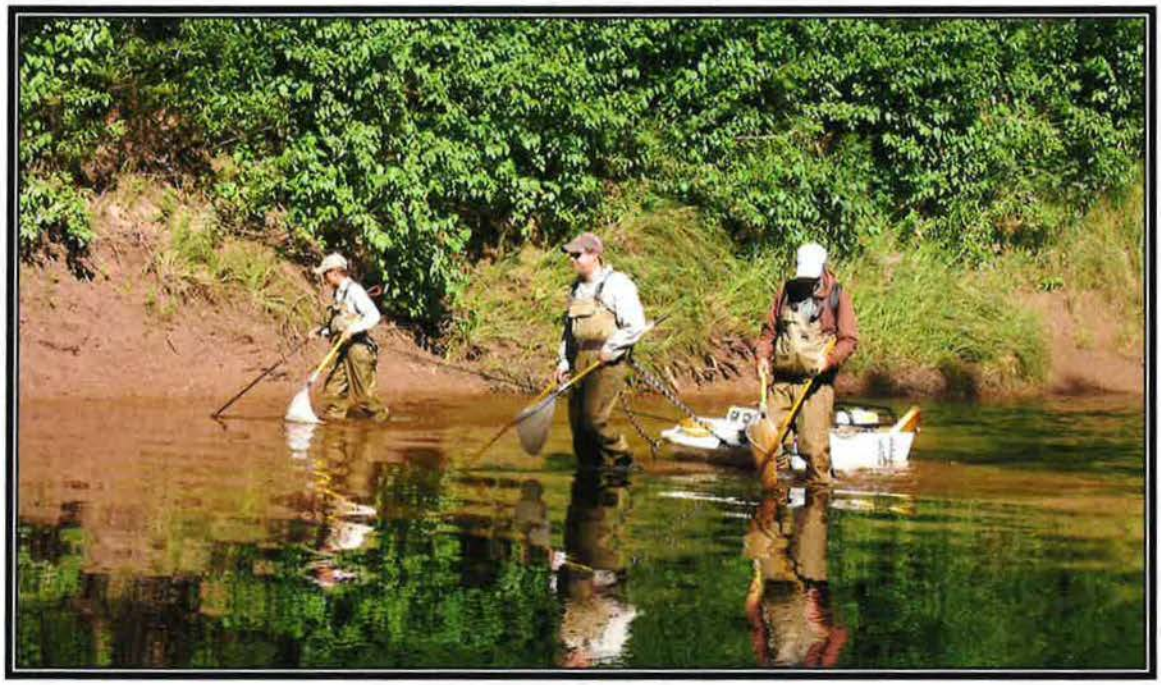
Appendix 7

Lower River Nemadji River - Douglas County Fish Community Survey
(Pertains to management action 6.05)

Lower Nemadji River – Douglas County
2015 Fish Community Survey Summary



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2015
Lower Nemadji River – Douglas County
Fish Community Survey
Aaron Nelson

1. Introduction

The Nemadji River is the second largest tributary to the Duluth/Superior Harbor located at the western end of Lake Superior. The total length of the Nemadji River is 65 miles from its origin in Pine County, Minnesota to its terminus when it reaches Superior Bay in the eastern end of the Duluth/Superior Harbor. The entire Nemadji River watershed was included in the St. Louis River Area of Concern due to Beneficial Use Impairments from Excessive Loading of Sediments and Nutrients; it is also listed on the Wisconsin and Minnesota 303d Impaired Waters List for Sediment/Total Suspended Solids as a Pollutant and Degraded Habitat as an Impairment. This report will summarize survey work completed by Wisconsin DNR Fisheries Management staff to assess the fish community present in the Wisconsin portion of the Nemadji River, specifically the Lower Nemadji River watershed, from the confluence with the Black River to its terminus.

2. Site Description

The project boundary for this survey spanned from the mouth of the Nemadji River upstream to County Highway C. The current Douglas County land-use zoning along the immediate riparian area of the Nemadji River is resource conservation meaning there is no human development permitted along the banks in these zoned areas. Away from the river's edge, county land use and zoning is dominated by agricultural use with minor residential development. Within the City of Superior, the land-use and zoning along the river and within the watershed is primarily suburban or family residential with a few areas of manufacturing or industrial land-use present, most notably the Enbridge Energy refinery on the west side of the river and Burlington Northern rail yard on the east side.

The project area was measured off in one-mile segments to assign one-mile non-wadable river stations. The segment from County Highway C upstream to Finn Rd was also included to allow for additional sampling with the mini-boom if the river channel was navigable and sufficient access was available to launch the mini-boom. This segment included two previous wadable stream stations that were surveyed at Finn Road and Highway 35. Stations numbers were assigned as Station 1, at the upstream most station, continuing downstream to Station 6, the last station selected. Any stations surveyed after the original stations were designated were assigned the number of the station upstream and a letter designation; for example Station 3A was sampled after Stations 3 and 4 had been sampled.

The only sufficient access to launch the mini-boom was at Grand Avenue, and due to low water levels during the sampling period, Mile-14 was assigned Station 3 as it was the furthest upstream that navigation with the mini-boom shocker was possible. Field reconnaissance downstream from County Highway C to the Duluth, Missabe & Iron Range railroad trestle crossing revealed that in low-flow conditions the majority of the stream channel width was wadable with water depths of 1.5 feet or less, however there were deep, non-wadable pools and bends that prevented sampling with the tow barge. The prolonged low-flow conditions prior to the sampling period may have limited areas where sampling with the mini-boom could occur, however it also led to significantly improved visibility of normally very turbid water. Secchi depths taken during the sampling period ranged from 1.8 to 2.25 feet.

3. Methods

The Quality Assurance Project Plan (QAPP) developed for the Lower Nemadji River Biological and Water Quality Monitoring project mentioned use of several types of gear for sampling the fish community of the Nemadji River based on judgement of Fisheries Management staff. Ultimately, electrofishing was chosen over several types of passive net gear (fyke net, minnow trap, gill net) because electrofishing would have less selectivity by eliminating bias from chosen net locations, mesh sizes or openings on nets or traps, or fish behavior and would allow for standardized Index of Biotic Integrity sampling. Using netting gear to sample fish in the Nemadji River could be considered in the future as a means of targeting specific groups of fish or areas, but isn't an unbiased means of gauging the health of the fish community present.

Wadable Stations

The wadable station sampled at County Highway C was surveyed with a tow barge stream electrofishing unit consisting of a Whitney fiberglass sled with three anodes (see report cover). A 240-volt AC generator supplied electrical current to the cathode and anodes via a SDC rectifier control box that transformed AC current to DC. A stainless steel plate affixed to the bottom of the sled acts as the cathode and the anodes are diamond shaped stainless steel hoops epoxied into the end of a fiberglass poles; the anodes then are connected to the SDC control box with power cable by series of connectors and a retractable cable reel. Each of the anodes is deployed by personnel carrying fiberglass handle dip nets and electrofishing commenced from the station start upstream with one crew member towing the barge behind them. Fish were captured as personnel swept the anodes over or around habitat features in the stream channel; any fish captured were transferred to an aerated tub onboard the tow barge.

Water Resources has the option to use best professional judgment and sample a maximum 400-meter station if the habitat, morphology, and substrate are relatively uniform within the 35xMSW station. Based on the mean stream width of 19 meters, a station of 665 meters would have been sampled at County Highway C, however a truncated station of 400 meters was sampled at County Highway C based on available habitat types (deep meanders, shallow inside bends), substrate (sand and clay mix), and morphology (run). While sampling the additional length of the station would have yielded more fish, the extra effort typically results in catching more of the same fish species that have already been captured. Another survey of a station at State Highway 35 was scheduled to be sampled this year, but this area was last sampled in 2011 and IBI scores are considered current for 5 years from the last sampling effort. The station at County Highway C was surveyed as single run fish community (all species collected) catch-per-effort only.

Non-Wadable Stations

The non-wadable stations downstream of County Highway C were surveyed with a mini-boom shocker comprised of an outboard motor propelled aluminum johnboat with a pulsed DC electrofishing unit powered by a 3500 watt AC generator (see report cover). An anode consisting of a standard aluminum "Wisconsin ring" with 16 cylindrical, 17-mm diameter stainless steel droppers was used to deliver electrical current to the water. Pulsed DC settings were set at a pulse rate of 60-hertz and 25% duty cycle; the voltage and amperage were set to maintain electrical output to the anode as close to 3000 watts as possible. This electrofishing unit requires a two person crew consisting of a driver/operator at the stern to run the outboard, generator and electrofishing system and a "dipper" is seated at the bow of the boat with a fiberglass long-handled dip net to capture fish as they are stunned. Electrofishing commenced at the upstream end of the station and the mini-boom shocker drifted with the current or, in slower current areas, run at idle speed downstream along the bank. Fish were drawn to the anode end of the electrical field and any fish captured were transferred to an aerated stock tank on board the mini-boom. Extra sampling effort beyond a simple downstream pass was used to capture additional fish near log jams, snags, or other habitat features as they occurred in the stream channel. All of the 1-mile non-wadable stations were surveyed as single run fish community (all species collected) catch-per-effort only. The distance shocked reflects the distance the mini-boom shocker travelled along the bank and around obstacles or other structure within the 1-mile station.

Fish Processing / Data Collection
All fish captured were identified by species. Gamefish and panfish species were measured to the nearest tenth of an inch and larger individuals were weighed. All other non-gamefish species were counted. All fish captured in the survey were released back to the river with the exception of any voucher species that were retained to confirm species identification.

4. Results/Discussion

Fish Community
The Nemadji River supports a fairly diverse fish assemblage; 24 different fish species were documented in the 6 stations assessed in 2015. (Table 1, Table 3) Based on relative abundance from the electrofishing surveys, cyprinids (minnows) were the most abundant and widely distributed fish species in the Nemadji River and were represented mainly by common shiners and emerald shiners. Silver redhorse, shorthead redhorse, rock bass, smallmouth bass and walleyes were also widely distributed throughout the Nemadji River, but didn't occur in the higher abundance seen in the minnow species.

Based on the gear and sampling methodology used, the fish communities from each station were scored and rated using the Lake Superior Warmwater IBI rating to determine if the site is degraded and to what extent. The summer thermal regime of the Nemadji River is too warm to support salmonids, so based on that criteria, the Warmwater IBI was selected instead of the Coolwater IBI for wadable stations (Lyons et al., 2001). Furthermore, the thermal preferences of the species captured reflect a warmwater system. Muskellunge were the only stenothermal primary coolwater fish species captured in the 2015 surveys. The remaining species captured include a number of secondary coolwater species that occur in both coolwater and warmwater streams, but they are classified as eurythermic meaning they have no thermal requirement or preference. If more primary coolwater or primary coldwater stenothermal species were present, the case could be made for using the Coolwater IBI to determine if the Nemadji River is a degraded coolwater system.

The wadable and non-wadable Lake Superior Warmwater IBI ratings also have scoring criteria calibrated for Lake Superior basin streams that have lower sucker, darter, and centrarchid panfish species richness relative to the other basins in the state (Lyons, 1992, Lyons et al., 2001). For each respective IBI rating system, the fish community receives a score from 0 to 100 points and the score is then assigned a corresponding qualitative rating from "Very Poor" to "Excellent". There is some variation between the two different IBI rating systems for the number of points required to receive the various qualitative ratings, but generally speaking, higher IBI scores receive better qualitative ratings.

Non-Wadable Stations
The IBI scores and ratings for the non-wadable stations are not complete because weight information was not collected for all of the fish captured. Personnel on the crew were unfamiliar with the non-wadable IBI procedures and mandatory requirement for weighing all fish to compute two of the metrics. Despite missing information for two of the metrics, using the scoring metrics with the available data, the sites that were assessed scored between 56.25 and 75 points and were rated from "Fair" to "Good". These scores and ratings should be considered minimum values; if weights had been collected, it's likely all of the sites would be rated "Good", with the possibility of some sites rating "Excellent".

Fish abundance based on the catches may seem low however there are a few factors that may have influenced our ability to capture fish. The first is turbid water conditions; at its best visibility was around 2 feet, so fish had to come up into the field to be visible for dippers to capture. Another factor was the depth of the bends and pools that were sampled, some bends and pools were estimated to be up to 15 feet deep based on readings from the fish finder on board one of the boats used. Capturing fish from these deeper areas of the stream channel with the mini-boom was difficult, if not impossible. Lastly was the efficiency of the mini-boom compared to the stream shocker. The stream shocker is a more efficient

means of capturing fish because multiple anodes and dippers can be used with one electrofishing unit to cover a shallow, wadable stream channel compared to one electrofishing unit with one dipper trying to cover the immediate area along one bank of a large, non-wadable stream channel with variable water depths.

Two scoring metrics required weights and they focused on the weight per unit effort and percent insectivore weight. As mentioned above, no scores for these metrics can be computed without weights collected from all fish captured. Lengths were only taken on gamefish species, so it isn't possible to extrapolate approximate weights for non-gamefish species. Based on the information collected, it is doubtful that any station would have received 10 points for >25kg of fish biomass; some of the stations may have received 5 points for falling into the range of 10 to 25kg of fish biomass. All of the stations would have likely met the criteria of 11 to 60-percent of the fish biomass being classified insectivores and thus would have received 5 points for this metric; it's highly unlikely that any site would have exceeded 60-percent insectivore biomass to receive 10 points.

The second metric that was scored was the number of native species; all but one station received a score of 10 points. The only station that didn't score 10 points had 9 native species present, one short of the 10 or more native species present required to receive the full score of 10 points.

The third metric scored was the number of intolerant species present; Stations 3A and 6 received scores of 10 points, while the remaining stations only received 5 points. The lower score for the stations and low abundance of intolerant species may be somewhat misleading. Lake Superior basin streams lack many of the intolerant sucker and darter species found in other basins in the state; out of 25 species listed as intolerant in Appendix A.1 of Lyons, et al. 2001, only 6 have been documented in the Nemadji River watershed and 1 of them is a coldwater species that would not be expected to be present in the mainstem river.

The one noticeable difference between the stations receiving 10 points and those receiving 5 points was the capture of muskellunge, and in stations where muskellunge were captured, only a single individual was captured. The ability to catch muskellunge with the mini-boom relies on effectively stunning them so the dipper could capture them. In particular, adult muskellunge are able to feel the electrical field earlier than smaller fish due to their larger body size, so they are able to evade the mini-boom or are partially stunned, but still manage to swim away. During the electrofishing some larger fish splashed near the boat, but weren't readily identified, so muskellunge could have been present in the station, but not captured. Station 6 had also had spottail shiner present as a fourth intolerant species. Spottail shiners are considered a large river species rather than a riverine species and were likely migrants from the Superior Bay harbor area.

The fourth metric was the number of obligate riverine species present; three stations scored 10 points and the remaining two scored 5 points. Four or more riverine species were required to obtain a score of 10 points and the stations that received 10 points had the minimum number required, the stations that didn't receive 10 points had 2 or 3 riverine species present. As with the intolerant species, the low abundance of riverine species may be misleading because many of the darter, sucker, and minnow species commonly found in other Wisconsin drainages that fit the criteria of riverine species are not found in Lake Superior basin streams. Out of 90 species listed in Appendix A.1 of Lyons, et al. 2001, 37 are known to be present in the Nemadji River watershed and only 10 are considered riverine or large river species.

The fifth metric was the percentage of fish with deformities, erosions, lesions and/or tumors (DELT); no fish at any sites had exhibited deformities, open sores or other abnormalities. The percent of fish with signs or symptoms of DELT are often attributed to industrial or sewage discharge, however most point source pollution from industrial or municipal sources in the state of Wisconsin was eliminated or heavily treated since passage of the Clean Water Act in the 1970's. This metric was left in the scoring criteria as an extra sensitive measure to detect potential future discharge from point source polluters.

The percent obligate riverine species was the sixth metric; four of the five stations received 10 points and the remaining site, Station 3A, received 5 points. The percent riverine species in Station 3A was 29-percent, only 7-percentage points below the lower threshold of 36-percent to receive 10 points. Out of the 23 fish species captured in the surveys, only 6 were considered riverine species and the most prevalent riverine species were emerald shiner and silver redhorse. These species were present in the station and if a few more individuals were captured, this would have raised the percentage and score.

The percent simple lithophilic spawners was the last remaining metric that could be scored and four of five stations received 10 points, the remaining station received 5 points. Station 5 received 5 points, but was only 8-percentage points away from the lower threshold of 69% to receive 10 points. Capture of additional emerald or common shiners in this station would have improved the percentage and score for this metric.

Wadable Stations

The two wadable sites used were County Highway C and the 2011 survey at State Highway 35. The IBI score for County Highway C was 87 points and rated as "Excellent"; the IBI score for State Highway 35 was 70 points and was also rated as "Excellent". Out of the 10 metrics used for scoring the fish community, both IBI scores had 5 metrics that received the highest score of 10 points. Overall, the score reflects excellent water quality, but the metrics that didn't receive scores of 10 points may reflect some of the issues with habitat in both stations.

The first metric where both IBI scores missed points was the number of sunfish plus yellow perch. None of the wadable stream stations sampled between 2006 through 2011 (Table 3) have had more than 2 species in this metric and beyond rock bass, the only other species of sunfish captured in any of the four previous surveys was one bluegill. Downstream in the five non-wadable stations (Table 1), the only sunfish species found were rock bass and two black crappies. The moderate current and lack of deep pools in both wadable stations may be limiting preferred habitat for sunfish species other than rock bass. There was also a noticeable lack of aquatic vegetation in the both the County Highway C and State Highway 35 stations that would serve as suitable sunfish habitat.

The 2011 survey at State Highway 35 also missed points for number of intolerant species and the percent of top carnivore species. The capture of two additional intolerant fish species found in other segments of the Nemadji River would have raised the score up to 10 instead of 5. As mentioned in the discussion of the non-wadable scores and ratings, Lake Superior basin streams lack many of the intolerant sucker and darter species that are prevalent in the warmwater streams in other Wisconsin basins.

At the County Highway C station, 20-percent of the fish captured were considered tolerant of degraded habitat and were represented by creek chub and white sucker. While the station at County Highway C didn't receive a score of 10 points, the lower score isn't cause for alarm. The percentage tolerant species was 1-percentage point over the upper threshold of 19 to receive 10 points and three intolerant species were found in this survey.

In the 2011 survey at State Highway 35, tolerant species comprised 37-percent of the fish community and were represented mainly by creek chubs with white sucker and fathead minnows present. Five years prior to the 2011 survey, a survey of this station yielded only 13 percent of the fish community considered tolerant and 2 intolerant fish species were present. The variation in these scores may reflect migration because of the timing of the surveys; the 2011 survey took place in mid-September, while the 2006 survey was conducted in early July.

Smallmouth bass and muskellunge are two intolerant species that were present in other stations assessed on the Nemadji River, but not captured in this station. Low quality habitat may be the reason they were

not present; notes from previous surveys indicate most of the stream channel was shallow, sandy, and generally lacking cover.

The absence of smallmouth bass and muskellunge also resulted in a low top carnivore percentage of only 5 percent and a score of 0 for the State Highway 35 station. In spite of the low percentage and score, this metric was only 2 percentage points away from a score of 2 and 3 percentage points away from the lower threshold for 5 points. If rock bass and smallmouth bass were present at the same level of abundance as the 2006 survey at State Highway 35, the percentage of top carnivore score could have been better. The lower percentage top carnivore may also reflect fish migration because of the mid-September timing of the 2011 survey.

The third metric that both IBI scores didn't receive 10 points for was the percent of simple lithophil spawners (species that lay their eggs on clean gravel or cobble substrate without building a nest or providing parental care). The fish community at both County Highway C and State Highway 35 were comprised of 49-percent simple lithophil spawners and received 5 points, but were only 2 percentage points below the lower threshold of 51-percent for receiving 10 points; 50-percent would have received a score of 7 points. The vast majority of the substrate in both stations was sand or clay and the lack of suitable rock or gravel in the station limits the amount of spawning habitat for simple lithophil species and, in some cases, preferred habitat for several simple lithophil species like suckers, darters and certain minnow species. Previous surveys at other locations have typically received 5 points with the percent simple lithophil spawners ranging from 37 to 49-percent; however the station at County Highway W is an exception. This area of the Nemadji River is higher gradient and has rock and gravel riffle habitat and those features are reflected by the percent of simple lithophil species present as well as presence of species like longnose dace, hornyhead chub, stonecat and log perch that tend to live in areas with gravel and cobble habitats.

IBI Rating Summary

Despite relatively poor instream and riparian habitat in the Lower Nemadji River and some difficulty sampling fish, the fish communities documented reflect good water quality. In some instances, the lower scores for the IBI metrics reflect lower fish diversity in the Lake Superior basin rather than environmental degradation. Intolerant fish species were found in all of the stations and there was little change in species composition between the stations surveyed. Based on the information collected, the wadable stations were all scored as "Excellent". The scores for the non-wadable stations assessed this year can only be considered minimum estimates due to missing weight information for two of the metrics, however available information for each station gives ratings between "Good" and "Fair". If weight information was collected, it's likely that the non-wadable stations would have rate between "Good" and "Excellent".

5. Gamefish Resource Management Considerations

The survey work in the Lower Nemadji River watershed revealed this segment of the river supports several warmwater and coolwater gamefish species, but may be an overlooked fishery because access to the river is limited to carry-in or road-side access for anglers fishing from a canoe or similar watercraft. The Nemadji River does receive some angling pressure in the upper reaches based on observations of lures and fishing line snagged in over-hanging trees and electrical cables at bridge crossings, but most of the recreational fishing effort probably occurs within the last two to three miles; during survey work our crew encountered a pair of anglers fishing from shore at the Grand Avenue launch and two boats with fishermen trolling for walleyes and muskellunge. Future survey work could be considered to better define the gamefish resource present in the Nemadji River and what functions it might serve within the Duluth/Superior Harbor and Lake Superior.

Northern Pike and Muskellunge

Northern pike or muskellunge were found at the wadable station and 4 of 5 non-wadable stations this year as well as previous wadable stream survey work upstream from County Highway C which suggests both species are relatively common in the Nemadji River.

There are some discrepancies with the muskellunge water classification for the Nemadji River. The current version of DNR Wisconsin Muskellunge Waters lists the Nemadji River downstream from CTH C as road only access, Class A1 (lower abundance, higher trophy potential) and Category 2 (combination of natural reproduction and stocking) muskellunge water. The Surface Water Data Viewer Muskellunge Waters layer shows the Nemadji River from the WI-MN border downstream to Lake Superior is Category 0 muskellunge water (unknown reproductive status, stocking occurs). These discrepancies should be clarified to represent the muskellunge fishery present; a Class A1 designation is appropriate and Category 2 would be appropriate based on the current known reproductive status of the musky population. Future survey work could be considered to better document the muskellunge fishery present and extent of natural reproduction in the lower Nemadji River. One young-of-year muskellunge was captured in Station 6, which could indicate adult muskellunge use the floodplain marshes near the mouth of the river for spawning.

There are no state classifications or designations for northern pike waters, but the Nemadji River has the potential to produce quality size (21"+) fish and the population appears to be sustained by natural reproduction.

Muskellunge angling regulations for the Nemadji River are now consistent with the St. Louis River regulation of an open season from the Saturday nearest Memorial Day through November 30th, with a 1 fish daily bag limit and a 50-inch minimum. The current angling regulation for northern pike in the Nemadji River is an open season from the Saturday 2 weeks prior to the Saturday nearest Memorial Day through March 1st the following year, with a 26-inch minimum and 2 fish daily bag limit. These regulations are appropriate as they maintain consistency with the same opportunities for harvest that are available in the adjoining waters of the St. Louis Estuary and Lake Superior.

Walleye

Walleye were found at the wadable station and 4 out of the 5 non-wadable stations this year, as well as previous wadable stream survey work upstream from County Highway C which suggests they are also relatively common in the Nemadji River. There were multiple year classes of walleyes present in the 2015 sampling, but it is unknown if there is natural reproduction occurring in the Nemadji River or if these fish are migrants from the St. Louis River; two tagged walleyes from the spring 2015 survey work on the St. Louis River were captured in the lower Nemadji River and reported by anglers. Additional survey work could be undertaken to determine the status of the walleye fishery in the Nemadji River.

The current set of regulations for walleye, sauger, and their hybrids is an open season from the Saturday 2 weeks prior to the Saturday nearest Memorial Day to March 1st with a 15-inch minimum and a daily bag limit of 2 fish. This regulation maintains consistency with the regulations for the St. Louis River and Superior Bay as well as the same length limit for Lake Superior and Lake Superior tributaries and sloughs; Lake Superior and the tributary streams and sloughs have different season structure and a daily bag limit of 5 walleyes, but only 1 fish over 20-inches is allowed.

Smallmouth and Largemouth Bass

Smallmouth bass are also very prevalent in the Lower Nemadji River, every site sampled this year had smallmouth bass present, however most individuals captured were young-of-year and juvenile fish less than 12-inches. The Lower Nemadji River could be added to the current state list of smallmouth bass waters as a non-wadable fine substrate smallmouth bass stream. This designation does not afford any additional environmental protection to the river, but serves to confirm the presence of smallmouth bass in the river and this segment of the river would appear in Smallmouth Bass Stream layer in the Surface Water Data Viewer that is accessible to department staff and the public.

Largemouth bass abundance in the Nemadji River appears to be very low; only one largemouth bass was captured in Station 6, near the confluence with Superior Bay.

There is no catch are release season for bass applied to the Nemadji River, the current set of regulations for smallmouth and largemouth bass is an open (harvest) season from the last Saturday in May through March 1st the following year, with a five fish daily bag limit and a minimum length limit of 12-inches. The length limit on bass could be increased to 14-inches for the sake of consistency with the length limit on the St. Louis River and Superior Bay.

Other notable species

Based on the electrofishing catch, panfish species occurred in relatively low abundance at all of the stations sampled this year. Rock bass and yellow perch were the most prevalent of the panfish species documented, but few of the individuals captured were large enough to be of interest to anglers.

One channel catfish was captured, no other catfish were observed or collected, but electrofishing with the mini-boom shocker is not the most efficient means of capturing catfish or other benthic species. If there is management interest in channel catfish in the St. Louis River estuary, a survey including the lower Nemadji River could be considered and hoop or trap nets or hook-and-line gear like a trot line or set line could be a more efficient means of capturing catfish.

Lake sturgeon are present in the St. Louis River and estuary, but sturgeon were not observed or collected during this survey work, so there remains no information to suggest sturgeon use any portion of the Nemadji River.

6. Literature and Data Cited

John Lyons, Randal R. Piette & Kent W. Niermeyer (2001) Development, Validation, and Application of a Fish-Based Index of Biotic Integrity for Wisconsin's Large Warmwater Rivers, Transactions of the American Fisheries Society, 130:6, 1077-1094

Lyons, John 1992. Using the index of biotic integrity (IBI) to measure environmental quality in warmwater streams of Wisconsin. General Technical Report NC-149. St. Paul, MN: U.S. Dept. of Agriculture, Forest Service, North Central Forest Experiment Station

Wisconsin Department of Natural Resources. 2015. Wisconsin Muskellunge Waters. PUB-FH-515(2015).

NON-WADABLE RIVER FISH COMMUNITY SUMMARY

STREAM: Nemadji River
WBIC: 2835300
COUNTY: Douglas
GEAR: 240V 3000W DCP Mini-Boom
16 Droppers, 1 Dipper

STATION:	DISTANCE SHOCKED (miles):	RIVER MILE AT STATION START:
3	1.08	14
3A	1.30	12
4	1.10	10
5	1.22	3
6	1.44	1

TAXONOMIC FAMILY / FISH SPECIES	Origin / Thermal Guild	Tolerance	Feeding	Habitat	Spawning Type	STATION NUMBERS 3	3A	4	5	6	Total Captured
Esocidae											8
MUSKELLUNGE	NCL	I	TC	O	O		1			1	2
NORTHERN PIKE	NEU	M	TC	O	O	1	1		4		6
Percidae											39
WALLEYE	NEU	M	TC	O	SL	1		2	1	6	10
YELLOW PERCH	NEU	M	IN	O	O	1	4	1	5	6	17
JOHNNY DARTER	NEU	M	IN	O	O		2				2
LOG PERCH	NEU	M	IN	O	SL	6	4				10
Centrarchidae											35
SM BASS	NEU	I	TC	O	O	3	9	1	1	3	17
LM BASS	NEU	M	TC	O	O					1	1
BLACK CRAPPIE	NEU	M	TC	O	O					2	2
ROCK BASS	NEU	I	TC	O	O	1	7	2	4	2	16
Cyprinidae											225
EMERALD SHINER	NEU	M	IN	L	SL	51	12	12	1	32	108
COMMON SHINER	NEU	M	IN	O	SL	15	30	8	5	1	59
SAND SHINER	NEU	M	IN	R	O	3	8		10	29	50
SPOTTAIL SHINER	NEU	I	IN	L	O					6	6
BLUNTNOSE MINNOW	NEU	T	OM	O	O				1		1
HORNYHEAD CHUB	NEU	M	IN	R	O		1				1
Ictaluridae											1
CHANNEL CATFISH	NEU	M	TC	O	O			1			1
Catostomidae											45
WHITE SUCKER	NEU	T	OM	O	SL	6					6
SILVER REDHORSE	NEU	M	IN	R	SL	5	3	4	7	6	25
SHORTHEAD REDHORSE	NEU	M	IN	O	SL	7	1	1	1	4	14
Moronidae											6
WHITE PERCH	EEU	M	TC	O	O				2	4	6
Percopsidae											1
TROUT-PERCH	NEU	M	IN	O	O	1					1
Sciaenidae											1
FRESHWATER DRUM*	EEU	M	IN	L	O	1					1
*Native to WI, but not Lake Superior Basin											
Station Total						102	83	32	42	103	

COMMENTS:

Origin / Thermal Guild - N - Native, E - Exotic / EU - Eurythermic, CL - Stenothermal Coolwater, CD - Stenothermal Coldwater

Tolerance - I-Intolerant, M-Moderately Intolerant, T-Tolerant

Feeding - Fi-Filter Feeder, Ge-Genral Feeder, He-Herbivore, Pa-Parasitic, O-Omnivore, I-Insectivore, TC-Top Carnivore,

Habitat - L-Large River, R-Riverine, O-Other, Spawning Habitat - SL-Simple Lithophilous, O-Other

Table 1. Catch-per-effort summary and species characteristics for all non-wadable stations surveyed on Nemadji River in 2015.

NON-WADABLE RIVER FISH COMMUNITY SUMMARY

	STATION:	DISTANCE SHOCKED (miles):	RIVER MILE AT STATION START:
STRFAM: **Nemadji River**	3	1.08	14
WBIC: **2835300**	3A	1.30	12
COUNTY: **Douglas**	4	1.10	10
GEAR: **240V 3500W DCP Mini-Boom**	5	1.22	3
16 Droppers, 1 Dipper	6	1.44	1

Station Name	3	3A	4	5	6
Survey Year	2015	2015	2015	2015	2015
Survey Date	8/3/15	8/4/15	8/3/15	8/5/15	8/5/15
Primary Survey Purpose	Fish Community Survey	Fish Community Survey	Fish Community Survey	Fish Community Survey	Fish Community Survey
Total Fish Collected:	102	83	32	40	98
WPUE (kg):	Minnows, suckers, and darters were counted but not weighed				
WPUE (kg) Score:	**Unable to compute scores without aggregate weights**				
Number Native Species:	14	13	9	11	13
Number Native Species Score:	**10**	**10**	**5**	**10**	**10**
Number Intolerant Species:	2	3	2	2	4
Number Intolerant Species Score:	**5**	**10**	**5**	**5**	**10**
Riverine Species:	4	4	2	3	4
Riverine Species Score:	**10**	**10**	**5**	**5**	**10**
% DELT (Deformity, Erosion, Lesion, Tumor):	0	0	0	0	0
% DELT (Deformity, Erosion, Lesion, Tumor) Score:	**10**	**10**	**10**	**10**	**10**
% Riverine Species (n):	59%	29%	50%	42%	74%
% Riverine Species Score	**10**	**5**	**10**	**10**	**10**
% Lithophils (n):	92%	70%	84%	61%	79%
% Lithophils (n) Score:	**10**	**10**	**10**	**5**	**10**
%Insectivore (wt):	Minnows, suckers, and darters were counted but not weighed				
%Insectivore (wt) Score:	**Unable to compute scores without aggregate weights**				
Score Subtotal	**55**	**55**	**45**	**45**	**60**
Correction Factor For Lower Sucker Richness in Lake Superior Basin Streams (Subtotal X 1.25)	1.25	1.25	1.25	1.25	1.25
Non-Wadable River Warmwater IBI Score	**≥68.75**	**≥68.75**	**≥56.25**	**≥56.25**	**≥75**
Non-Wadable River Warmwater IBI Integrity Rating	**Good**	**Good**	**Fair**	**Fair**	**Good**

Table 2. IBI scores and ratings for all non-wadable stations surveyed on Nemadji River in 2015. Ratings and scores for all of the stations are not complete due to missing weight information and should be considered minimum estimates.

WADABLE RIVER FISH COMMUNITY SUMMARY

STREAM: Nemadji River	YEAR:	STATION:	DISTANCE SHOCKED (meters):	RIVER MILE AT STATION START:
WBIC: 2835300	2006	STH 35	645	18.4
COUNTY: Douglas	2008	Finn Rd	425	20
GEAR: 240V Tow Barge	2009	CTH W	440	31.2
	2011	STH 35	855	18.4
	2015	CTH C	400	11.75

TAXONOMIC FAMILY / FISH SPECIES	Origin / Thermal Guild	Tolerance	Feeding	Spawning Type	STATION NUMBERS / SURVEY YEAR: STH 35 2006	Finn Rd 2008	CTH W 2009	STH 35 2011	CTH C 2015
Esocidae									
MUSKELLUNGE	NCL	I	TC	O			1		1
NORTHERN PIKE	NEU	M	TC	O				2	
Percidae									
WALLEYE	NEU	M	TC	SL	1	1	3	1	1
YELLOW PERCH	NEU	M	IN	O					2
JOHNNY DARTER	NEU	M	IN	O	2	1	11	3	6
LOG PERCH	NEU	M	IN	SL	3	1	70	8	1
Centrarchidae									
SM BASS	NEU	I	TC	O	4	2	3		6
ROCK BASS	NEU	I	TC	O	39	29	25	16	12
BLUEGILL	NEU	M	IN	O				1	
Cyprinidae									
EMERALD SHINER	NEU	M	IN	SL					4
COMMON SHINER	NEU	M	IN	SL	53	29	54	150	36
SAND SHINER	NEU	M	IN	O		11	1	4	1
HORNYHEAD CHUB	NEU	M	IN	O	27	22	78	8	7
LONGNOSE DACE	NEU	M	IN	SL			38		
CREEK CHUB	NEU	T	GE	O	23	4	3	78	21
FATHEAD MINNOW	NEU	T	O	O				14	
BRASSY MINNOW	NCL	M	HE	O	3				
Ictaluridae									
STONECAT	NEU	M	IN	O	5	2	7	2	
Catostomidae									
WHITE SUCKER	NEU	T	O	SL		2	18	8	1
SILVER REDHORSE	NEU	M	IN	SL	2	6	38	2	
SHORTHEAD REDHORSE	NEU	M	IN	SL	22	7		5	13
Percopsidae									
TROUT-PERCH	NEU	M	IN	O		7	4	22	1
Umbridae									
CENTRAL MUDMINNOW	NEU	M	IN	O				12	1
Petromyzonidae									
LAMPREYS	UNKNOWN	I	FI / PA	O		1			
No indication of life stage or species - can't assign origin, thermal guild or feeding									
Station Total					184	125	354	336	114

COMMENTS:

Origin / Thermal Guild - N - Native, E - Exotic / EU - Eurythermic, CL - Stenothermal Coolwater, CD - Stenothermal Coldwater

Tolerance - I-Intolerant, M-Moderately Intolerant, T-Tolerant

Feeding - FI-Filter Feeder, Ge-Genral Feeder, He-Herbivore, Pa-Parasitic, O-Omnivore, I-Insectivore, TC-Top Carnivore,

Spawning Habitat - SL-Simple Lithophilous, O-Other

Table 3. Catch-per-effort summary and species characteristics for all wadable stations surveyed on Nemadji River.

WADABLE RIVER FISH COMMUNITY SUMMARY

STREAM: **Nemadji River**	YEAR:	STATION:	DISTANCE SHOCKED (meters):	RIVER MILE AT STATION START:
WBIC: **2835300**	2006	STH 35	645	18.4
COUNTY: **Douglas**	2008	Finn Rd	425	20
GEAR: **240V Tow Barge**	2009	CTH W	440	31.2
	2011	STH 35	855	18.4
	2015	CTH C	400	11.75

Station Name	STH 35	Finn Rd	CTH W	STH 35	CTH C
Survey Year	2006	2008	2009	2011	2015
Survey Begin Date	7/7/06	7/29/08	8/19/09	9/15/11	8/4/15
Survey End Date	7/7/06	7/31/08	9/11/09	9/15/11	8/4/15
Primary Survey Purpose	WATERSHED CLEAN WATER ACT	NATURAL COMMUNITY REFERENCE	SPECIAL STUDY	NATURAL COMMUNITY REFERENCE	FISH COMMUNITY SURVEY
Mean Stream Width (meters)	18.4	13.8	17.5	24.4	19
Total Fish Count Sum	184	124	354	355	114
Number Native Species	12	14	15	18	16
Number of Native Species Score Lake Superior	5	5	10	10	10
Number Darter Madtom Sculpin Species	2	2	2	2	2
umber Darter Madtom Sculpin Species Lake Superior Score	10	10	10	10	10
Number Sucker Species	2	3	2	3	2
Number Sucker Species Score Lake Superior	10	10	10	10	10
Number Sunfish Yellow Perch Species	1	1	1	2	2
Number Sunfish Yellow Perch Species Score Lake Superior	5	5	5	5	5
Number Intolerant Species	2	2	3	1	3
Number Intolerant Species Score Lake Superior	5	5	10	5	10
Percent Tolerant	13	5	6	37	20
Percent Tolerant Score	10	10	10	5	7
Percent Omnivore	0	2	5	6	1
Percent Omnivore Score	10	10	10	10	10
Percent Insectivores	62	69	85	66	63
Percent Insectivore Score	10	10	10	10	10
Percent Top Carnivore	24	26	9	5	18
Percent Top Carnivore Score	10	10	5	0	10
Percent Simple Lithophile	44	37	62	49	49
Percent Simple Lithophile Score	5	5	10	5	5
Abundance Correction Factor	75	83	227	79	68
Percent DELT	-	-	-	-	-
Warmwater IBI Score Lake Superior	80	80	90	70	87
Warmwater IBI Score Lake Superior Corrected	80	80	90	70	87
Warmwater IBI Integrity Rating Lake Superior	Excellent	Excellent	Excellent	Excellent	Excellent

Table 4. IBI scores and ratings for all wadable stations surveyed on Nemadji River.

Appendix I.
Electrofishing Station Maps

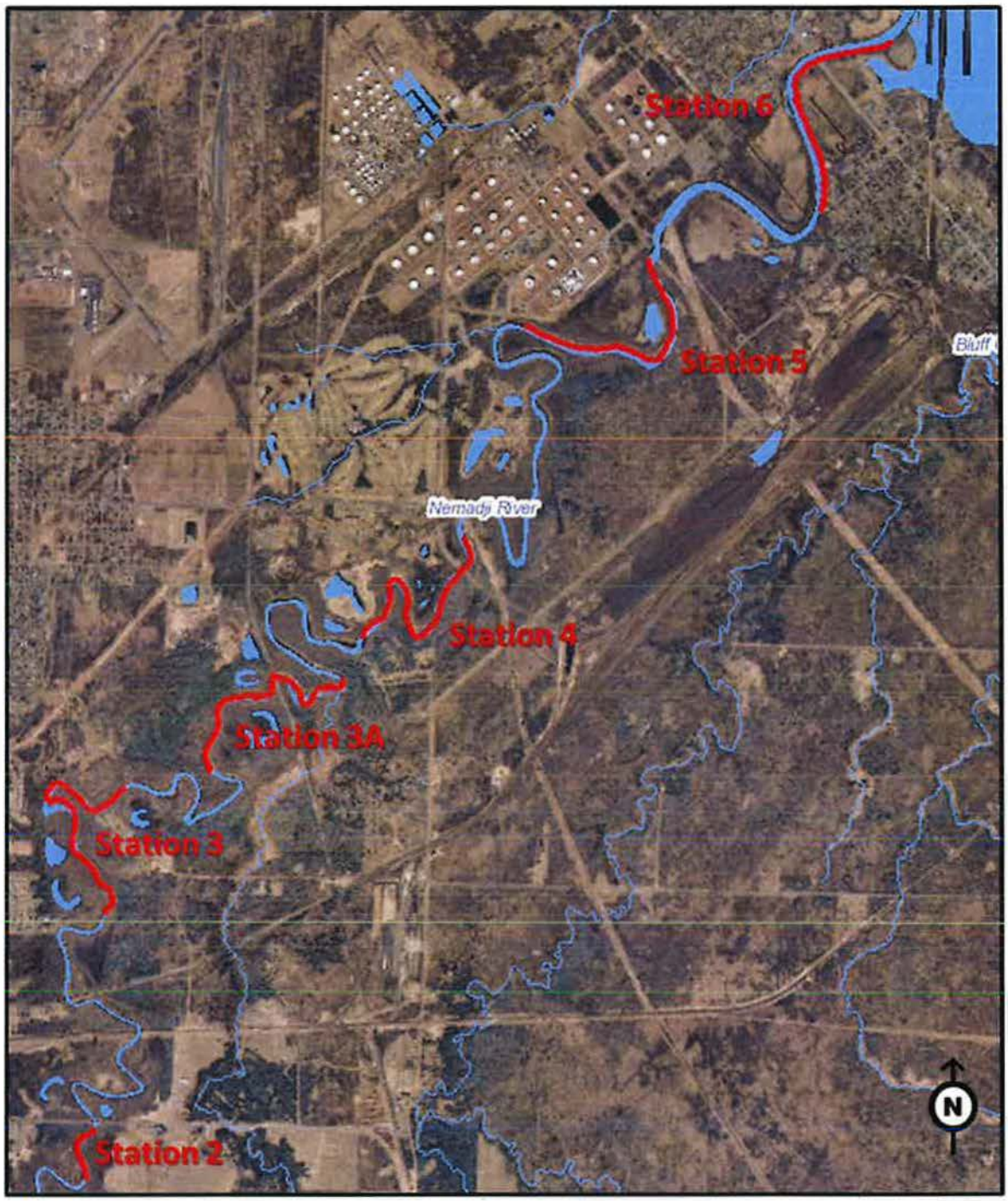
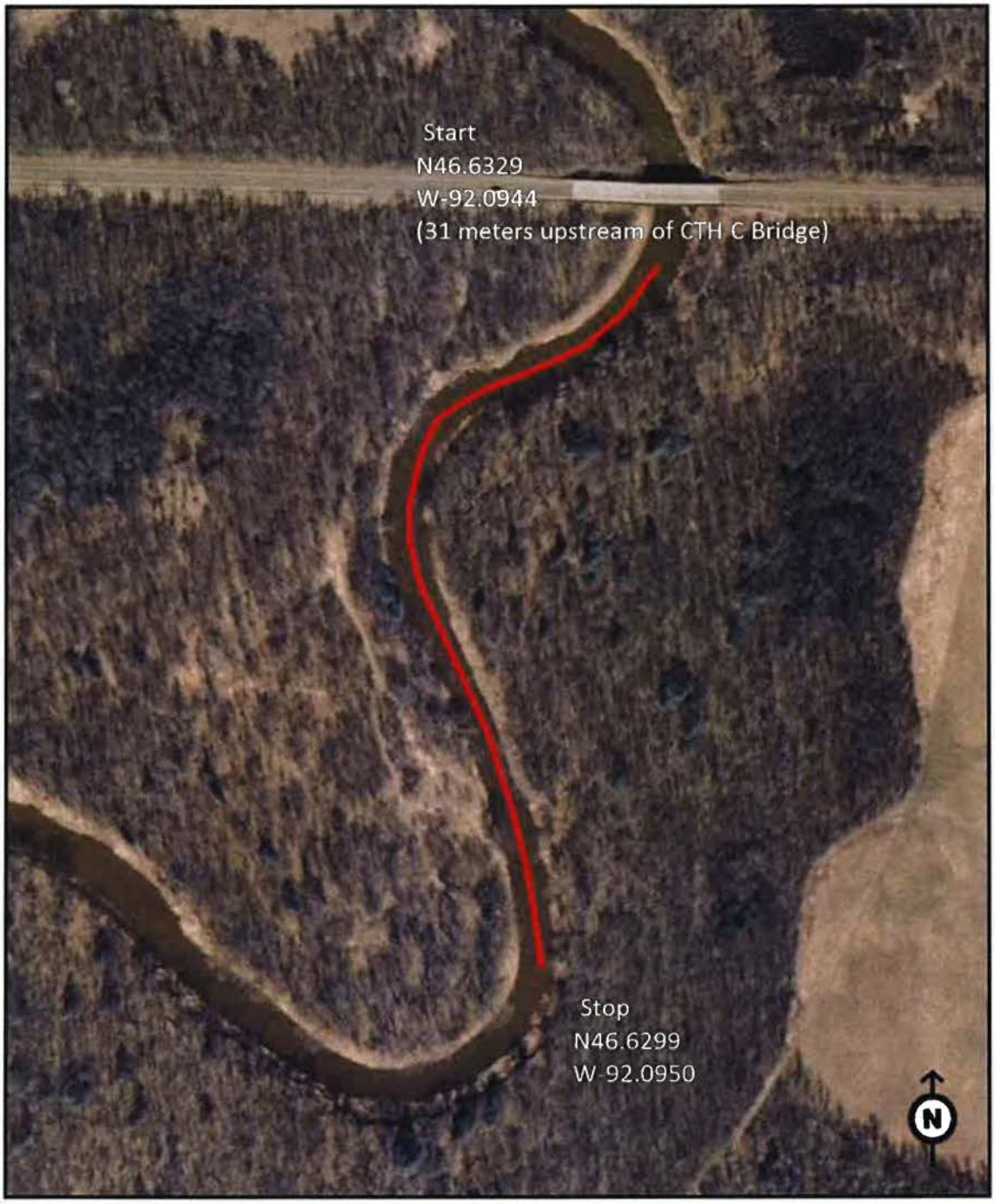


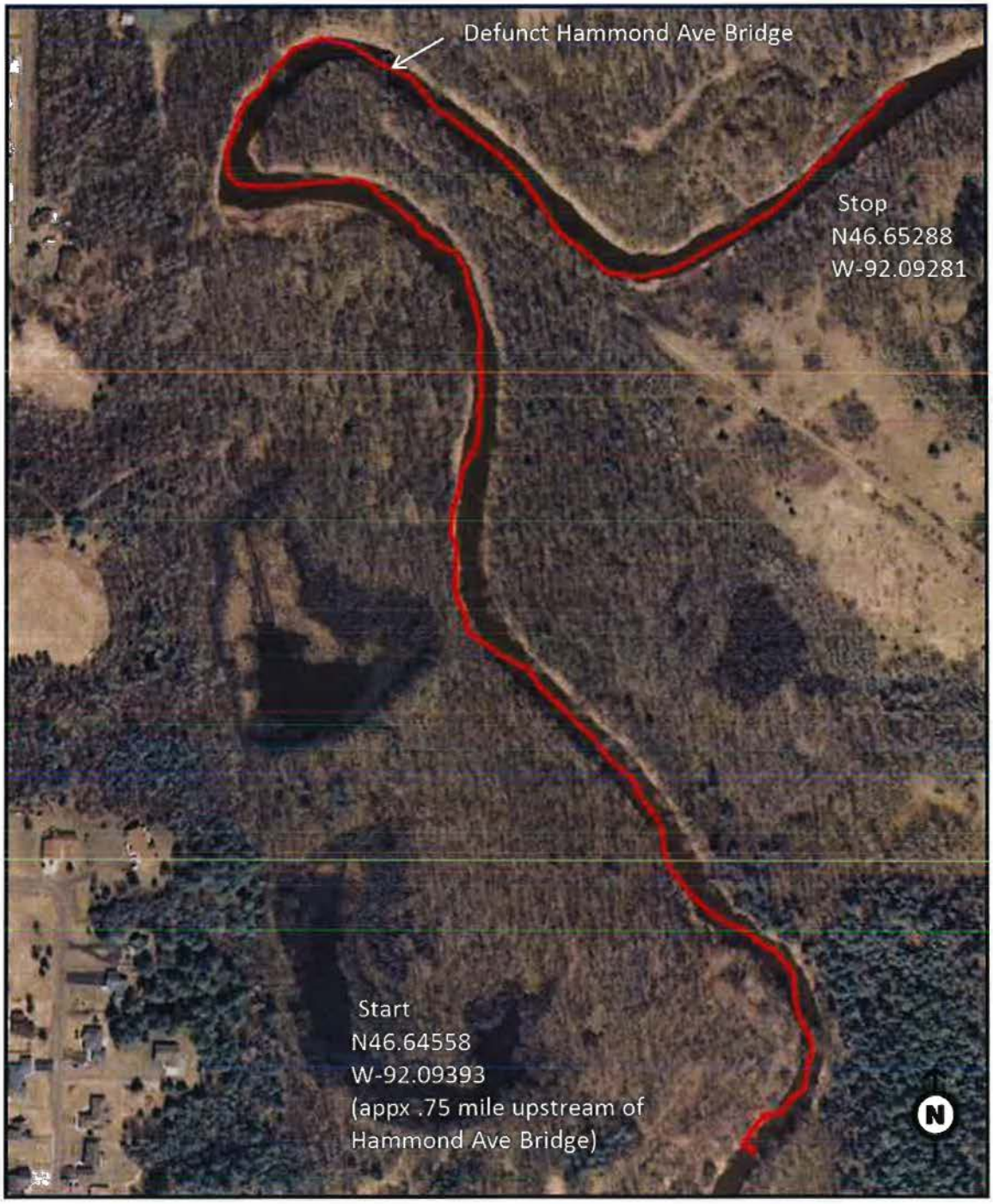
Figure 1. Map of electrofishing stations assessed during 2015 fish community sampling on Nemadji River, Douglas County, WI.



Nemadji River, Douglas County
Wadable River Electrofishing
Fish Community Survey
Station 2

Legend
— Station (400 meters)

WISCONSIN DEPT. OF NATURAL RESOURCES



Nemadji River, Douglas County
Non-Wadable River Electrofishing
Fish Community Survey
Station 3

Legend
— Station (1.08 miles)

WISCONSIN
DEPT. OF NATURAL RESOURCES

Legend

— Station (1.30 miles)

Nemadji River, Douglas County

Non-Wadable River Electrofishing
Fish Community Survey
Station 3A

WISCONSIN DEPT OF NATURAL RESOURCES

Legend

— Station (1.10 miles)

Nemadji River, Douglas County

Non-Wadable River Electrofishing

Fish Community Survey

Station 4

Legend

— Station (1.22 miles)

Nemadji River, Douglas County

Non-Wadable River Electrofishing
Fish Community Survey
Station 5

WISCONSIN DEPT OF NATURAL RESOURCES

Nemadji River, Douglas County

Non-Wadable River Electrofishing
Fish Community Survey
Station 6

Legend

— Station (1.44 miles)

WISCONSIN DEPT. OF NATURAL RESOURCES

Appendix 8

Nemadji River and Tributaries Water Quality Assessment
(Pertains to management action 6.05)

Nemadji River and Tributaries
Water Quality Assessment

Craig Roesler – DNR, Spooner (3/24/14)

Introduction

Monitoring of the Nemadji River and several of its tributaries was conducted during 2008 to 2010 by Superior office staff to assess water quality conditions, and to help determine if these streams should be placed on Wisconsin's 303d list of impaired waters. Sites monitored are shown in figures 1 and 2. Streams monitored were the Nemadji River, Crawford Creek, Black River, Balsam Creek, Clear Creek, and Mud Creek.

The Wisconsin portion of the Nemadji River watershed is located in Douglas County in the northwest corner of the state. The upstream half of the watershed is located in Minnesota. The Nemadji River flows into Superior Bay on the south side of the City of Superior.

Crawford Creek was previously placed on the 303d list in 1998. The impairment identified is chronic aquatic toxicity. Pollutants identified at that time were creosote and PAH's. Dioxins are also present. Koppers Industries operated a wood treatment facility that discharged to the creek and contaminated sediments in the creek and its floodplain. The Department is working with the responsible party to better define the degree and extent of sediment contamination and to work toward the clean-up of the creek and flood plain soils.

Erodible clay soils interspersed with sands and silts are present in the Crawford Creek watershed. Flows are "flashy" with high peak flows during runoff events, and low base flows between runoff events. Eroding stream banks, high turbidity, high suspended solids concentrations, and fine sediment bed load are other concerns for this stream.

Much of the Nemadji River watershed also has erodible clay soils interspersed with sands and silts. Erosion of stream banks and drainageways to streams provides most of the sediment load to the Nemadji River. The river carries a large load of both suspended sediment and bed load sediment. The Nemadji River is estimated to deliver 127,000 tons of sediment per year to Superior Bay and Lake Superior. The Army Corps of Engineers removes about 33,000 tons of sediment (mostly sand) per year near the mouth of the river to maintain the navigation channel. The river has high turbidity and high suspended solids concentrations.

The Nemadji River was added to the 303d list in 2010. The high sediment load was judged to exceed the narrative water quality standard found in NR102.04 (a) of the Wisconsin Administrative Code, which states "Substances that will cause objectionable deposits on the shore or in the bed of a body of water, shall not be present in such amounts as to interfere with public rights in waters of the state." Minnesota placed the Nemadji River on its 303d list in 2004 due to exceedences of their turbidity standard (25 ntu), and began developing a TMDL in 2008. Wisconsin's listing in 2010 was based in large part on Minnesota's listing since conditions in the Minnesota and Wisconsin reaches are similar, although Wisconsin does not have a standard for turbidity or total suspended solids. The median turbidity measured in the Nemadji River at CTH C (Wisconsin) is 27.5 ntu (2006-2012), which exceeds Minnesota's turbidity standard. Including the Nemadji River on Wisconsin's 303d list would allow the two states to work together to develop a comprehensive TMDL that would benefit the entire watershed.

The other consideration that contributed to the listing decision was that creosote and PAH's from Crawford Creek are a continuing source of pollutants to the Nemadji River.

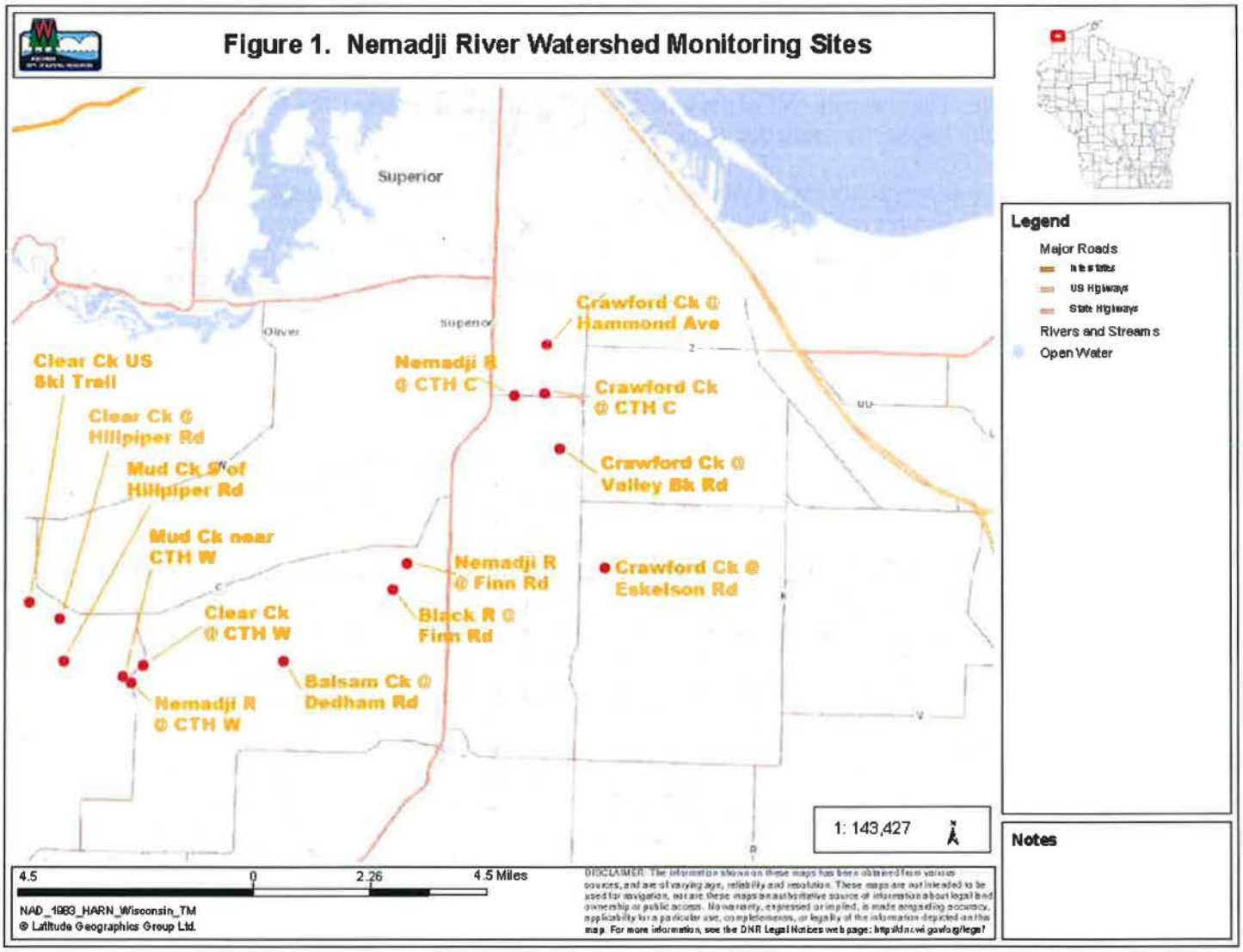
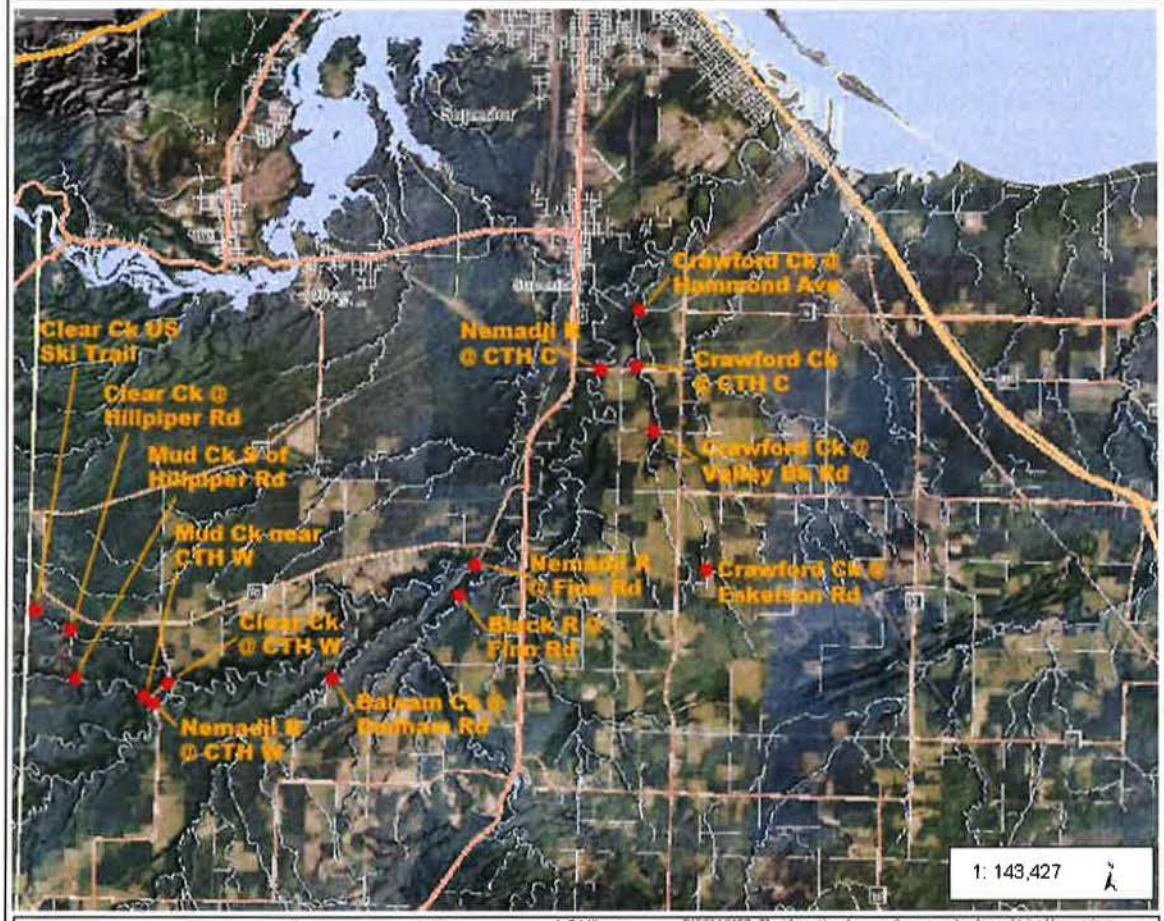


Figure 2. Nemadji River Watershed Monitoring Sites with 2008 Airphoto Background



Legend

Major Roads
- Interstates
- US Highways
- State Highways

Rivers and Streams

Open Water

Notes

DISCLAIMER: The information shown on these maps has been obtained from various sources, and are of varying age, reliability and resolution. These maps are not intended to be used for navigation, nor are these maps an authoritative source of information about legal land ownership or public access. No warranty, expressed or implied, is made regarding accuracy, applicability for a particular use, completeness, or legality of the information depicted on this map. For more information, see the DNR Legal Notices web page: http://dnr.wi.gov/org/legal

There are four point source discharges going directly or indirectly to the Nemadji River:

- Burlington Northern R.R. Co.; a taconite loading and storage facility (direct)
- Lakehead Pipeline Co.; oil storage tanks (direct)
- Superior Sewage Disposal System; municipal wastewater (direct)
- Four Corners School; school wastewater (indirect via unnamed tributary and Copper Creek to the Nemadji River)

The three direct discharges all enter the Nemadji River downstream of the Nemadji River monitoring site at CTH C. Monitoring the Nemadji River downstream of CTH C is difficult due to lack of access points and periodic backflows caused by Lake Superior seiches. The Four Corners School discharge is very small and located far upstream.

The data collected from this monitoring project does not support 303d listing of any of the other streams monitored (see discussion on p. 18).

Methods

One or more sites were monitored on each of the six streams:

- Nemadji River – 3 sites
- Crawford Creek – 3 sites
- Black River – 1 site
  Balsam Creek 1 site
- Clear Creek – 3 sites
- Mud Creek – 2 sites

Monitoring was done for fish and macroinvertebrate communities, water chemistry, and stream habitat. The range of monitoring at each site varied (table 1).

Fish communities were assessed by electrofishing with a single anode backpack shocker on small stream sites, and a double anode tow barge stream shocker on larger stream sites. As many fish as possible were captured with a single upstream pass. Station lengths were 35 times the mean stream width, with a minimum length of 100 meters. Fish captured were counted and identified to species. Fish community data was used to determine the natural community of the stream, and to calculate potentially appropriate biotic indices.

Macroinvertebrate communities were assessed by collecting kick samples from riffles, using a 500 um mesh D-frame net. Samples were preserved in 85% ethanol and were processed by UW – Stevens Point's Aquatic Biomonitoring Lab. Macroinvertebrates were counted and identified to the lowest possible taxa. Biotic indices and other statistics were generated.

Water samples were collected and field parameters were measured following standard DNR protocols. Water samples were preserved, as needed, and shipped on ice to the Wisconsin State Lab of Hygiene for analysis. Field parameters measured were:

- Temperature
- pH
- Dissolved Oxygen
- Conductivity
- Transparency (using a transparency tube)

Table 1. Nemadji River Watershed Monitoring Site Information

Site					Types of Monitoring Done			
Nemadji River	**Swims ID**	**WBIC**	**Latitude**	**Longitude**	**Water**	**Macroinvertebrates**	**Fish**	**Habitat**
Nemadji R @ CTH C	163003	2835300	46.63327	-92.09419	x			
Nemadji R @ Finn Rd	163233	2835300	46.58611	-92.13708	x		x	x
Nemadji R @ CTH W	163047	2835300	46.55017	-92.24740	x	x	x	x
Crawford Creek								
Crawford Ck @ Hammond Ave	10015464	2835500	46.64780	-92.08208	x		x	x
Crawford Ck @ CTH C	10031796	2835500	46.63416	-92.08542		x		
Crawford Ck @ Vally Brook Rd	10032010	2835500	46.61938	-92.07584		x		
Crawford Ck @ Eskelson Rd	10031826	2835800	46.58692	-92.05675		x	x	x
Black River								
Black R @ Finn Rd	10030272	2836900	46.57887	-92.14029		x	x	x
Balsam Creek								
Balsam Ck @ Dedham Rd	10007599	2841400	46.55773	-92.18507	x	x	x	x
Clear Creek								
Clear Ck @ CTH W	10030271	2842800	46.55563	92.24165	x	x	x	x
Clear Ck @ Hillpiper Rd	10031879	2842800	46.56770	-92.27605		x		x
Clear Ck US Ski Trail	10031940	2842800	46.57161	-92.28800	x	x	x	x
Mud Creek								
Mud Ck near CTH W	10030270	2843000	46.55028	-92.24752	x	x	x	x
Mud Ck South of Hillpiper Rd	10031880	2843000	46.55594	-92.27283		x		

Lab parameters were:

- Total Phosphorus
- Ammonia – N
- Total Kjeldahl N
- Nitrate plus Nitrite – N
- Total Suspended Solids
- Turbidity
- Chlorophyll a
- Dissolved phosphorus

Findings and Discussion

Fish Communities

Complete fish survey results are contained in appendix A. Fish survey IBI's (index of biotic integrity) are summarized in table 2.

Fish community indices of biotic integrity (IBI) ranged from excellent to fair. Sites on the Nemadji River, Black River, Balsam Creek, and Mud Creek had IBI ratings of excellent. Clear Creek had IBI ratings of good.

The Crawford Creek sites had the lowest IBI ratings of fair. The percent of fish that are tolerant of environmental disturbances exceeded 75% at these sites (92-100%; table 2). Seventy-five percent tolerant fish is the maximum typically expected in a Cool-Cold Headwater stream. The high percentages of intolerant fish at the Crawford Creek sites probably reflects the influence of erodible clay soils in a small watershed with moderate development, limited fish habitat, and very low base flows.

The stream sites varied in size and natural stream community types (table 2-2), from Cool-Cold Headwaters to Warm Mainstems. Clear Creek upstream of the ski trail was the only site where a coldwater species was found (mottled sculpin; appendix A).

Macroinvertebrate Communities

Macroinvertebrate IBI (MIBI) ratings were excellent or good at all but one site (table 3). The MIBI for Crawford Creek at CTH C was rated as fair. MIBI ratings were generally similar to the IBI's for the fish communities. One exception was the Crawford Creek site at Eskelson Road, where the MIBI was excellent, while the fish IBI was only fair.

Hilsenhoff biotic index (HBI) ratings ranged from good to excellent. HBI's are mostly influenced by organic matter loading and the resultant dissolved oxygen concentrations. The HBI's suggest dissolved oxygen stress to macroinvertebrates is minimal.

Table 2. Nemadji River and Tributaries Fish IBI Summary

Stream Site	Date	Small Stream IBI	Small Stream IBI Rating	Cool - Warm IBI	Cool - Warm IBI Rating	Warmwater L. Superior IBI	Warmwater L. Superior IBI Rating	% Tolerant	> 25 Fish?
Nemadji R @ Finn Rd	06/31/2008					80	excellent	30	yes
Nemadji R @ CTH W	09/11/2009					90	excellent	21	yes
Crawford Ck @ Hammond Ave	07/07/2006	40	fair					92	no, 23
Crawford Ck @ Eskelson Rd	08/23/2010	40	fair					100	yes
Black R @ Finn Rd	09/11/2009			80	excellent			30	yes
Balsam Ck @ Dedham Rd	09/21/2010			90	excellent			38	yes
Clear Ck @ CTH W	08/05/2009	60	fair	60	good			59	yes
Clear Ck US Ski Trail	08/24/2010	90	good					77	yes
Mud Ck near CTH W	09/01/2009	90	good	100	excellent			35	yes

TABLE 2-2. NEMADJI RIVER AND TRIBUTARIES NATURAL STREAM COMMUNITIES

Stream Site	Natural Stream Community*
Nemadji R @ Finn Rd	**Warm Mainstem**
Nemadji R @ CTH W	**Warm Mainstem**
Crawford Ck @ Hammond Ave	**Cool-Cold or Cool-Warm Headwater**
Crawford Ck @ Eskelson Rd	**Cool-Cold or Cool-Warm Headwater**
Black R @ Finn Rd	**Cool-Warm Mainstem**
Balsam Ck @ Dedham Rd	**Cool-Warm Mainstem**
Clear Ck @ CTH W	**Cool-Warm Mainstem**
Clear Ck US Ski Trail	**Cool-Cold or Cool Warm Headwater**
Mud Ck near CTH W	**Cool-Warm Mainstem**

*as indicated by sampled fish populations

Table 3. Nemadji River Watershed Macroinvertebrate Sample Summary*

Site	Station No.	Date	MIBI	MIBI Rating	HBI	HBI Rating	Species Richness (No. species)	% EPT (genera/individuals)
Nemadji R @ CTH W	163047	11/05/2009	8.64	excellent	3.83	very good	47	43/63
Crawford Ck @ CTH C	10031796	10/19/2010	3.84	fair	4.12	very good	20	30/55
Crawford Ck @ Valley Brook Rd	10032010	11/04/2010	6.03	good	5.36	good	28	18/33
Crawford Ck @ Eskelson Rd	10031826	10/19/2010	9.03	excellent	4.48	very good	25	40/73
Black R @ Finn Rd	10030272	11/12/2009	9.55	excellent	4.39	very good	46	41/65
Balsam Ck @ Dedham Rd	10007599	11/12/2009	8.27	excellent	3.23	excellent	40	49/79
Clear Ck @ CTH W	10030271	11/05/2009	5.88	good	3.27	excellent	27	44/62
Clear Ck @ Hillpiper Rd	10031879	10/07/2010	9.27	excellent	3.62	very good	44	39/70
Clear Ck US Ski Trail	10031940	10/25/2010	8.58	excellent	3.31	excellent	25	58/72
Mud Ck near CTH W	10030270	11/12/2009	5.13	good	2.28	excellent	28	46/84
		10/25/2010	5.25	good	2.66	excellent	23	57/89
Mud Ck South of Hillpiper Rd	10031880	10/07/2010	6.61	good	3.69	very good	37	40/60

*MIBI = macroinvertebrate index of biotic integrity
HBI = Hilsenhoff biotic index
% EPT = percent Ephemeroptera, Plecoptera, Trichoptera
US = upstream

Water Chemistry

Water chemistry data for the Nemadji River and sampled tributaries is shown in table 4. Sampling frequency and duration varied by site making comparisons between sites tentative. No water samples were collected from the Black River.

Only the two Nemadji River sites had more than one sample collected for nutrients. Median concentrations of total phosphorus (TP) and total nitrogen (TN)(total Kjeldahl nitrogen plus nitrate and nitrite nitrogen) were low to moderate at these sites (table 4-2). TP concentration medians ranged from 30 – 46 ug/l. The percent of total phosphorus present in the dissolved form was low, with a median concentration of 3 ug/l. TN concentration medians ranged from 0.59 – 0.63 mg/l. More than 92% of the total nitrogen was present in an organic form.

The Nemadji River sites had low concentrations of ammonia and nitrate plus nitrite. Ammonia concentration medians were less than 0.015 mg/l. Nitrate plus nitrite concentration medians ranged from 0.029 – 0.038 mg/l.

All sites had fairly high total suspended solids (TSS) concentrations, fairly high turbidity, and fairly low transparency. The three Nemadji River sites and the Clear Creek site had the lowest turbidity medians (19-35 ntu), and the highest transparency medians (25-42 cm). The Mud Creek site had the highest turbidity median (57 ntu), and the lowest transparency median (17 cm).

Daytime dissolved oxygen (D.O.) concentrations were generally good. Only two concentrations were less than 5 mg/l. The Nemadji River at CTH C had a D.O. concentration of 4.5 mg/l on one date. Crawford Creek at Hammond Avenue had a D.O. concentration of 2.4 mg/l on one date.

Median conductivities ranged from 195 – 520 umhos/cm. Conductivity was highest in Clear Creek (median 520 umhos/cm), probably as a result of more groundwater discharge to this stream. pH median values ranged from 7.5 to 8.0

TABLE 4. NEMADJI RIVER AND TRIBUTARY STREAMS WATER QUALITY MONITORING DATA

Nemadji R. @ CTH C Station 163004

	Lab parameters								Field parameters					
Date	NH3 (mg/l)	NO3+2 (mg/l)	TKN (mg/l)	TP (ug/l)	TSS (mg/l)	Turbidity (NTU)	Chl. A (ug/l)	DP (ug/l)	Temp (C)	D.O. (mg/l)	D.O.sat. (%)	pH (SU)	Cond. umhos/cm	Transp. (cm)
10/03/2006		ND		20	4	8.2	0.62	ND	12.2	8.5		7.8		
03/27/2007	0.09	0.172	1.13	234	162				0.1	4.5			83	7
04/25/2007	0.026	0.207	0.97	132	129				8	11.4			128	9
05/10/2007		0.031		47	17	27.5	2.54	6						
05/29/2007	ND	0.02	0.8	46	16				15.3	8.8		7.2		36.6
06/13/2007		ND	0.91	49	11		1.61	8						
06/27/2007	ND	0.036	0.624	38	17				23	9.8		7.9	255	30
07/16/2007		ND	0.51	22	8		1.81	ND						
07/24/2007	ND	0.031	0.41	40	18				24.3	7.6		7.8	292	23
08/30/2007	ND	ND	0.35	22	7			4	20.1	8.7		8		56
09/26/2007	ND	ND	0.61	54	31				14.1	10.3		7.9	237	21
09/18/2008		0.031	0.99	56	12		1.47	9						
05/20/2009		ND	0.81	47*	20		0.73	3						
06/23/2009	ND	ND	0.62	34*	16	17.2			18.7	8.7	93	7.8	227	38
06/30/2009		0.028	0.5	30	9		1.66	2						
07/29/2009					25	37.3			20.8	9.2	102.8	8.1	230	27
08/20/2009					548	1070			14.6	8.4	82.4	7.6	133	1.5
09/14/2009					10	15			21.7	8.4	95.4	8	274	64
09/29/2009					3	8.3			10.9	11	99.8	7.9	315	107
10/07/2009					46	45.3			8.6	13.2	112.7	7.6	200	20.5
10/30/2009					68	89.2			6.4	12.3	99.6	7.3	148	12
08/26/2010		0.055	1.42	79	51		1.19	10	17.9	7.9		7.7		20
07/18/2012		0.073	0.57	139*	15			17						
08/20/2012		0.029	0.4	36*	6			2						
09/18/2012		ND	0.22	30*	4			ND						
10/22/2012		0.035	ND	22*	4			ND	8.6	12.1	104	8.3	185	80
11/15/2012		0.097	0.26	19	5			3						

TABLE 4. NEMADJI RIVER AND TRIBUTARY STREAMS WATER QUALITY MONITORING DATA (CONT.)

Nemadji R. @ CTH C (cont.) Station 163004

	Lab parameters								Field parameters					
	NH3 (mg/l)	NO3+2 (mg/l)	TKN (mg/l)	TP (ug/l)	TSS (mg/l)	Turbidity (NTU)	Chl. A (ug/l)	DP (ug/l)	Temp (C)	D.O. (mg/l)	D.O.sat. (%)	pH (SU)	Cond. umhos/cm	Transp. (cm)
Range =	ND-.09	ND-.207	ND-1.42	19-234	3-548	8-1,070	0.6-2.5	ND-17		4.5-13.2		7.2-8.3	83-315	1.5-107
Median =	ND	0.029	0.62	46	16	27.5	1.54	3		8.8		7.8	227	25
May-Oct* TP range =				22-139										

TABLE 4. NEMADJI RIVER AND TRIBUTARY STREAMS WATER QUALITY MONITORING DATA (CONT.)

Nemadji R. @ Finn Rd. Station 163234

	Lab parameters						Field parameters					
Date	NH3 (mg/l)	NO3+2 (mg/l)	TKN (mg/l)	TP (ug/l)	TSS (mg/l)	Turbidity (NTU)	Temp (C)	D.O. (mg/l)	D.O.sat. (%)	pH (SU)	Cond. (umhos/cm)	Transp. (cm)
10/09/2007	0.029	0.06	2.24	292*	326		12.3	10.3		7.9	162	5
11/06/2007	ND	0.075	0.76	36	15		3.7	13.7		7.9	197	42
12/04/2007	0.049	0.182	0.55	33	8		0	15.8		7.8	271	53
01/03/2008	ND	0.206	0.45	30	6		0	15.1		7.7	251	60
02/06/2008	ND	0.272	0.15	25	7		0	15		7.6	304	47
03/05/2008	0.033	0.313	0.2	25	6		0.6	13.2		7.7	298	55
04/03/2008	0.081	0.281	0.64	90	28		0.6	15.2		7.8	181	20
05/07/2008	0.022	ND	0.85	93*	89		10.5	11.1		7.6	124	13
06/04/2008	0.017	ND	0.78	61*	12		11.8	10.3		7.8	197	30
07/09/2008	ND	ND	0.51	28*	9		25.6	7.8		7.9		50
08/11/2008	ND	ND	0.19	23*	4		21	8.7		8		66
09/10/2008	ND	ND	0.32	27*	6		15.4	10.2		8	274	55
06/23/2009	ND	ND	0.61	29	16	17	20.8	8.5	95.2	8	265	41
07/29/2009					21	34.6	20.6	9.6	106.5	8.1	238	29
08/20/2009					592	935	14.5	8.9	86.8	7.5	141	2.5
09/14/2009					7	11.4	21	8.7	99.8	7.9	232	65
09/29/2009					4	9.3	11.4	11.1	101.7	7.9	319	96
10/07/2009					48	54.8	8.4	13.7	116.9	7.7	227	18
10/30/2009					93	106	6.1	12.6	101.7	7.4	159	10
Range =	ND-.081	ND-.313	.15-2.24	23-292	4-592	9.3-935		7.8-15.8		7.4-8.1	124-319	2.5-66
Median =	ND	0.038	0.55	30	12	34.6		11.1		7.8	232	42
May-Oct*												
TP range =				23-292								

TABLE 4. NEMADJI RIVER AND TRIBUTARY STREAMS WATER QUALITY MONITORING DATA (CONT.)

Nemadji R. @ CTH W Station 163048

	Lab parameters						Field parameters					
Date	NH3 (mg/l)	NO3+2 (mg/l)	TKN (mg/l)	TP (ug/l)	TSS (mg/l)	Turbidity (NTU)	Temp (C)	D.O. (mg/l)	D.O.sat. (%)	pH (SU)	Cond. (umhos/cm)	Transp. (cm)
06/23/2009	ND	0.02	0.73	25	7	11.2	23.4	9.8	114.7	8.4	231	60.5
07/29/2009					11	26.8	20.3	10.8	119.4	8.3	223	40
08/20/2009					454	505	14.6	9.1	90.2	7.7	165	3
09/14/2009					5	11.5	20.8	9.3	104	8	255	78
09/29/2009					3	8.8	11.4	11	101.7	7.9	292	95
10/07/2009					28	46.2	8.3	12.9	110	7.5	210	22
10/30/2009							6.5	12.5	101.3	7.5	142	8
Range =					3-454	8.8-505		9.1-12.9		7.5-8.4	142-292	3-95
Median =					9	19.2		10.8		7.9	223	40

Crawford Ck. @ Hammond Ave Station 10015466

	Lab						Field					
Date	NH3 (mg/l)	NO3+2 (mg/l)	TKN (mg/l)	TP (ug/l)	TSS (mg/l)	Turbidity (NTU)	Temp (C)	D.O. (mg/l)	D.O.sat. (%)	pH (SU)	Cond. (umhos/cm)	Transp. (cm)
06/23/2009	ND	ND	1.25	106	23	35.6	14.7	6.9	68.6	7.3	337	21
07/29/2009					7	12	16.1	8.4	86	7.7	367	76
08/20/2009					81	152	15	8.4	84.3	7.7	170	7
09/14/2009					14	12.5	17.6	6.1	64.1	7.7	296	40
10/07/2009					5	8.6	8.6	2.4	20.7		392	83
10/30/2009					37	155	7.1	11.2	92.7	7.1	164	8.5
08/09/2010					39	100	20.2	7.8	85.5	7.1		13
Range =					5-81	8.6-155		2.4-11.2		7.1-7.7	164-392	7-83
Median =					23	35.6		7.8		7.5	316.5	21

TABLE 4. NEMADJI RIVER AND TRIBUTARY STREAMS WATER QUALITY MONITORING DATA (CONT.)

Balsam Ck. @ Deadham Rd. Station 10007599

	Lab parameters						Field parameters					
Date	NH3 (mg/l)	NO3+2 (mg/l)	TKN (mg/l)	TP (ug/l)	TSS (mg/l)	Turbidity (NTU)	Temp (C)	D.O. (mg/l)	D.O.sat. (%)	pH (SU)	Cond. (umhos/cm)	Transp. (cm)
06/23/2009	ND	ND	0.57	42	21	46	22.4	8.9	102.8	8.3	206	17
07/29/2009					22	53.4	19.5	10.2	111.3	8.3	195	19.5
08/20/2009					222	228	14.6	9.7	95.6	7.7	97	5
09/14/2009					14	39.7	19.6	8.9	97.6	7.9	253	30
10/30/2009					65	138	7.1	12.2	100.4	7.4	123	9.5
08/09/2010					16	38.8	23.2	8.1	91.5	8		24
10/23/2010					10	35.3	9.3	7.8	68.3	7.7		25
Range =					10-222	35.3-228		7.8-12.2		7.4-8.3	97-253	5-30
Median =					21	46		8.9		7.9	195	19.5

Clear Ck. @ CTH W Station 10030272

	Lab parameters						Field parameters					
Date	NH3 (mg/l)	NO3+2 (mg/l)	TKN (mg/l)	TP (ug/l)	TSS (mg/l)	Turbidity (NTU)	Temp (C)	D.O. (mg/l)	D.O.sat. (%)	pH (SU)	Cond. (umhos/cm)	Transp. (cm)
06/23/2009	ND	ND	0.45	35	20	21.6	21	9.4	106	8.3	523	38
07/29/2009					23	30.7	18.1	11.3	119.7	8.2	517	35
08/20/2009					364	303	14.4	9.7	95	7.6	250	4
09/14/2009					9	16.2	18.2	10.2	107.1	7.7	566	55
09/29/2009					6	14.3	10.8	10.2	100.9	7.6	614	65
10/07/2009					30	41.4	8.3	13.4	114.4	7.8	530	25
10/30/2009					145	193	6.8	12.2	100.6	7.7	305	7
08/09/2010					10	21.5	22	9.1	104.3	8.4		34.5
08/16/2010					22	49.4	17.6	9.5	99.9	8.1	438	22
08/18/2010					1120	933	17.2	9.1	94.7	7.8	781	4.5
10/06/2010							9.3	7.9	68.5	8		55
10/25/2010					22	31.5	8.1	11.8	100.2	8.2	426	28

TABLE 4. NEMADJI RIVER AND TRIBUTARY STREAMS WATER QUALITY MONITORING DATA (CONT.)

Clear Ck. @ CTH W (cont.) Station 10030272

	Lab parameters						Field parameters					
	NH3	NO3+2	TKN	TP	TSS	Turbidity	Temp	D.O.	D.O.sat.	pH	Cond.	Transp.
	(mg/l)	(mg/l)	(mg/l)	(ug/l)	(mg/l)	(NTU)	(C)	(mg/l)	(%)	(SU)	(umhos/cm)	(cm)
Range =					6-1120	14.3-933		7.9-13.4		7.6-8.4	250-781	4-65
Median =					22	31.5		10.0		7.9	520	31.3

Clear Ck. US Ski Trail Station 10031940

	Lab parameters						Field parameters					
	NH3	NO3+2	TKN	TP	TSS	Turbidity	Temp	D.O.	D.O.sat.	pH	Cond.	Transp.
Date	(mg/l)	(mg/l)	(mg/l)	(ug/l)	(mg/l)	(NTU)	(C)	(mg/l)	(%)	(SU)	(umhos/cm)	(cm)
10/25/2010					29	32.4	8.2	8.1	96.5		402	35

TABLE 4. NEMADJI RIVER AND TRIBUTARY STREAMS WATER QUALITY MONITORING DATA (CONT.)

Mud Ck. @ CTH W Station 10030271

Date	Lab NH3 (mg/l)	NO3+2 (mg/l)	TKN (mg/l)	TP (ug/l)	TSS (mg/l)	Turbidity (NTU)	Field Temp (C)	D.O. (mg/l)	D.O.sat. (%)	pH (SU)	Cond. (umhos/cm)	Transp. (cm)
06/23/2009	ND	ND	0.85	41	24	38.3	22.1	9.1	104.1	8.2	455	26
07/29/2009					29	91.6	19.1	10.1	109.3	8.1	355	15
08/20/2009					396	409	14.5	9.8	95.5	7.5	178	1.5
09/14/2009					19	27.4	18.9	7.9	84.2	7.8	466	46
09/29/2009					7	14.8	10.9	9	89.4	7.7	553	69
10/07/2009					24	54.5	8.7	12.6	108	7.8	420	18
10/30/2009					203	250	6.6	12.4	101.6	7.5	207	6
08/09/2010					5	12.6	23.6	8.6	101.6	8.3		61
08/16/2010					20	59.6	18.7	7.9	84.7	8	364	16
08/18/2010					140	191	18.4	8.7	91.7	7	310	5
10/06/2010					11	36.3	9.7	7.5	65.8	7.7		45
10/25/2010					46	60.9	7.8	10.6	97.9	8.1	353	15
Range =					7-396	12.6-409		7.5-12.6		7-8.3	178-466	1.5-69
Median =					24	57		9		8	360	17

TABLE 4-2. MEDIAN WATER CHEMISTRY VALUES FOR NEMADJI RIVER AND TRIBUTARY SITES

	NH3 (mg/l)	NO3+2 (mg/l)	TKN (mg/l)	TP (ug/l)	TSS (mg/l)	Turbidity (NTU)	Chl. A (ug/l)	DP (ug/l)	Transp. (cm)	pH (SU)	Cond. (umhos/cm)
Nemadji R @ CTH C	<0.015	0.029	0.62	46	16	27.5	1.5	3	25	7.8	227
Nemadji R @ Finn Rd	<0.015	0.038	0.55	30	12	34.6			42	7.8	232
Nemadji R @ CTH W					9	19.2			40	7.9	223
Crawford Ck @ Hammond Ave					23	35.6			21	7.5	316
Balsam Ck @ Deadham Rd					21	46			19.5	7.9	195
Clear Ck @ CTH W					22	31.5			31.3	7.9	520
Mud Ck near CTH W					24	57			17	8.0	360

Conclusions

The sites monitored in Wisconsin's portion of the Nemadji River watershed are diverse, with natural stream communities ranging from Cool-Cold headwaters to Warm mainstems. Erodible clay soils interspersed with sands and silts dominate the drainage areas for most sites. Erosion of stream banks and drainageways are the dominant source of sediment loads. Common stream concerns in this area include:

- High peak flows resulting from rapid runoff from clay soils.
- Low base flows resulting from limited groundwater discharge.
- Scouring of stream bed, and bank erosion resulting from high peak flows.
- High bed loads of sand and silt, reducing the substrate quality for fish and macroinvertebrates. .
- High TSS and turbidity, and low transparency resulting from erosion of clay soils.

Most of the Black River watershed extends south of the red clay plain area and has soils dominated by stony and sandy loams, and organic wetland soils. Water quality is likely to be better in that stream, but water sampling was not done at the Black River monitoring site. Both the fish IBI and the macroinvertebrate IBI for this site were excellent.

The Nemadji River and Crawford Creek have already been placed on Wisconsin's 303d list of impaired waters, as discussed in the introduction section. The data collected during this project does not provide any further supporting information for having these streams on the list.

WISCALM guidance (2014) indicates at least two samples of one biological assemblage (fish or macroinvertebrates) collected in different calendar years and having "poor" ratings are required to list a stream as impaired. Neither the Nemadji River nor Crawford Creek had any poor ratings for fish IBI's or macroinvertebrate IBI's (table 5).

Total phosphorus (TP) concentrations can also be used toward listing a stream as impaired. Six monthly samples collected from May to October are needed for this assessment. The lower bound of the 90% confidence interval of the mean must exceed 75 ug/l to list a stream as impaired. Only two sites on the Nemadji River (CTH C and Finn Road) had the needed samples collected (table 5). At both sites the 75 ug/l TP threshold is not exceeded.

The data collected during this project for the other streams does not support 303d listing. No poor ratings for fish IBI's or macroinvertebrate IBI's were found. Sampling for TP concentrations was inadequate to determine if the 75 ug/l threshold is exceeded (table 5).

TABLE 5. NEMADJI RIVER AND TRIBUTARIES TOTAL PHOSPHORUS AND IBI SUMMARY*							
					Small	Cool warm	Warmwater
	SWIMS				Stream	Transitional	L. Superior
STREAM /SITE	ID NO.	Year	May-Oct TP	MIBI	IBI	IBI	IBI
Nemadji R @ CTH C	163003	2006-12	lower 90% C.I.< 75ug/l	no sample			no survey
Nemadji R @ Finn Road	163233	2007-9	lower 90% C.I.< 75ug/l	no sample			excellent
Nemadji R @ CTH W	163047	2009	insufficient samples	excellent	good	excellent	
Crawford Ck @ Hammond Ave	10015464	2006-10	insufficient samples	no sample	fair		
Crawford Ck @ CTH C	10031796	2010	no samples	fair	no survey		
Crawford Ck @ Vally Brook Rd	10032010	2010	no samples	good	no survey		
Crawford Ck @ Eskelson Rd	10031826	2010	no samples	excellent	fair		
Black R @ Finn Road	10030272	2009	no samples	excellent		excellent	
Balsam Ck @ Dedham Road	10007599	2009	insufficient samples	excellent		excellent	
		2010					
Clear Ck @ CTH W	10030271	2009	insufficient samples	good	fair	good	
Clear Ck @ Hillpiper Rd	10031879	2010	no samples	excellent	no survey	no survey	
Clear Ck US Ski Trail	10031940	2010	insufficient samples	excellent	good		
Mud Ck near CTH W	10030270	2009	insufficient samples	good	good	excellent	
		2010		good			
Mud Ck South of Hillpiper Road	10031880	2010	no samples	good	no survey	no survey	

*May-October total phosphorus sample sufficiency considers all samples collected since 2006.
MIBI = macroinvertebrate index of biotic integrity
IBI = index of biotic integrity

APPENDIX A. FISH SURVEY DATA FOR NEMADJI RIVER AND TRIBUTARIES

(HW = headwater, IBI = index of biotic integrity, DS = downstream, US = upstream)

Nemadji R @ Finn Rd 06/31/2008
SWIMS sta. no. 163233 Station length 425m

Fish Species	Number	Thermal	Size	Tolerance
common shiner	29	warmwater	medium	tolerant
creek chub	4	transient	small	tolerant
hornyhead chub	22	warmwater	medium	intermediate
johnny darter	1	transient	medium	intermediate
lamprey	1			
logperch	1	warmwater	large	intermediate
rock bass	29	warmwater	large	intolerant
sand shiner	11	warmwater	large	intermediate
shorthead redhorse	7	warmwater	large	intermediate
silver redhorse	6	warmwater	large	intermediate
smallmouth bass	2	warmwater	large	intolerant
stonecat	2	warmwater	medium	intermediate
troutperch	7	transient	large	intermediate
walleye	1	transient	large	intermediate
white sucker	2	transient	medium	tolerant
Total number	124			

% Coldwater	0	% small	3	% intolerant	26
% Transitional	13	% medium	48	% intermediate	44
% Warmwater	87	% large	49	% tolerant	30

Model-predicted natural community - Warm mainstem
Does sampled population include > 25 fish? - yes
Does sampled population support predicted community? - yes, but
% medium slightly less than 50-100%
Warmwater Lake Superior basin IBI: 80 = excellent

APPENDIX A. FISH SURVEY DATA FOR NEMADJI RIVER AND TRIBUTARIES (CONT.)

(HW = headwater, IBI = index of biotic integrity, DS = downstream, US = upstream)

Nemadji R @ CTH W 09/11/2009
SWIMS sta. no. 163048 Station length 440m

Fish Species	Number	Thermal	Size	Tolerance
common shiner	54	warmwater	medium	intermediate
creek chub	3	transient	small	tolerant
hornyhead chub	78	warmwater	medium	intermediate
johnny darter	11	transient	medium	intermediate
logperch	70	warmwater	large	intermediate
longnose dace	38	transient	medium	intermediate
muskellunge	1	transient	large	intolerant
rock bass	25	warmwater	large	intolerant
sand shiner	1	warmwater	large	intermediate
silver redhorse	38	warmwater	large	intermediate
smallmouth bass	3	warmwater	large	intolerant
stonecat	7	warmwater	medium	intermediate
troutperch	4	transient	large	intermediate
walleye	3	transient	large	intermediate
white sucker	18	transient	medium	tolerant
Total number	354			

% Coldwater	0	% small	1	% intolerant	8
% Transitional	22	% medium	58	% intermediate	71
% Warmwater	78	% large	41	% tolerant	21

Model-predicted natural community - Cool-Warm HW
Does sampled population include > 25 fish? - yes
Does sampled population support predicted community? - no
Sampled population indicates a Warm mainstem community
Small stream (intermittent) IBI: 80 = good
Warmwater Lake Superior basin IBI: 90 = excellent

APPENDIX A. FISH SURVEY DATA FOR NEMADJI RIVER AND TRIBUTARIES (CONT.)

(HW = headwater, IBI = index of biotic integrity, DS = downstream, US = upstream)

Crawford Ck @ Hammond Ave 07/07/2006
SWIMS sta. no. 10015464 Station length 210m

Fish Species	Number	Thermal	Size	Tolerance
central mudminnow	2	transient	small	tolerant
creek chub	10	transient	small	tolerant
golden shiner	1	warmwater	medium	tolerant
muskellunge	1	transient	large	intolerant
troutperch	1	transient	large	intermediate
white sucker	8	transient	medium	tolerant
Total number	23			

% Coldwater	0	% small	52	% intolerant	4
% Transitional	96	% medium	39	% intermediate	4
% Warmwater	4	% large	9	% tolerant	92

Model-predicted natural community - Cool-Cold HW
Does sampled population include > 25 fish? - no
Does sampled population support predicted community? - yes, but < 25 fish and % tolerant > 75
Small stream (intermittent) IBI: 40 = fair
Cool-Cold IBI: 20 = poor

APPENDIX A. FISH SURVEY DATA FOR NEMADJI RIVER AND TRIBUTARIES (CONT.)

(HW = headwater, IBI = index of biotic integrity, DS = downstream, US = upstream)

Crawford Ck @ Eskelson Rd 08/23/2010

SWIMS sta. no. 10031826 Station length 107m

Fish Species	Number	Thermal	Size	Tolerance
brook stickleback	11	transient	small	tolerant
central mudminnow	12	transient	small	tolerant
creek chub	24	transient	small	tolerant
western blacknose dace	2	transient	small	tolerant
white sucker	23	transient	medium	tolerant
Total number	72			

% Coldwater	0	% small	68	% intolerant	0
% Transitional	100	% medium	32	% intermediate	0
% Warmwater	0	% large	0	% tolerant	100

Model-predicted natural community - Cool-Cold HW

Does sampled population include > 25 fish? - yes

Does sampled population support predicted community? - yes but % tolerant > 75

Small stream (intermittent) IBI: 40 = fair

Cool-Cold IBI: 30 = fair

APPENDIX A. FISH SURVEY DATA FOR NEMADJI RIVER AND TRIBUTARIES (CONT.)

(HW = headwater, IBI = index of biotic integrity, DS = downstream, US = upstream)

Black R @ Finn Rd 09/11/2009

SWIMS sta. no. 10030272 Station length 470m

Fish Species	Number	Thermal	Size	Tolerance
common shiner	258	warmwater	medium	intermediate
creek chub	76	transient	small	tolerant
hornyhead chub	41	warmwater	medium	intermediate
johnny darter	11	transient	medium	intermediate
logperch	6	warmwater	large	intermediate
northern pike	1	transient	small	intermediate
rock bass	2	warmwater	large	intolerant
sand shiner	43	warmwater	large	intermediate
shorthead redhorse	7	warmwater	large	intermediate
smallmouth bass	6	warmwater	large	intolerant
troutperch	3	transient	large	intermediate
walleye	3	transient	large	intermediate
western blacknose dace	1	transient	small	tolerant
white sucker	90	transient	medium	tolerant
Total number	548			

% Coldwater	0	% small	14	% intolerant	2
% Transitional	34	% medium	73	% intermediate	68
% Warmwater	66	% large	13	% tolerant	30

Model-predicted natural community - Cool-Warm mainstem

Does sampled population include > 25 fish? - yes

Does sampled population support predicted community? - yes

Cool-Warm IBI: 80 = excellent

APPENDIX A. FISH SURVEY DATA FOR NEMADJI RIVER AND TRIBUTARIES (CONT.)

(HW = headwater, IBI = index of biotic integrity, DS = downstream, US = upstream)

Balsam Ck @ Dedham Rd 09/21/2010

SWIMS sta. no. 10007599 Station length 370m

Fish Species	Number	Thermal	Size	Tolerance
brassy minnow	6	transient	small	intermediate
common shiner	207	warmwater	medium	intermediate
creek chub	155	transient	small	tolerant
hornyhead chub	46	warmwater	medium	intermediate
johnny darter	6	transient	medium	intermediate
logperch	4	warmwater	large	intermediate
longnose dace	18	transient	medium	intermediate
northern redbelly dace	1	transient	small	intermediate
rock bass	6	warmwater	large	intolerant
shorthead redhorse	6	warmwater	large	intermediate
stonecat	2	warmwater	medium	intermediate
troutperch	38	transient	large	intermediate
western blacknose dace	19	transient	small	tolerant
white sucker	30	transient	medium	tolerant
Total number	544			

% Coldwater	0	% small	33	% intolerant	1
% Transitional	50	% medium	57	% intermediate	61
% Warmwater	50	% large	10	% tolerant	38

Model-predicted natural community - Cool-Warm mainstem

Does sampled population include > 25 fish? - yes

Does sampled population support predicted community? - yes

Cool-Warm IBI: 90 = excellent

APPENDIX A. FISH SURVEY DATA FOR NEMADJI RIVER AND TRIBUTARIES (CONT.)

(HW = headwater, IBI = index of biotic integrity, DS = downstream, US = upstream)

Clear Ck @ CTH W 08/05/2009
SWIMS sta. no. 10030271 Station length 255m

Fish Species	Number	Thermal	Size	Tolerance
bluntnose minnow	1	warmwater	medium	tolerant
common shiner	102	warmwater	medium	intermediate
creek chub	115	transient	small	tolerant
hornyhead chub	10	warmwater	medium	intermediate
johnny darter	6	transient	medium	intermediate
logperch	1	warmwater	large	intermediate
longnose dace	17	transient	medium	intermediate
sand shiner	2	warmwater	large	intermediate
shorthead redhorse	1	warmwater	large	intermediate
troutperch	28	transient	large	intermediate
western blacknose dace	40	transient	small	tolerant
white sucker	87	transient	medium	tolerant
Total number	410			

% Coldwater	0	% small	38	% intolerant	0
% Transitional	72	% medium	54	% intermediate	41
% Warmwater	28	% large	8	% tolerant	59

Model-predicted natural community - None
Does sampled population include > 25 fish? - yes
Does sampled population support predicted community? - none predicted
Sampled population indicates a Cool-Warm mainstem, but also close to Cool-Warm HW
Cool-Warm **IBI:** 60 = good
Small stream (intermittent) **IBI**: 60 = fair

APPENDIX A. FISH SURVEY DATA FOR NEMADJI RIVER AND TRIBUTARIES (CONT.)

(HW = headwater, IBI = index of biotic integrity, DS = downstream, US = upstream)

Clear Ck US Ski Trail		08/24/2010			
SWIMS sta. no. 10031940		Station length 153m			
Fish Species	Number	Thermal	Size	Tolerance	
brook stickleback	6	transient	small	tolerant	
common shiner	9	warmwater	medium	intermediate	
creek chub	134	transient	small	tolerant	
hornyhead chub	1	warmwater	medium	intermediate	
johnny darter	21	transient	medium	intermediate	
longnose dace	2	transient	medium	intermediate	
mottled sculpin	27	coldwater	small	intolerant	
western blacknose dace	57	transient	small	tolerant	
white sucker	1	transient	medium	tolerant	
Total number	258				
% Coldwater	10	% small	87	% intolerant	10
% Transitional	86	% medium	13	% intermediate	13
% Warmwater	4	% large	0	% tolerant	77

Model-predicted natural community - None
Does sampled population include > 25 fish? - yes
Does sampled population support predicted community? - none predicted
Sampled population indicates Cool-Warm HW or Cool-Cold HW
Small stream (intermittent) IBI: 90 = good
Cool-Warm IBI: 80 = excellent
Cool-Cold IBI: 50 = good

APPENDIX A. FISH SURVEY DATA FOR NEMADJI RIVER AND TRIBUTARIES (CONT.)

(HW = headwater, IBI = index of biotic integrity, DS = downstream, US = upstream)

Mud Ck near CTH W 09/01/2009
SWIMS sta. no. 10030270 Station length 152m

Fish Species	Number	Thermal	Size	Tolerance
common shiner	108	warmwater	medium	intermediate
creek chub	102	transient	small	tolerant
hornyhead chub	55	warmwater	medium	intermediate
johnny darter	3	transient	medium	intermediate
logperch	3	warmwater	large	intermediate
longnose dace	1	transient	medium	intermediate
muskellunge	1	transient	large	intolerant
pearl dace	1	transient	small	intermediate
rock bass	1	warmwater	large	intolerant
sand shiner	23	warmwater	large	intermediate
smallmouth bass	4	warmwater	large	intolerant
troutperch	22	transient	medium	tolerant
walleye	1	transient	large	intermediate
western blacknose dace	4	transient	small	tolerant
white sucker	12	transient	medium	tolerant
Total number	341			

% Coldwater	0	% small	31	% intolerant	2
% Transitional	43	% medium	53	% intermediate	63
% Warmwater	57	% large	16	% tolerant	35

Model-predicted natural community - Cool-Warm HW
Does sampled population include > 25 fish? - yes
Does sampled population support predicted community? - no
Sampled population indicates Cool-Warm mainstem
Station sampled is very close to confluence with Nemadji R
Cool-Warm IBI: 100 = excellent
Small stream (intermittent) IBI: 90 = good

Appendix 9

Lower Nemadji River Water Quality and Macroinvertebrate Community Assessment (Pertains to management action 6.05)

LOWER NEMADJI RIVER WATER QUALITY AND MACROINVERTEBRATE COMMUNITY ASSESSMENT, 2015

Craig Roesler – WI Dept. of Natural Resources, Spooner

Introduction

The Nemadji River watershed is located in northwest Wisconsin and northeast Minnesota (figure 1). The watershed is included in the St. Louis River Area of Concern (AOC). Both Wisconsin and Minnesota have listed the Nemadji River as an impaired water. Wisconsin added the Nemadji River to the 303d list of impaired waters in 2010. The high sediment load was judged to exceed the narrative water quality standard found in NR 102.4 (a) of the Wisconsin Administrative Code, that states, "Substances that will cause objectionable deposits on the shore or in the bed of a body of water, shall not be present in such amounts as to interfere with public rights in waters of the state." Other considerations that contributed to the listing decision were:

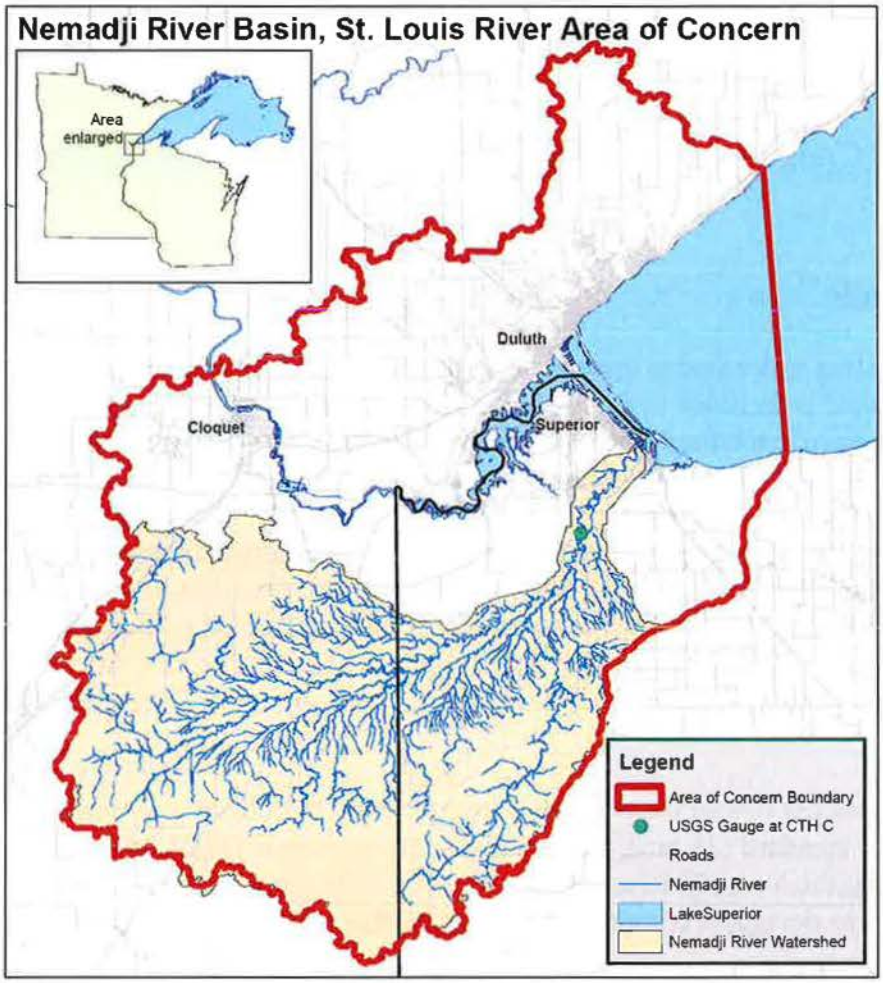
- Creosote and PAH's from Crawford Creek are a continuing source of pollutants to the Nemadji River.

- Minnesota has placed the Nemadji River on their 303d list due to exceedences of their turbidity standard (25 ntu), and has begun developing a TMDL to address turbidity. Including the Nemadji River on Wisconsin's 303d list will allow the two states to work together to develop a comprehensive TMDL that will benefit the entire watershed.

- The median turbidity measured in the Nemadji River at CTH C during 2006-2012 was 27.5 ntu, which exceeds Minnesota's turbidity standard.

Nemadji River turbidity results from the erosion of clay rich soils in the lower portion of the watershed. The majority of the suspended clay in the river is derived from channel and bank erosion in the river, tributaries, and drainageways. Despite the high turbidity, biological assessments have shown good quality fish and macroinvertebrate communities are present at previous locations monitored (Roesler 2014).

Figure 1.



However, there has been a lack of monitoring in the lower reach of the river in the past. Lake Superior seiche influence, which causes partial backflow in the lower 8.8 miles of the river, has discouraged water quality monitoring. The most downstream water quality data was collected at CTH C, 11.9 miles above the river mouth.

Deep water and lack of coarse substrate has discouraged macroinvertebrate sampling. The most downstream macroinvertebrate sample previously collected was at CTH W, 31.2 miles above the river mouth.

Higher percentages of urban and agricultural land use are present in the lower portion of the watershed. Three intermittent point source outfalls are also present. This suggests poorer water quality and macroinvertebrate communities may be present in the lower river. Monitoring of water quality, and macroinvertebrate sampling were done in 2015 to allow an initial evaluation of conditions in the lower river. Fish community monitoring in 2015 was also done in a separate project (Nelson 2016).

Methods

Water Quality

Water quality monitoring was conducted at three sites (fig. 2 and below), monthly from May to October. Monitoring was scheduled for the second Wednesday of each month to provide a systematic random distribution of samples.

Site Description	SWIMS Station No.	Coordinates
Nemadji R. @ CTH C	163003	46.6333, -92.0942
Nemadji R. @ Woodlawn Rd.	10037076	46.6662, -92.0642
Nemadji R. @ USH 2/53	163049	46.6966, -92.0346

Water samples were collected and field parameters were measured following standard DNR protocols. Samples at the two downstream sites were collected with a Kemerrer sampler which was lowered from the bridge near the river center. This was done to avoid any direct influence from backflows caused by Lake Superior seiches. During the periodic backflows, water was observed moving upstream near the stream banks, but continued to move downstream near the stream center.

Water samples were preserved, as needed, and most were shipped on ice to the Wisconsin State Lab of Hygiene for analysis. E. coli samples were delivered on ice to the Lake Superior Research Institute at UW-Superior for analysis so that holding time requirements could be met.

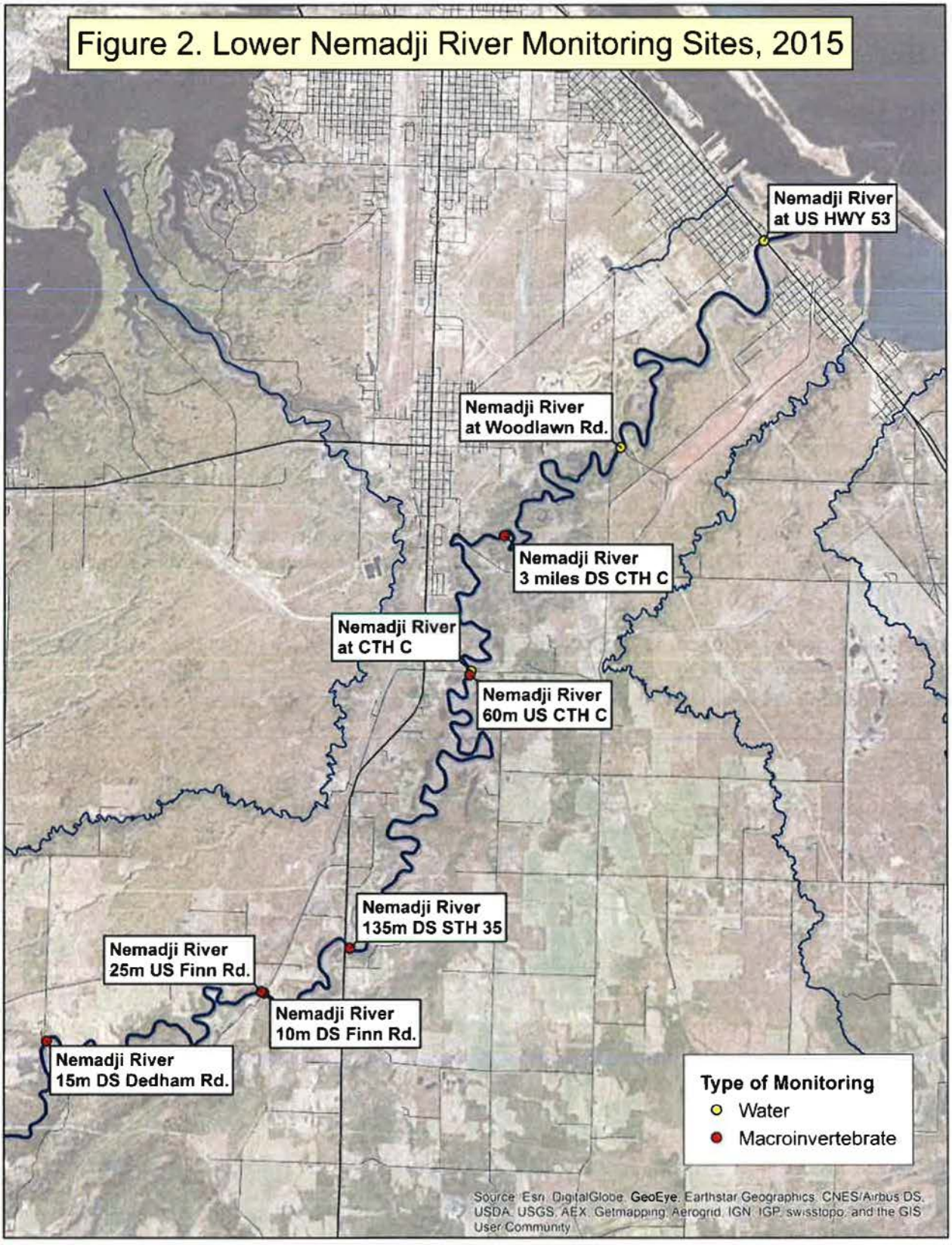


Figure 2. Lower Nemadji River Monitoring Sites, 2015
Nemadji River at US HWY 53
Nemadji River at Woodlawn Rd.
Nemadji River 3 miles DS CTH C
Nemadji River at CTH C
Nemadji River 60m US CTH C
Nemadji River 135m DS STH 35
Nemadji River 25m US Finn Rd.
Nemadji River 10m DS Finn Rd.
Nemadji River 15m DS Dedham Rd.
Type of Monitoring
Water
Macroinvertebrate
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Field parameters measured were:

- Temperature
- pH
- Dissolved Oxygen
- Conductivity
- Transparency (using a transparency tube)

Lab parameters were:

- Total Phosphorus
- Dissolved Ortho Phosphorus
- Ammonia – N
- Total Kjeldahl N
- Ammonia-N
- Nitrate plus Nitrite – N
- Total Suspended Solids
- Turbidity
- E. coli

Macroinvertebrate Sampling

The six macroinvertebrate sampling sites are shown in figure 2.
Macroinvertebrate communities were assessed by collecting kick samples using a 500 um mesh D-frame net. Due to the lack of riffles and scarcity of coarse substrate (gravel/cobble), all but one sample were collected from woody debris draped with leaf packs and other vegetative debris. One sample, just upstream of Finn Road was collected from cobble substrate to allow a comparison to a sample just downstream of Finn Road collected from woody debris/leaf snags.

Samples were preserved in 85% ethanol and were processed by UW – Superior's Aquatic Biomonitoring Lab. Macroinvertebrates were counted and identified to the lowest possible taxa. Biotic indices and other statistics were generated.

Results and Discussion

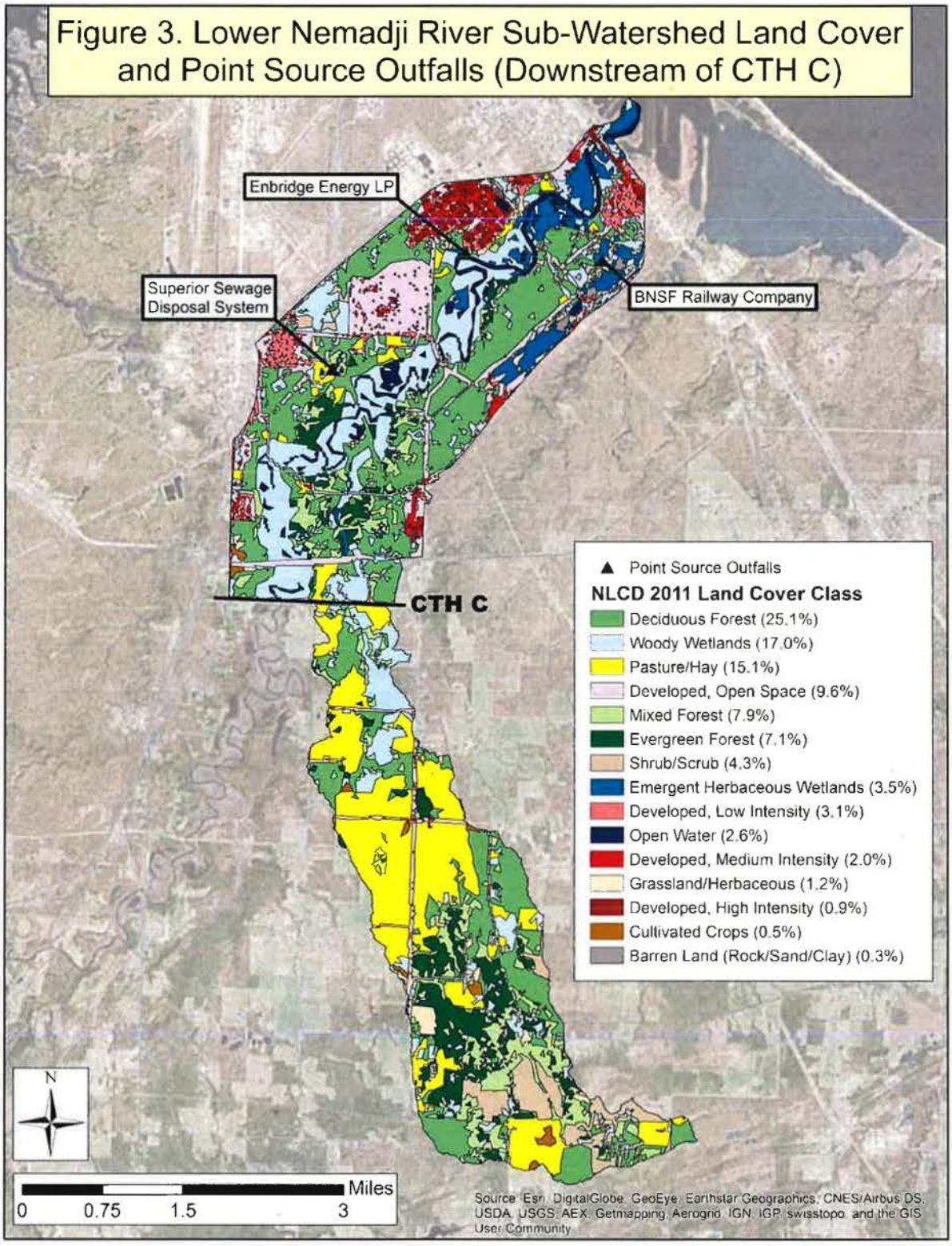
Sub-watershed characteristics

Land Cover

The sub-watershed and land cover for the lower Nemadji River is shown in figure 3. This is the drainage area that contributes water to the river downstream of CTH C. The three point source outfalls in the sub-watershed are also shown. The sub-watershed has an area of 16.2 mi^2, which is only 3.7% of the total Nemadji River watershed.

Undeveloped land covers occupy 69% of the sub-watershed. Developed agricultural land covers occupy 15.6%, with 15.1% being pasture or hay, and only 0.5% being cultivated crops. Developed urban land covers also occupy 15.6%, with most (12.7%) being developed open space, and low intensity development.

The remainder of the watershed for the Nemadji River, upstream of CTH C, has 87.3% undeveloped land covers. Developed agricultural land covers occupy 9.6%, and developed urban land covers occupy 3%.



Point Sources

There are three point source outfalls in the sub-watershed (figure 3):

- Superior Sewage Disposal System; combined sewer treatment plant (CSTP5)
- Enbridge Energy LP
- BNSF Railway Company

The characteristics of the three point sources and their potential influence on the Lower Nemadji River are discussed in the Water Quality section below.

Water Quality

Water Quality Monitoring Results for 2015

Results of water quality monitoring are shown in table 1.
Dissolved oxygen (D.O.) concentrations range from 6.3 to 11.5 mg/l. All values exceed the 5 mg/l water quality standard for fish and aquatic life.

Conductivity ranges from 93 to 275 umhos/cm. Conductivity tends to be lower when flows are higher since the surface runoff contributing to the high flows tends to have lower conductivity. Transparency (measured with a transparency tube) ranges from 3 to 65 cm. Lowest transparencies occur during highest flows. Erosion of clay from stream and drainageway channels is greatest during high flows. A transparency of 104 cm was measured at the CTH C site during macroinvertebrate sampling on October 22nd, following an extended period of dry weather.

Total phosphorus (TP) concentrations are low to high, ranging from 33 to 501 ug/l. TP concentrations are highest when flows are highest due to watershed runoff and channel scouring. Median TP concentrations at the three sites (49 – 56.3 ug/l) were below Wisconsin's stream water quality standard of 75 ug/l. The upper 90% confidence limit of the median ranged from 133.5 – 159 ug/l. Wisconsin DNR 2016 WisCALM guidance indicates these sites "may meet" the 75 ug/l standard since the median is below, but the 90% upper confidence limit is above the standard.

Dissolved ortho phosphorus (DOP) concentrations are low, ranging from <1.7 – 13 ug/l. The percent of TP as DOP ranges from 2.2 – 25%. There is a tendency for DOP to comprise a smaller percentage of TP when flows are higher, and more particulate bound TP is present.

Total Kjeldahl nitrogen (TKN) concentrations are moderate, ranging from 0.56 to 1.62 mg/l. TKN concentrations are higher when flows are higher due to watershed runoff. Ammonium-nitrogen (NH_4-N) and Nitrate plus nitrite-nitrogen (NO_{3+2}-N) concentrations are very low. NH_4-N concentrations range from <0.0150 – 0.0303 mg/l. NO_{3+2}-N concentrations range from <0.0190 – 0.0868 mg/l.

Table 1.

LOWER NEMADJI RIVER WATER QUALITY DATA, 2015 (Sites listed from upstream to downstream)

Nemadji River at CTH C (163003)

Date	Temp. (°C)	D.O. (mg/l)	pH (s.u.)	Conductivity (umhos/cm)	Transp. (cm)	Total P (ug/l)	Diss. ortho P (ug/l)	TKN (mg/l)	NH_4-N (mg/l)	NO_{3+2}-N (mg/l)	TSS (mg/l)	Turbidity (n.t.u.)	E. coli (cfu/100ml)	Mean daily flow (cfs)
05/12/2015	6.5	11.3	6.9	147	5	350	8	1.42	0.03	0.0868	344	374	980	1,490
06/10/2015	19.9	8.6	6.9	149	29	53	4	0.751	0.0154	0.037	30.7	26.3	35.9	265
07/08/2015	18.2	8.1	7	93	10	161	10	1.37	0.0243	<0.0190	130	121	648.8	1,580
08/06/2015	19.5	8.2	6.8	275	54	33	2	0.584	<0.0150	<0.0190	6.2	6.98	57.6	50
09/09/2015	19.1	8.4	7.8	194	32	54	7	0.72	0.0193	0.0411	24.8	23.5	81.6	140
10/14/2015	9.1	10.6	7.7	182	65	36/35	8/7	0.638/0.653	<.0150/<.0150	<.0190/<.0190	6.0/6.2	9.73/10.7	22.8/37.3	132
median	18.7	8.5	7.0	165.5	30.5	53.5	7.3	0.736	0.0174	0.0233	27.8	24.9	69.6	202.5

Nemadji River at Woodlawn Rd. (10037076)

Date	Temp. (°C)	D.O. (mg/l)	pH (s.u.)	Conductivity (umhos/cm)	Transp. (cm)	Total P (ug/l)	Diss. ortho P (ug/l)	TKN (mg/l)	NH_4-N (mg/l)	NO_{3+2}-N (mg/l)	TSS (mg/l)	Turbidity (n.t.u.)	E. coli (cfu/100ml)
05/12/2015	6.7	11.5	6.8	162	4	436	11	1.62	0.0272	0.0823	346	520	980
06/10/2015	19.9	8.4	6.7	153	31	49	5	0.753	0.0189	0.0368	17.4	28.3	20
07/08/2015	18.6	8	6.8	97	8	186	12	1.41	0.0236	<0.0190	161	142	980.4
08/06/2015	21	7.6	6.7	267	60	33	<1.7	0.698	<0.0150	<0.0190	7	6.47	24.6
09/09/2015	20.7	7.4	7.7	209	30	49	7	0.738	0.0161	0.047	11.8	22.2	83.6
10/14/2015	10.5	9.5	7.6	202	52	34/33	6/6	0.557/0.557	<.0150/<.0150	<.0190/<.0190	5.8/6.2	11.6/12.2	24.3/27.5
median	19.3	8.2	6.8	182.0	30.5	49	6.5	0.746	0.0175	0.0232	14.6	25.3	54.8

Nemadji River at USH 2/53 (163049)

Date	Temp. (°C)	D.O. (mg/l)	pH (s.u.)	Conductivity (umhos/cm)	Transp. (cm)	Total P (ug/l)	Diss. ortho P (ug/l)	TKN (mg/l)	NH_4-N (mg/l)	NO_{3+2}-N (mg/l)	TSS (mg/l)	Turbidity (n.t.u.)	E. coli (cfu/100ml)
05/12/2015	6.7	11.5	6.6	186	3	501	11	1.51	0.0296	0.0866	392	729	1120
06/10/2015	19.8	8.2	6.9	173	30	46	4	0.732	0.021	0.0337	15.2	26.6	37.3
07/08/2015	18.4	7.5	6.8	101	9	164	13	1.3	0.029	0.022	106	138	866.4
08/06/2015	21.5	7.1	6.9	240	47	43	2	0.736	0.0191	0.0407	10.4	7.1	9.7
09/09/2015	21.4	6.3	7.5	229	29	69	10	0.85	0.0303	0.0461	10.2	27.2	98.7
10/14/2015	11.6	8.5	7.6	225	62	35/37	9/9	0.532/0.571	<.0150/<.0150	<.0190/.0200	5.8/5.8	10.9/10.9	15.6/8.6
median	19.1	7.9	6.9	206	30	57.5	9.5	0.793	0.0293	0.0372	12.8	26.9	68.0

Total suspended solids (TSS) concentrations and turbidity are moderate to high. TSS concentrations range from 5.8 – 393 mg/l. Turbidities range from 7.1 – 729 ntu. Both parameters are much higher during high flows due to watershed runoff and channel erosion of clay.

Minnesota has a stream turbidity standard of 25 ntu's, which Wisconsin is using as one reason for designating the Nemadji River as an impaired stream. Median turbidities at the three sites are very close to the 25 ntu standard, ranging from 24.9 to 26.9 ntu's (table 1).

E. coli concentrations are low to high, ranging from 9.7 to 1,120 cfu/100ml. Concentrations are much higher during high flows due mostly to watershed runoff. Wisconsin does not currently have an E. coli standard from streams, but it does apply EPA E. coli standards to swimming beaches. An "advisory" standard of 235 cfu/100ml results in a caution sign being placed at a beach to warn of an increased risk of exposure to fecal bacteria and viruses. A "closure"

standard of 1,000 cfu/100ml results in beach closure. Only the two dates with flows >1,000 cfs (May 12th and July 8th) have E. coli concentrations > 235 cfu/100ml. The samples collected on May 12th had concentrations very close to the "closure" standard (980,980, 1,120 cfu/100ml).

Potential Influences on Water Quality Differences at the Three Monitoring Sites

A substantial amount of water quality data from other sources is available for the Nemadji River at CTH C. A USGS gaging station is also operated at that location. No previous water quality data was available for the two downstream sites, Woodlawn Road and USH 2/53. Monitoring the two downstream sites simultaneously with the CTH C site was intended to allow an initial comparison between the sites, and provide some sense of additional inputs to the Nemadji River not being measured at the CTH C site.

There are multiple potential sources of influence on water quality in the Lower Nemadji River that need to be considered. These include the Lake Superior seiche effect, runoff from the Lower Nemadji River sub-watershed, Crawford Creek inflow, and point source discharges.

Lake Superior Seiche Effect

Lake Superior seiches cause backflows up the Nemadji River for about 8.8 miles upstream. The distance the backflows move upstream was determined by observing the lack of, or presence of, vegetative debris snagged on submerged wood during the Fall. Where backflow pulses occured regularly, wood was free of vegetative debris. Beyond the extent of backflows, flow is unidirectional (downstream) and vegetative debris was retained on wood.

During the periodic backflows, water is observed moving upstream near the stream banks, but continues to move downstream near the stream center. The backflows have the effect of providing another water source to the lower Nemadji River. The water backflowing up the river is derived mostly from the St. Louis River Estuary (SLRE), with additional contributions from Lake Superior.

St. Louis River estuary water quality is compared to Lower Nemadji River water quality below:

Parameter	St. Louis River Estuary (median)*	Lower Nemadji River (median)**
Total phosphorus (ug/l)	27.2	53.3
Total nitrogen (ug/l)	912	786
NO_x-N (ug/l)	182	27.9
NH_4-N (ug/l)	35.2	21.4
Total suspended solids (mg/l)	9.9	18.4

*average of May-October 2012 and 2013 medians for St. Louis River estuary harbor zone (downstream of USH 2), in Bellinger 2015

**average of May-October 2015 medians from the three sites on the Lower Nemadji River

The Superior entrance to Superior Bay is in close proximity to the mouth of the Nemadji River (0.6 miles). This makes it uncertain how adequately SLRE water quality represents backflow water, since Lake Superior water may be a larger component of SLRE water in that area.

The SLRE has lower TP and TSS concentrations, roughly similar TN and NH_4-N concentrations, and higher NO_{3+2}-N concentrations. Backflow of SLRE water into the lower Nemadji River would be expected to contribute to lower TP and TSS concentrations, and higher NO_{3+2}-N concentrations.

Conductivity, temperature and dissolved oxygen (D.O.) data for the SLRE is available from the Barker's Island continuous monitoring station operated by the Lake Superior National Estuarine Research Reserve System (NERR). If this data is representative of backflow water, it indicates backflows are contributing to Nemadji River water quality for all of these three parameters:

- Conductivity was higher in the SLRE than in the Nemadji River on five of six dates and so may have contributed to conductivity increases between CTH C and USH 2/53 on those five dates. On the sixth date (August 6th), SLRE water was lower in conductivity and so may have contributed to the decline in the Nemadji River.

- Temperature was higher in the SLRE than in the Nemadji River on five of six dates and so may have contributed to temperature increases between CTH C and USH 2/53 on those five dates. On the sixth date (June 10th), SLRE water was lower in temperature and so may have contributed to the slight temperature drop in the Nemadji River.

- On four of five dates when Nemadji River D.O. declined between CTH C and USH 2/53, D.O. was lower in the SLRE than in the Nemadji River and so may have contributed to the declines.

Lower Nemadji River Sub-watershed runoff

Some sense of possible total phosphorus contributions from the lower Nemadji River sub-watershed runoff can be obtained as follows:

- The sub-watershed is 3.7% of the total watershed.
- Developed land covers are 18.6% higher in the sub-watershed than in the remaining watershed upstream of CTH C.
- Developed land covers can be roughly assumed to export 0.7 kg/ha/yr of TP and undeveloped land covers can be assumed to export 0.1 kg/ha/yr of TP. The weighted average TP export rate for the upper watershed would then be 0.18 kg/ha/yr. Developed land cover TP export in the lower sub-watershed (0.7 kg/ha/yr) is 3.9 times higher than the weighted average for the upper watershed.
- The increase in TP loading to the Nemadji River from the lower sub-watershed would then be - 3.7% x 18.6% x 3.9 = 2.7% increase.

This suggests that increased concentrations or loads of total phosphorus from sub-watershed runoff would be less than 3%.

Increased concentrations or loads of nitrogen and TSS due to runoff from the lower Nemadji River sub-watershed are also likely to be small. However, nitrogen concentrations or loads are typically poorly correlated with land cover. TSS concentrations or loads in the Nemadji River have been shown to be mostly derived from channel and drainageway erosion and so are unlikely to be predictable from land cover.

Crawford Creek

Crawford Creek flows into the Nemadji River between CTH C and Woodlawn Road. Its watershed is about half the area of the Lower Nemadji River sub-watershed, and about 1.8% of the total Nemadji River watershed. Crawford Creek is contaminated with creosote and PAH's from a former wood preserving facility. Crawford Creek conductivities at Hammond Road during 2009-10 had a median of 316.5 umhos/cm. This is higher than the Nemadji River (166 umhos/cm at CTH C) and so would be expected to cause a slight increase in downstream Nemadji River conductivities.

Point Sources

Superior Sewage Disposal System

The Superior combined sewer treatment plant discharges to the Nemadji River between CTH C and Woodlawn Road (figure 3). The plant only discharges intermittently following heavy rainfalls, when Nemadji River flows are usually high, and so considerable dilution capacity is usually available. During 2015 discharges occurred on 5 days during the May to October Nemadji River monitoring period (July 6,7,8th and September 24,25th).

Discharges can at times have high concentrations of BOD_5 (2-60 mg/l), E. coli (100-250,000cfu/100ml), ammonia (0.2-5.36 mg/l), total phosphorus (40-793 ug/l), and total suspended solids (9-189 mg/l). Maximum reported discharge rate in July was 4.6 cfs. Nemadji River flow on July 8th was 1,580 cfs (table 1).

E. coli increases in the Nemadji River from this point source may be one of the more distinguishable impacts. E. coli concentrations were higher at the two sampling sites downstream of this source than at the upstream sampling site on July 8th (table 1).

BOD_5 concentrations from this point source may contribute to reduced dissolved oxygen concentrations downstream, as was observed on July 8th (table 1). Deposition of oxygen-demanding solids on the stream bottom might contribute to delayed, chronic, oxygen demand.

Enbridge Energy

Enbridge Energy discharges to the Nemadji River between Woodlawn Road and USH 2/53 (figure 3). Enbridge Energy typically has occasional discharges of water used to pressure test

tanks and pipelines. Pressure test water is tested for a range of petroleum related compounds to assure permit limits are met. Pressure test water is treated with carbon filtration prior to release, when necessary.

During 2015 a much larger than usual pipeline pressure test occurred that resulted in discharge of water during most of October. Discharge averaged about 4.6 cfs, with a mean TP concentration of 238 ug/l. The Nemadji sampling site downstream of the discharge point had a 1.5 ug/l higher TP concentration than the upstream sampling site on October 14th (table 1).

Average concentrations of other parameters from the three outfall sites in October were:

- BOD_5, 3 – 8.7 mg/l.
- Ammonia, 0.54 – 1.3 mg/l
- TSS, 5.3 – 16.9 mg/l

These concentrations are unlikely to produce measureable impacts in the Nemadji River. Conductivity of the discharges is not reported, so they are a possible contributor to higher conductivities in the Nemadji River.

BNSF Railway Company

BNSF Railway Company discharges to the Nemadji River between Woodlawn Road and USH 2/53 (figure 3). BNSF Railway Company discharge is mostly taconite storage pile runoff that is treated in a retention/settling pond. Small amounts of maintenance washwater pass through a grit chamber, an oil/water separator, and a concrete lagoon, before also entering the retention/settling pond. Pond discharge was generally continuous during April through October of 2015 and averaged 1.5 cfs. Average concentrations of water quality parameters in past years were:

- TP, 120 ug/l (one sample)
- TSS, 8.9 mg/l
- BOD5, 2 mg/l
- Chloride, 104 mg/l
- Iron, 0.5 mg/l

With the exception of chloride, this point source appears unlikely to produce measureable impacts to the Nemadji River. Nemadji River samples were not tested for chloride in 2015. Chloride does contribute strongly to conductivity, which was tested. Conductivity was higher at the downstream monitoring site (USH 2/53) than the upstream monitoring site (Woodlawn Rd.) on 5 of the 6 sampling dates.

Water Quality Differences at the Three Monitoring Sites

Water quality parameter differences between the upstream site (CTH C) and the downstream site (USH 2/53) are shown in table 2, below. Some parameters show changes that appear to be significant. The previous discussion on potential influences on water quality identifies some possible explanations for occasional differences. Due to the limited data collected and the complexity of the inputs that occur, further interpretations are difficult or speculative.

Potential influence of backflows on conductivity, temperature, and dissolved oxygen (D.O.) are discussed above (Lake Superior Seiche Effect). Other potential influences on temperature and D.O. include:

- The Nemadji River widens, deepens, and slows between CTH C and USH 2/53. Solar radiation inputs may also be a contributor to the increases.

- Reduced oxygen solubility due to temperature increases may also contribute to the decreases (up to 1 mg/l). Sediment oxygen demand might be higher in the lower river if temporary deposition of organic solids is occurring due to reduced stream velocities. This could also contribute to D.O. decreases.

Total phosphorus increases on five of six dates, but two of the five increases are $\leq$ 3 ug/l and not significant. Turbidity increases on all six dates, but half of the increases are less than 1 n.t.u. and probably not significant. Transparency decreases on five of six dates, although most decreases are 3 cm. or less and may not be significant.

Table 2.

Unit change and % Change from CTH C to USH 2/53

Date	Temp. (oC) unit ch.	Temp. (oC) % ch.	D.O. (mg/l) unit ch.	D.O. (mg/l) % ch.	pH (s.u.) unit ch.	pH (s.u.) % ch.	Conductivity (umhos/cm) unit ch.	Conductivity (umhos/cm) % ch.	Transp. (cm) unit ch.	Transp. (cm) % ch.	Total P (ug/l) unit ch.	Total P (ug/l) % ch.	Mean daily flow (cfs)
05/12/2015	0.2	3.1	0.2	1.8	-0.3	-4.3	39	26.5	-2	-40.0	151	43.1	1,490
06/10/2015	-0.1	-0.5	-0.4	-4.7	0	0.0	24	16.1	1	3.4	-7	-13.2	265
07/08/2015	0.2	1.1	-0.6	-7.4	-0.2	-2.9	8	8.6	-1	-10.0	3	1.9	1,580
08/06/2015	2	10.3	-1.1	-13.4	0.1	1.5	-35	-12.7	-7	-13.0	10	30.3	50
09/09/2015	2.3	12.0	-2.1	-25.0	-0.3	-3.8	35	18.0	-3	-9.4	15	27.8	140
10/14/2015	2.5	27.5	-2.1	-19.8	-0.1	-1.3	43	23.6	-3	-4.6	0.5	1.4	132
mean =	1.2	8.9	-1.0	-11.4	-0.1	-1.8	19	13.4	-2.5	-12.3	28.8	15.2	

Table 2 (cont.)

Date	Dissolved ortho P (ug/l) unit ch.	Dissolved ortho P (ug/l) % ch.	TKN (mg/l) unit ch.	TKN (mg/l) % ch.	NH4-N (mg/l) unit ch.	NH4-N (mg/l) % ch.	NO3+2-N (mg/l) unit ch.	NO3+2-N (mg/l) % ch.	TSS (mg/l) unit ch.	TSS (mg/l) % ch.	Turbidity (n.t.u.) unit ch.	Turbidity (n.t.u.) % ch.	E. coli (cfu/100ml) unit ch.	E. coli (cfu/100ml) % ch.
05/12/2015	3.00	37.5	0.090	6.3	-0.0004	-1.3	-0.0002	-0.2	48	14.0	355	94.9	140	14.3
06/10/2015	0	0	-0.019	-2.5	0.0056	36.4	-0.0033	-8.9	-15.5	-50.5	0.3	1.1	1.4	3.9
07/08/2015	3	30	-0.070	-5.1	0.0047	19.3	ND	ND	-24	-18.5	17	14.0	217.6	33.5
08/06/2015	0	0	0.152	26.0	ND	ND	ND	ND	4.2	67.7	0.12	1.7	-47.9	-83.2
09/09/2015	3	42.9	0.130	18.1	0.011	57.0	0.005	12.2	-14.6	-58.9	3.7	15.7	17.1	21.0
10/14/2015	1.5	20	-0.093	-17.2	ND	ND	ND	ND	-0.3	-4.9	0.7	6.7	-17.9	-59.7
mean =	1.8	21.7	0.032	4.3	not calc.	not calc.	not calc.	not calc.	-0.4	-8.5	62.8	22.4	51.7	-11.7

Macroinvertebrate Samples

Initial plans to collect samples at multiple sites downstream of CTH C had to be altered due to flow conditions in that section of the river. A relatively wide and deep channel, and low discharge rates in late summer and early fall resulted in inadequate current velocities (< 0.3 ft/sec) to meet sampling protocols for applying Wisconsin DNR macroinvertebrate biotic indices for streams (>0.5 ft/sec) or rivers (>0.3 ft/sec). Furthermore, the periodic backflows prevented any accumulation of leaf packs or other vegetative debris on the woody debris present that would have provided a suitable sampling substrate.

The most downstream site with suitable flow conditions was 3 miles downstream of CTH C. This site was just upstream of the extent of seiche influence and had leaf packs on woody debris.

Summarized macroinvertebrate sample results are shown in table 2. Very healthy macroinvertebrate communities were found at all six sites. All samples had high macroinvertebrate index of biotic integrity (MIBI) values that are rated as excellent. Hilsenhoff biotic index (HBI) values ranged from good to excellent, indicating oxygen availability is consistently good and little organic pollution is present.

Species richness is fairly high ranging from 19 to 41. Percent EPT individuals is high (40-75%), and percent Chironomidae individuals is low (2-21%), which both also suggest good water quality.

The two Finn Road samples were collected from different substrates for comparison. The downstream sample was collected from leaf packs snagged on woody debris, while the upstream sample was collected from cobble. The cobble had fairly heavy coatings of periphyton and silt. The sample from cobble had a similar MIBI, a poorer HBI, higher species richness, a lower percent EPT, and a higher percent Chironomids. The coatings of periphyton and entrapped silt on the cobble substrate were probably a major reason for these differences.

The high quality of the macroinvertebrate community found is consistent with past findings for the Nemadji River. One of the conclusions of "The Red Clay Project Final Report Summary" (Andrews et al. 1979) was that "number of macroinvertebrates per unit area, total number of taxa, diversity, and biomass are not significantly affected by clay turbidity and siltation within the Nemadji River system".

Table 3. Lower Nemadji River Macroinvertebrate Sample Results

Site	SWIMS station #	Date	Macroinvertebrate Index of Biotic Integrity (MIBI)	MIBI Condition Category	Hilsenhoff Biotic Index (HBI)	HBI Condition Category
Nemadji R. 15 m DS Dedham Rd.	10044435	11/02/2015	8.75	excellent	3.99	Very good
Nemadji R. 25 m US Finn Rd.	163233	10/22/2015	9.04	excellent	4.96	Good
Nemadji R. 10 m DS Finn Rd.	163233	10/22/2015	9.32	excellent	2.78	Excellent
Nemadji R. 135 m DS STH 35	163048	11/02/2015	8.69	excellent	3.85	Very good
Nemadji R. 60 m US CTH C	163003	10/22/2015	11.62	excellent	3.73	Very good
Nemadji R. 3 mi. DS CTH C	10044397	10/22/2015	11.34	excellent	3.61	Very good

Table 3.(cont.) Lower Nemadji River Macroinvertebrate Sample Results				
Site	Species Richness	% EPT* Individuals	% EPT* Genera	% Chironimidae Individuals
Nemadji R. 15 m DS Dedham Rd.	39	60	46	13
Nemadji R. 25 m US Finn Rd.	36	40	41	21
Nemadji R. 10 m DS Finn Rd.	19	75	78	2
Nemadji R. 135 m DS STH 35	41	52	38	15
Nemadji R. 60 m US CTH C	33	69	61	13
Nemadji R. 3 mi. DS CTH C	35	72	58	9
*EPT = ephemeroptera (mayflies), plecoptera (stoneflies), trichoptera (caddisflies) Complete sample result information is available on WI DNR's SWIMS data base.				

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Appendix 10

Nemadji River Watershed Implementation Planning Report
(Pertains to management action 6.05)

January 15th, 2017

Final Project Report

Project Name: Nemadji Watershed Implementation Planning

Name and Contact Information: Christine Ostern, Douglas County Land Conservation Department

Project Deliverables Summary

The following activities have been completed.

Phase 1 Activities: December 2015 – June 2016

- Develop a Nemadji Watershed Implementation Strategy
- Compile landowner database
- Compile natural resource information for the watershed
- Compile watershed maps
- Develop a newsletter and mail to resident landowners (approximately 1600 residents)
- Conduct a Watershed Informational Workshop
- Coordinate with Carlton County Soil and Water Conservation District

Phase 2 Activities: July 2016 – December 31st, 2017

Implement the Nemadji Implementation Plan developed during Phase 1.

Convene and coordinate a Wisconsin stakeholder group

- Convene a minimum of 4 meetings of this group

A watershed stakeholder group was formed that included 10 watershed residents. This group included three county board members and one town elected official. Other participants included staff from City of Superior Environmental Services, Douglas County Ag/Horticulture Extension and Carlton County Soil and Water Conservation District. This group met four times at the Superior Town Hall from 6-8pm.

Develop informational workshops to provide information on water quality issues.

- Target audience of at least 30 watershed residents

Topics of informational workshops that were developed included stormwater management, public and private landowner forest management strategies, land stewardship, watershed management and erosion control.

Identify a minimum of 3-5 landowners who agree to explore cost-share opportunities to implement best management practices on their property.

- Schedule site visits and provide technical assistance to landowners to apply for relevant cost-share opportunities.
- Schedule follow-up site visits for landowners with other natural resource professionals.

Site visits were conducted on 5 properties (see attached map). Referrals were made to NRCS for 3 of the sites which have been followed up with site visits by NRCS staff.

Maintain communication with Carlton County SWCD to identify ways to continue to collaborate on outreach activities.

Carlton County staff attended several of our staff planning meetings, participated in the watershed tours, provided resources/handouts to workshop participants and invited staff to attend several of the CC SWCD meetings.

Summary of Activities Completed

Activity	Target #	Actual #	# Contacts	Comments
Implementation plan	Completed	Completed		
Compilation of landowner database	Completed	Completed		
Compilation of natural resource information and maps	Completed	Completed		
Stakeholder committee members	10	10		
Stakeholder committee meetings	3	4		
Workshops/Informational Sessions	4	5	119	
Landowner site visits	3-5	5		3 have submitted NRCS applications
Coordination with Carlton County SWCD	-	On-going		
Newsletter	-	1	1600	
Mailings	1	2	1640	

Supporting documents

- Implementation Plan
- Map of landowner parcels who scheduled site visits
- Stakeholder committee contacts
- Stakeholder committee meeting agenda
- Open House Flyer
- Workshop Invitation
- Landowner site visit form
- Newsletter
- Photos

Nemadji Watershed Implementation Strategy

Douglas County has increased the local capacity for addressing watershed issues in the Nemadji River through the engagement of landowners, community leaders and local decision-makers. Educational workshops have increased stakeholder knowledge of water resource problems and provided information on best management practices to reduce runoff and facilitate the implementation of projects that will improve watershed health.

Year One Activities

Information on the Nemadji River Watershed organized

Landowner database compiled

- A Wisconsin landowner database was compiled and organized into categories for mailings and other contacts. These categories included full-time residents, part-time residents, in-state or out-of-state landowners and non-resident landowners.

Natural resource information for watershed compiled

- Information on the Nemadji River watershed (WI and MN) was compiled into a database including planning, research and management documents that are relevant to watershed issues, research, modeling and best management practices.

Watershed maps compiled

- The open lands update for the watershed (2014) was incorporated into Douglas County Lake Superior basin information. The current information on wetland functions and potentially restorable wetlands were identified in the Nemadji watershed and updated. The high priority subwatersheds in the Nemadji watershed were identified from the watershed-based plan for the County.

Outreach Programming Developed

Outreach programs were developed and implemented for several target audiences: Wisconsin (and Minnesota) landowners, local government officials and a focused watershed group of Wisconsin stakeholders. Information and outreach materials were developed for each audience targeted and included:

- A newsletter was developed and mailed to Wisconsin landowners. It was developed in coordination with Carlton County Soil and Water Conservation District (SWCD) staff. The content was watershed-wide and had information on both the Carlton County SWCD and the Douglas County Land Conservation Department management efforts in the Nemadji River watershed. In addition to several short articles it also included map(s), contact information a short survey to identify landowners who are interested in receiving additional information and attending an informational workshop.

- Watershed informational workshop
  Landowners responding to the survey (sent in the newsletter) were invited to attend an informational workshop in April. This workshop provided information on watershed health, wetland functions in watersheds and current management activities to reduce erosion. Information and recommendations about the watershed-based plan developed for Douglas County were presented with specific information related to the Nemadji River watershed. Contact lists were developed from the list of attendees at this workshop in order to identify stakeholders who have an interest in receiving additional information.

- Wisconsin stakeholder meeting (#1)
  Recruitment for the stakeholder watershed planning committee commenced with this workshop. The goal was to recruit 15 stakeholders to participate in the next phase of planning. City and County local government officials and other community leaders whose jurisdictions include areas within the watershed were contacted and invited to participate. This planning committee participated in several meetings and workshops and stakeholders who committed to participating in the Nemadji watershed planning committee were eligible for the stipend available through this project and expectations for participation were discussed (minimum number of meetings, workshops). The focus of this stakeholder group was to learn about watershed issues, best management practices for reducing runoff and erosion and initiated the development of a 'slow the flow' outreach strategy for the watershed. They learned more about opportunities for funding and cost-share for individual implementation projects. The first workshop was scheduled summer 2016.

- Presentations and outreach materials were developed for Wisconsin landowners and focused on watershed health issues. They included the work recently completed by the Douglas County Lake Superior Basin watershed-based plan that includes identification of wetland restoration and preservation priorities, riparian restoration and forest management best management practices to slow stormwater runoff. Watershed (HUC 12) and subwatershed (HUC 14) maps depicting high priority areas for implementation of these practices in the Nemadji River watershed were discussed. Additional land use/land cover information available for the watershed was also included in these presentations.

- Watershed-wide outreach
  A watershed-wide (Wisconsin and Minnesota) watershed tour was coordinated in the spring with the Carlton County Soil and Water Conservation District (SWCD). This tour included site visits to areas where current management practices are being implemented. Information on best management practices to reduce erosion were discussed and outreach materials containing information to assist landowners in implementation of BMPs on their land were distributed.

Coordination with other Nemadji Watershed projects

Staff coordinated outreach efforts with groups involved with Nemadji Watershed research and outreach that included in-person meetings, phone conversations and/or other means of communication on the progress of the project. These contacts included the following: Andy Robertson (SMUMN), Carlton County Soil and Water Conservation District, modeling scenarios (TetraTech), TMDL modeling (MPCA), Army Corps of Engineers (sediment modeling) and Lake Superior Basin Restoration Partnership (WDNR).

Year Two Activities

Outreach Programming

Workshops were scheduled for the WI Nemadji Watershed committee in order to assist participants in the identification of best management practices that could be implemented at a variety of scales in the watershed to reduce runoff and minimize erosion. This included on-site water retention and infiltration practices, riparian restoration, streambank stabilization techniques and landowner forest management programs. Participants learned about cost-share opportunities for implementation of these projects. Local and regional experts were available to present and discuss the types of practices currently being utilized.

The goals of these workshops were to 1) develop an increased understanding of how watershed issues impact individual landowners and the cumulative impacts of land use practices on aquatic resources and 2) increase local capacity for implementing projects through engagement of local government, community leaders and stakeholders.

Workshops for the WI. Nemadji Watershed committee were scheduled as follows:

- Workshop #3: October 2016
- Workshop #4: April 2017

Meetings were coordinated to assist participants in the development of an outreach and implementation strategy for other landowners and community leaders in the watershed. These stakeholders were encouraged to disseminate their experiences of implementing BMPs to neighbors and community members. Topics were determined by the watershed planning committee and included discussions of the goals, development of successful outreach strategies and long-term strategies for stakeholder engagement.

WI Nemadji Watershed committee meetings were scheduled as follows:

- Meeting #1 : September 2016
- Meeting #2: January 2017
- Meeting #3: March 2017

Watershed-wide outreach

A second watershed tour was coordinated with the SWCD in May/June 2017. Additional events were developed through discussions with SWCD staff.

Next Steps

Primary considerations for next steps beyond this grant effort will include:

- Identifying needs for project design assistance, cost share and other support for implementing best management practices for reducing runoff and erosion
- Strategies for continuing funding and outreach efforts in the watershed
- Expanding watershed partnerships to include groups such as (for example) Northern Institute of Applied Climate Science, West Wisconsin Land Trust, Wisconsin Towns Association, Wisconsin Farmers Union, Ruffed Grouse and American Woodcock Society. This will form the foundation for a coalition with the capacity to further develop and implement watershed protection, restoration and participation into the future and beyond any one grant-funded project.

Timeline

Activities	Q1 12/2015 – 3/2016	Q2 4/2016 – 6/2016	Q3 7/2016 – 9/2016	Q4 10/2016 – 12/2016	Q5 1/2017 – 3/2017	Q6 4/2017 – 6/2017	Q7 7/2017 – 9/2017	Q8 10/2017 – 12/2017
Develop and finalize Implementation Plan								
Organize information on the Nemadji River Watershed								
Compile landowner database								
Compile watershed maps								
Develop Outreach Programming								
Design and send newsletter *(2nd newsletter sent through other funding)*								
Landowner informational workshop								
Stakeholder Meetings								
Stakeholder workshops								
Watershed tours and outreach coordination with MN partners								

Stakeholder committee contact information

Nemadji Watershed Planning Committee

Planning committee members	Affiliation	email
Doug and Donna Hank	Watershed resident	joshua4340@yahoo.com
Susi Pakes	Watershed resident	badger5757@gmail.com
Mary McConnell	Watershed resident	aandeg1952@gmail.com
Janet Dalbec	Town of Superior	jan4joy@centurytel.net
Pat Ryan	Douglas County Board	pat.ryan@douglascountywi.org
Dan Corbin	Douglas County Board	dandcorbin@gmail.com
Jeff O'Flanagan	Watershed resident	jeffrey.oflanagan@wisconsin.gov
Kay McKenzie	Watershed resident	kamck@chartermi.net
Terry White	Douglas County Board	twmillwhite@yahoo.com
Jane Anklam	Douglas County Extension	jane.anklam@ces.uwex.edu
Andrea Crouse	City of Superior	crousea@ci.superior.wi.us
Melanie Bormier	Carlton County SWCD	melanie.bomier@carltonswcd.org
Nicky Martin	WDNR	nichol.martin@wisconsin.gov

Example meeting agenda

Nemadji Watershed Planning Committee Meeting Superior Town Hall
February 2, 2017

Attendees:

Mary McConnell	Kay McKenzie
Mark McConnell	Jeff O'Flanagan
Doug Hank	Donna Hank
Andrea Crouse	Jane Anklam
Christine Ostern	Mike Gardner
Sue O'Halloran	

Agenda Items

- Restate purpose of planning group
- Site visits, reviewed form for participation
- Stipends
    - Discussed stipends available to committee; described process for sign-up
- Proposed outreach activities: workshop or open house in spring 2017
    - Venue, date, potential topics, etc. for workshop/open house

Committee members expressed interest in an open house
General theme: stewardship planning, watershed practices to protect/improve water quality, wildlife habitat
Types of presentations and displays:
Venue

- Superior Town Hall, Saturday afternoon in April

Audience

- Postcard/invitations sent to landowners; City of Superior; local elected officials
- PR for event; Andrea will provide her contact list

Speakers

- 2-3 presentations, each ~ 15 mins. plus Q&A
- Scheduled through the afternoon

Displays

- Stewardship planning
- Managing land/forests for wildlife and water quality
- Native plants, pollinators and gardens
- Forest management on public/private land
- Erosion control
- Groundwater

Provide refreshments, native plants for sale, tree seedling give-away?
Survey for attendees: interest in receiving information, site visit, etc.

Additional discussion items: Mary Mc. discussed her project with UWS: native plant guide, handed out plant guide

Landowner Site Visit Form

Nemadji Watershed Implementation Planning Project

Landowner Application for Implementation Information

Landowner and Property Information:

Name_____

Address_____

Phone_____ **Email**_____

Property location if different from address:

Size of property (acres):

Landowner interests (circle all that apply):

agriculture practices	erosion control	forest planning & management
pollinator habitat	preservation/conservation easements	
shoreland management	water quality in watershed	wetland planning & management
wildlife habitat		
others (please list):		

Interested in cost-sharing? yes no

Landowner Request

I wish to implement conservation practices on my property that will address my interests. I also wish to meet with a conservation specialist at my property to evaluate how my interests can be met implementing various practices utilizing programs that are available through multiple agencies. I understand that this determination does not obligate me to participate in a program nor does it obligate the Douglas County Land Conservation Department to provide cost share assistance to me.

Landowner signature:

_____**date**_____

County Conservationist signature:

_____**date**_____

- for internal use only –

Site visit notes:

Recommended programs/agency contacts (check all that apply):

__ County wetland program
__ County cost-share program
__ NRCS (standard programs and LSHRP)
__ WDNR Wildlife Prog.
__ WDNR MFL
__ WDNR WFLGP
__ USFWS Private Landowner Prog.
__ Other(s):

Follow-up correspondence to landowner done and filed:

Date and initials _____

Appendix 11

Total Phosphorus Data for Western Lake Superior Site Su19

Total Phosphorus Data for Western Lake Superior Site Su19
USEPA Biology Monitoring Program (1996-2015) Data Source: https://cdx.epa.gov/

Season	Year	Type (1)	Station Depth (m)	TP (ug/L)
Spring	1996	INT	183.02	3.9
Summer	1996	INT	185.00	4.5
Spring	1997	INT	192.94	2.1
Summer	1997	INT	191.41	3.0
Spring	1998	INT	192.02	8.0
Summer	1998	INT	188.37	4.2
Summer	1999	DCL	191.40	2.4
Spring	1999	INT	193.00	2.8
Summer	1999	INT	191.40	2.8
Spring	2000	INT	188.70	1.5
Summer	2000	INT	186.00	1.3
Spring	2001	INT	192.30	1.0
Summer	2001	INT	192.30	3.8
Spring	2002	INT	194.00	3.1
Summer	2002	INT	186.30	2.3
Summer	2003	DCL	187.70	1.1
Spring	2003	INT	184.60	2.5
Summer	2003	INT	187.70	1.2
Summer	2004	DCL	185.80	1.9
Spring	2004	INT	187.80	1.5
Summer	2004	INT	185.80	2.5
Summer	2005	DCL	186.80	2.3
Spring	2005	INT	187.70	1.9
Summer	2005	INT	186.80	2.5
Summer	2006	DCL	185.80	2.9
Spring	2006	INT	187.70	2.4
Summer	2006	INT	185.80	2.5

Season	Year	Type (1)	Station Depth (m)	TP (ug/L)
Summer	2007	DCL	191.10	2.9
Spring	2007	INT	187.70	1.6
Summer	2007	INT	191.10	2.9
Summer	2008	DCL	185.80	1.8
Spring	2008	INT		2.0
Summer	2008	INT		2.4
Summer	2009	DCL	185.80	1.4
Spring	2009	INT		1.6
Summer	2009	INT		2.0
Summer	2010	DCL	185.80	3.2
Spring	2010	INT	187.70	3.0
Summer	2010	INT	185.80	3.0
Summer	2011	DCL	185.80	2.2
Spring	2011	INT	187.80	1.9
Summer	2011	INT	185.80	2.7
Summer	2012	DCL	185.80	3.5
Spring	2012	INT	186.80	3.3
Summer	2012	INT	185.80	1.9
Summer	2013	DCL	181.20	2.2
Spring	2013	INT	183.00	2.1
Summer	2013	INT	181.20	3.9
Summer	2014	DCL	186.00	3.0
Spring	2014	INT	183.20	2.2
Summer	2014	INT	186.00	2.8
Summer	2015	DCL	179.00	1.7
Spring	2015	INT	183.00	2.4
Summer	2015	INT	179.00	3.3
			Mean from 1996 to 2015	**2.6**
			Range from 1996 to 2015	**1.0 - 8.0**

(1)
INT = integrated sample collected from the whole water column (spring) or the epilimnion (summer)
DCL = Deep Chlorophyll Layer, a discrete sample from the summer deep chlorophyll maximum

Appendix 12

Public Involvement Process and Letters of Support

BUI 6 Technical Team Members (2016-2019)

Jane Anklam	Wisconsin Landmark Conservancy
Richard Axler	UMN Natural Resource Research Institute
Donalea Dinsmore	Wisconsin Department of Natural Resources
Kari Hedin	Fond du Lac Band
Joel Hoffman	US Environmental Protection Agency
Richard Kiesling	US Geological Survey
Tonia Kittelson	City of Superior
Diane Nelson	City of Superior
Christine Ostern	Douglas County
Hannah Ramage	Lake Superior Reserve
Euan Reavie	UMN Natural Resource Research Institute
Craig Roesler	Wisconsin Department of Natural Resources
Shon Schooler	Lake Superior Reserve
Michele Wheeler	Wisconsin Department of Natural Resources
Ashley VandeVoort	Douglas County

Huberty, Barbara (MPCA)

From:	ntf5418@lakeconnections.net
Sent:	Friday, March 20, 2020 5:46 PM
To:	Huberty, Barbara (MPCA)
Cc:	Matthew.Steiger@wisconsin.gov; Kris Eilers
Subject:	Comments of Proposal to Remove SLRAOC the Excessive Sediment and Nutrient Loading Beneficial Use Impairment
Attachments:	20200318 NTF SED-NUT BUI REMOVAL COMMENTS.pdf

Barb,

I am pleased to provide you with my thoughts regarding the proposal to remove the SLRAOC Sediment and Nutrient Impairment.

I fully concur with the fact that identified "Legacy" issues have been addressed and the acknowledgment that full implementation of existing and any future regulatory programs must be conducted to ensure long term protection of the St. Louis River Estuary and Lake Superior. 3 of 9 impairments removed! Congratulations on a job well done! Looking forward to the removal of 6 more impairments and full SLRAOC delisting by 2025!

My 10 cents worth for what it is worth.

Congrats on the continued forward progress.

Regards,

Nelson T. French
The Boulders
5418 Rocky Wall Road
Silver Bay, MN 55614-4245

Mobile: 612.237.5171
Email: ntf5418@lakeconnections.net

"In the shadow of a nation
That once had its head so high
Hope for generations
Where white doves did fly

Now fear and dread are upon us
The soul of the land on a thread
Freedom's disappearing
Lady Liberty hangs her head

The halls of justice are bleeding
Illusion and lies swirl around

Right and wrong a talk show game
Truth cannot be found

A deceiver is among us
Spinning nightmares in our head
Fear and division his trademark
Fanning the flames to spread"

Scarlet Rivera
Lady Liberty

COMMENT FORM

for

St. Louis River Area of Concern

Removing the Excessive Sediment and Nutrient Loading Beneficial Use Impairment

Your feedback is very important to the four agencies implementing the St. Louis River Area of Concern Program: the Fond du Lac Bank of Lake Superior Chippewa, the Minnesota Department of Natural Resources, the Minnesota Pollution Control Agency, and the Wisconsin Department of Natural Resources. In the space below, please provide your comments regarding the proposal to remove the Excessive Sediment and Nutrient Loading Beneficial Use Impairment (BUI).

Please complete this form and return it by email to: barbara.huberty@state.mn.us or mail it to: Barb Huberty (MPCA) at the mailing address on the back of the form. ***All comments must be submitted before 5:00 pm on Tuesday, March 24, 2020.*** You may attach additional pages if needed.

To submit comments or petitions to the AOC agencies through the mail or email, you must state:

1) Name and address
2) The action you wish the AOC agencies to take, including specific references to the section of the draft BUI removal you believe should be changed.
3) The reasons supporting your position, stated with sufficient specificity as to allow the AOC agencies to investigate the merits of the position.

Please print clearly:

Name: Nelson T. French

Mailing address: 5418 Rocky Wall Rd., Silver Bay, MN 55614

Email address: ntf5418@lakeconnections.net

Actions desired (please reference pages of the BUI removal document that pertain) & supporting reasons:

I have reviewed the proposed Removal Recommendation for Excessive Loading of Sediment and Nutrients Beneficial Use Impairment ~~in the St. Louis River Area of Concern and am pleased to inform you that I fully concur with said proposal as the SLRAOC Team~~ has fully documented that the conditions for removal have been met.

~~Specifically I concur with the articulation in the Executive Summary on pages ii-v that the team has documented that the following~~ conditions have been met:

1. All federal, state, and local point source and nonpoint source discharge permits in the AOC are in compliance with regard to controlling sources of nutrients (particularly nitrogen and phosphorous), organic matter, and sediment.
2. Total phosphorus concentrations in the Lake Superior portion of the AOC do not exceed 0.010 mg/L (upper limit of oligotrophic range)
3. ~~There are no exceedances of the most protective water quality standard for either state in the western basin of~~ Lake Superior due to excessive inputs of organic matter or algal growth attributed to loadings from wastewater ~~overflows into the St. Louis River~~
4. Total phosphorus concentrations within the St. Louis River portion of AOC do not exceed an interim guide of 0.030 mg/L (upper limit of ~~esotrophic range) or the most restrictive water quality standards. This ensures that anthropogenic sources and activities in the St. Louis~~ River AOC do not result in excessive productivity and nuisance conditions within the St. Louis River Estuary.

Additional Comments:

I found the use of paleolimnological data documenting historic trends from "presettlement" through present a particularly useful analysis. Not only did this study document the success of environmental regulations implemented in the 1970's, but also provideded ~~a better understanding about the pre-industial conditions present in the natural system. I fully concur with the fact that identified~~ "Legacy" issues have been addressed and the acknowledgment that full implementation of existing and any future regulatory ~~programs must be conducted to ensure long term protection of the St. Louis River Estuary and Lake Superior. 3 of 9 impairments~~ removed! Congratulations on a job well done! Looking forward to the removal of 6 more impairments and full SLRAOC delisting by 2025!

Thank you for your feedback!

ST. LOUIS RIVER ALLIANCE
PROTECT, RESTORE AND ENHANCE
Working together to protect, restore, and enhance the St. Louis River

St. Louis River Alliance
394 Lake Avenue S, Suite 208
Duluth, Minnesota 55802-2338
Phone: 218-733-9520

April 3, 2020

Barb Huberty
St Louis River Area of Concern Coordinator
Minnesota Pollution Control Agency
Remediation Division
525 Lake Avenue South Suite 400
Duluth, MN 55802

Re: Support for Proposal to remove the St Louis River Area of Concern Excessive Loading of Sediments and Nutrients BUI

Dear Ms. Huberty,

On behalf of Board of Directors of the St. Louis River Alliance I am pleased to inform you that our board has reviewed the information provided by your agency and we are in agreement with the recommendation put forward by the Wisconsin Department of Natural Resources (WDNR), Minnesota Department of Natural Resources (MNDNR), Minnesota Pollution Control Agency (MPCA) and the
Fond du Lac Band of Lake Superior Chippewa to request to the United States Environmental Protection Agency (USEPA) Great Lakes National Program Office's (GLNPO) to approve removal of the St. Louis River Area of Concern Excessive Loading of Sediments and Nutrients BUI.

As you know, the St. Louis River Alliance was actively involved in the development of the St. Louis River Remedial Action Plan and has been participating in the discussions of the specific actions that have been fully completed by the Area of Concern Coordinators. Completion of this work and documentation that all actions have been taken is a tangible milestone for the delisting of the St. Louis River Area of Concern. This is a major accomplishment and we thank you for your work and commitment to this process.

We look forward to our continuing work together to remove the remaining 6 beneficial use impairments and to the eventual delisting of the St. Louis River Area of Concern.

Sincerely,

Kristi S Eilers
Executive Director
St. Louis River Alliance