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Office of Water
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National Lakes Assessment 2017

Field Operations Manual

Version 1.1, April 2017

NOTICE

The intention of the National Lakes Assessment 2017 (NLA 2017) project is to provide a comprehensive "State of the Lakes" assessment for lakes, ponds, and reservoirs across the United States. The complete documentation of overall project management, design, methods, and standards and Quality Assurance/Quality Control (QA/QC) measures is contained in this document and companion documents, including:

National Lakes Assessment 2017: Quality Assurance Project Plan (QAPP) (EPA 841-B-16-003)
National Lakes Assessment 2017: Site Evaluation Guidelines (SEG) (EPA 841-B-16-001)
National Lakes Assessment 2017: Laboratory Operations Manual (LOM) (EPA 841-B-16-004)

These documents together comprise the integrated set of QAPP documents. This document (*Field Operations Manual [FOM]*) contains a brief introduction and procedures to follow at the base location and on-site, including methods for sampling water chemistry (grabs and *in situ*), phytoplankton, zooplankton, sediment contaminants, bacteria, fish eDNA, algal toxins, benthic macroinvertebrates, physical habitat, and dissolved gases. These methods are based on both the guidelines developed and followed in the Western Environmental Monitoring and Assessment Program (Baker, et. al., 1997), methods employed by several key states that were involved in the planning phase of this project and the National Lakes Assessments 2007 and 2012. Methods described in this document are to be used specifically in work relating to the NLA 2017. All Project Cooperators should follow these guidelines. Mention of trade names or commercial products in this document does not constitute endorsement or recommendation for use. Details on specific methods for site evaluation and sample processing can be found in the appropriate companion document.

The suggested citation for this document is:

> USEPA. 2017. National Lakes Assessment 2017. Field Operations Manual. EPA 841-B-16-002. U.S. Environmental Protection Agency, Washington, DC.

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ACRONYMS/ABBREVIATIONS

ANC	acid neutralizing capacity
CO_2	carbon dioxide
CPR	cardiopulmonary resuscitation
DBH	diameter at breast height
DI	deionized
DO	dissolved oxygen
DOC	dissolved organic carbon
EMAP	Environmental Monitoring and Assessment Program
ETOH	ethyl alcohol
FOM	Field Operations Manual
GIS	geographic information system
GPS	global positioning system
HDPE	high density polyethylene
H_2S	hydrogen sulfide
HQ	Headquarters
IM	Information Management
LOM	Laboratory Operations Manual
MPCA	Minnesota Pollution Control Agency
NALMS	North American Lakes Management Society
NARS	National Aquatic Resource Surveys
NH_4	ammonium
NHD	National Hydrography Dataset
NIST	National Institute of Standards and Technology
NLA	National Lakes Assessment
NO_3	nitrate
OSHA	Occupational Safety and Health Administration
PAH	polycyclic aromatic hydrocarbon
PCBs	polychlorinated biphenyls
PDOP	Position Dilution of Precision
PETG	polyethylene terephthalate glycol
PFD	Personal Flotation Device
PHab	physical habitat
QA	quality assurance
QAPP	Quality Assurance Project Plan
QA/QC	quality assurance/quality control
QCCS	quality control check solution
QRG	Quick Reference Guide
SEG	Site Evaluation Guidelines
SOPs	Standard Operating Procedures
TN	total nitrogen

TOC	total organic carbon
TP	total phosphorus
TSS	total suspended solids
TVS	total volatile solids
USEPA	United States Environmental Protection Agency
UL	Underwriters Laboratory
USGS	United States Geological Survey
UTM	Universal Transverse Mercator

1.0 BACKGROUND

This manual describes field protocols and daily operations for crews to use in the 2017 National Lakes Assessment (NLA2017). The NLA 2017 is a statistical assessment of the condition of our nation's lakes, ponds, and reservoirs (subsequently referred to in this manual as "lakes") and is designed to:

- Assess the condition of the nation's lakes;
- Establish a baseline to compare future surveys for trends assessment and evaluate change in condition since the NLA 2007 and the NLA 2012; and
- Help build State and Tribal capacity for monitoring and assessment and promote collaboration across jurisdictional boundaries.

The NLA 2017 is one of a series of water surveys being conducted by states, tribes, the U.S. Environmental Protection Agency (USEPA), and other partners. In addition to lakes, partners will also study coastal waters, wadeable streams, rivers, and wetlands in a revolving sequence. The purpose of these surveys is to generate statistically-valid reports on the condition of our nation's water resources and identify key stressors to these systems.

The goal of the NLA 2017 is to address two key questions about the quality of the nation's lakes:

- What percent of the nation's lakes are least, moderately, and most disturbed for key indicators of trophic state, ecological health, and human use (recreation)?
- What is the relative importance of key stressors such as nutrients and pathogens?

The NLA 2017 is designed to be completed during the summer growing season before lake turnover (June through September). Field crews will collect a variety of measurements and indicators from an "index site" located at the deepest point of the lake up to 50 meters (or near the middle of the lake if the lake is a reservoir), and document conditions of the littoral zone and shoreline from stations around the lake.

1.1 Selection of Sampling Locations

USEPA selected sampling locations using a probability based survey design (Stevens and Olsen, 2004). Sample surveys have been used in a variety of fields (e.g., election polls, monthly labor estimates, and forest inventory analysis) to determine the status of populations or resources of interest using a representative sample of relatively few members or sites. Using this survey design allows data from the subset of sampled lakes to be applied to the larger target population and assessments with known confidence bounds to be made.

With input from the states and other partners, USEPA used the following framework to guide the site selection process:

- The National Hydrography Dataset (NHD) was used to derive a list of lakes for potential inclusion in the NLA 2017.
- For purposes of this survey, "lakes" refers to natural and man-made freshwater lakes, ponds, and reservoirs greater than one hectare (approximately 2.5 acres) in the conterminous U.S., excluding the Great Lakes.

  Mine ponds, retention basins, cooling ponds, and saline lakes due to salt water intrusion were

*excluded from this study. For more information on the site exclusion criteria refer to the National Lakes Assessment 2017: Site Evaluation Guidelines (EPA 841-B-*16-001*16-001).*

- The sample size was set to include 1,000 lake sampling events.

The result was the inclusion of 904 discrete lakes, with 96 of the lakes to be scheduled for revisit (a second sampling event during the same field season). The 904 lakes consist of three sets of lakes. The first set includes 226 lakes that were originally sampled in NLA 2007, resampled in NLA 2012 and will be resampled again in NLA 2017. Of these, 43 lakes will be sampled twice in NLA 2017. The second set includes 218 lakes originally sampled in NLA 2012 that will be resampled again in NLA 2017. Of these, 53 lakes will be sampled twice in NLA 2017. The third set includes 460 new lakes that will be sampled for the first time in NLA 2017, none of which are scheduled to be sampled twice in NLA 2017. An "oversample" list of additional lakes was also generated to allow for replacement of non-target or otherwise unsampleable sites. The oversample list will also accommodate any state wishing to conduct a state scale survey.

Lakes selected for the NLA 2017 are distributed among six size class categories and are spatially distributed across the lower 48 states and nine aggregated Omernik Level 3 ecoregions (USEPA, 2013).

Related NLA 2017 documents include the following:

National Lakes Assessment 2017: Quality Assurance Project Plan (EPA 841-B-16-003)

National Lakes Assessment 2017: Site Evaluation Guidelines (EPA 841-B- 16-001)

National Lakes Assessment 2017: Laboratory Operations Manual (EPA 841- 16-004)

These documents are available at: http://www.epa.gov/national-aquatic-resource-surveys/national-lakes-assessment.

1.2 Selection and Description of Survey Indicators

As part of the indicator selection process, USEPA and the NLA 2017 Steering Committee evaluated indicators used in NLA 2012, refined methodologies, and identified new indicators for NLA 2017. The Steering Committee, comprised of state representatives from each of the EPA regions, provided advice and recommendations to the Agency on matters related to the NLA 2017. Key screening and evaluation criteria included indicator applicability on a national scale, the ability of an indicator to reflect various aspects of ecological condition, and cost-effectiveness (e.g., Kurtz et al., 2001). EPA used the Committee's recommendations to refine methods and develop final documents.

The remainder of this section briefly describes the indicators that the NLA 2017 will use to assess trophic status, ecological integrity, human use value, and lake characteristics (Table 1.1). Some indicators provide a basis for evaluating more than one category. For example, an assessment of zooplankton allows for an examination of ecological integrity and trophic status, and to a certain extent, human use.

1.2.1 Trophic Status and Water Quality Indicators

Lakes are classified according to their trophic state. "Trophic" means nutrition or growth. A eutrophic ("well-nourished") lake has high nutrients and high plant growth. An oligotrophic lake has low nutrient concentrations and low plant growth. Mesotrophic lakes fall somewhere in between eutrophic and oligotrophic lakes.

Chlorophyll-a, total phosphorus, and Secchi disk transparency are most often used to estimate biomass and define the trophic state of a particular lake. Other variables are measured in conjunction with the trophic state variables to supplement and enhance understanding of lake processes that affect primary productivity.

1.2.1.1 Chlorophyll-a

Chlorophyll is the pigment that allows plants (including algae) to use sunlight to convert simple molecules into organic compounds via the process of photosynthesis. Of the several kinds of chlorophyll, chlorophyll-*a* is the predominant type found in green plants and algae. Measuring chlorophyll-*a* concentrations in water is a surrogate for actually measuring algae biomass and it is used to estimate trophic status.

1.2.1.2 Secchi Disk Transparency

A Secchi disk is a black and white patterned disk commonly used to measure the clarity of water based on the distance the disk can be seen when it is lowered into the water column. The Secchi disk measurement is used to estimate the euphotic zone depth in the field which is generally defined as two-times the Secchi disk depth.

1.2.1.3 Vertical Profile Measurements

Depth profiles for temperature, pH, and dissolved oxygen (DO) are taken with a calibrated water quality probe meter or multi-parameter probe sonde from the index site in each lake. This information is used to determine the extent of stratification and the availability of the appropriate temperature range and level of DO necessary to support aquatic life.

1.2.1.4 Water Chemistry and Associated Measurements

Water chemistry measurements are used to determine the acidic conditions, trophic state and nutrient enrichment, and water chemistry type.

1.2.2 Biological Indicators

Ecological integrity describes the ecological condition of a lake based on different assemblages of the aquatic community and their physical habitat (PHab). The indicators include zooplankton, benthic macroinvertebrates, sediment contaminants, and the physical habitat of the shoreline and littoral zones.

1.2.2.1 Benthic Macroinvertebrate Assemblage

Benthic macroinvertebrates are bottom-dwelling animals without backbones ("invertebrates") that are large enough to be seen with the naked eye ("macro"). Examples of macroinvertebrates include: crayfish, snails, clams, aquatic worms, leeches, and the larval and nymph stages of many insects, including dragonflies, mosquitoes, and mayflies. Populations in the benthic assemblage respond to a wide array of stressors in different ways so that it is often possible to determine the type of stress that has affected a macroinvertebrate assemblage (e.g., Klemm et al., 1990). Because many macroinvertebrates have relatively long life cycles of a year or more and are relatively immobile, the structure and function of the macroinvertebrate assemblage is a response to exposure of present or past conditions. For the NLA, the benthic macroinvertebrate assemblage occupying the littoral zone will be assessed, rather than the profundal assemblage occupying the deeper regions of lakes.

1.2.2.2 Zooplankton Assemblage

Zooplankton are animal microorganisms that consist of crustaceans (e.g., copepods and cladocerans), rotifers ("wheel-animals"), pelagic insect larvae (e.g., phantom midges), and aquatic mites. The zooplankton assemblage constitutes an important element of the food web, where zooplankton transfer energy from algae (primary producers) to larger invertebrate predators and fish. The zooplankton assemblage responds to environmental stressors such as nutrient enrichment and acidification (e.g.,

Stemberger and Lazorchak 1994, Dodson et al. 2005). The effects of these environmental stressors on zooplankton can be detected through changes in species composition, abundance, and body size distribution.

1.2.3 Physical Habitat Characterization

The characterization of shoreline and littoral zone (the nearshore areas of a lake) physical habitat (PHab) conditions serves three purposes. First, habitat information is essential to the interpretation of expected lake ecological condition in the absence of human disturbance (anthropogenic impacts). Second, the habitat evaluation is a reproducible, quantified estimate of habitat condition, serving as a benchmark against which to compare future habitat changes that might result from anthropogenic activities. Third, the specific selections of habitat information collected aid in the diagnosis of probable causes of ecological degradation in lakes.

In addition to information collected in the field by the shoreline and littoral surveys, the physical habitat description of each lake includes many map-derived variables such as lake surface area, shoreline length, and shoreline complexity. Furthermore, an array of information, including watershed topography and land use, supplements the physical habitat information. The shoreline and littoral characterization concentrates on information best derived "on the ground". As such, these results provide the linkage between large watershed-scale influences and those influences that directly affect aquatic organisms day to day. Together with water chemistry, the habitat measurements and observations describe the variety of physical and chemical conditions that are necessary to support biological diversity and foster long-term ecosystem stability. These characteristics of lakes and their shorelines are the very aspects that are often changed as a result of anthropogenic activities.

1.2.4 Human Use Indicators

Human use indicators address the ability of the lake population to support recreational uses such as swimming, fishing, and boating. The protection of these uses is one of the requirements of the Clean Water Act under Section 305(b). The extent of algal toxins (microcystins and cylindrospermopsin), bacteria (*E. coli*) and atrazine pesticides will serve as the primary indicators of human use.

1.2.4.1 Algal toxins (microcystins and cylindrospermopsin)

Cyanobacteria are microscopic organisms found naturally at low concentrations in freshwater systems. Under optimal conditions (such as high light and calm weather, usually in summer), these blue-green algae occasionally form blooms, or dense aggregation of cells, that float on the surface of the water. At higher concentrations, blooms may be so dense that they resemble bright green paint that has been spilled in the water. These blooms potentially affect water quality as well as human health (e.g., *Microcystis* can produce microcystin, a liver toxin) and natural resources. Decomposition of large blooms can lower the concentration of DO in the water, resulting in hypoxia (low oxygen) or anoxia (no oxygen) which may result in fish kills.

1.2.4.2 Bacteria (E.coli)

E. coli are bacteria whose presence indicates that water may be contaminanted by human or animal wastes. Microbes in these wastes can cause short term effects, such as diarrhea, cramps, nausea, headaches, or other symptoms. They may pose a special health risk for infants, young children, and people with severely compromised immune systems.

1.2.4.3 Atrazine Pesticide Screen

Atrazine pesticides are herbicides used to control the growth of weeds. Although applied to the land, these chemicals can enter lakes via transport in water (e.g., runoff, groundwater, etc.) or atmospheric transport. This screen will provide information about the occurrence and concentration of atrazine pesticides in water samples from lakes across the nation.

1.2.5 Other Indicators

1.2.5.1 Lake Characterizations

Observations and impressions about the lake and its surrounding catchment by field crews will be useful for ecological value assessment, development of associations and stressor indicators, and data verification and validation.

1.2.5.2 Dissolved Gases

Dissolved gases will be sampled for the purpose of informing the USEPA's research on the magnitude of methane, carbon dioxide, and nitrous oxide emissions from lakes and reservoirs in the U.S.

1.2.5.1 Fish Environmental DNA

A water sample will be collected and analyzed for fish environemtnal DNA (eDNA). This research measure will be used to explore indicator development for future NLAs.

1.2.5.2 Sediment Contaminants, TOC, and Grain Size

Lake sediments are often repositories of persistent chemical contaminants (e.g. heavy metals, PCB's, pesticides and herbicides) that enter lakes from their watersheds. These contaminants can be transferred to the food chain via uptake by organisms living in the sediments which can affect higher trophic levels such as fish, other wildlife, and humans. Sediment core samples will be obtained to measure sediment composition (e.g., grain size and percent moisture, organic content, etc.), and contaminant chemistry as an indicator of sediment quality.

Table 1.1 Summary table of indicators

Indicator Type	Indicator	Specifications/Location in Lake		
		Desktop Evaluation	**Index Site**	**Littoral Site**
Trophic Indicators	Vertical profile measurements (DO, Temperature, pH)		X	
	Secchi Disk transparency		X	
	Water chemistry (NH_4, NO_3), major anions and cations, alkalinity (ANC), DOC, TSS, silica, conductivity		Integrated water sample	
	Nutrients		Integrated water sample	
	Chlorophyll-*a* (and see human use)		Integrated water sample	
Biological	Benthic macroinvertebrate assemblage			10 stations
	Zooplankton assemblage (composition, structure, and size distribution)		Vertical tow (2 mesh sizes) through water column	
Physical Habitat	Physical habitat characterization			10 stations
Human use	Bacteria (*E. coli*)		Grab sample	
	Chlorophyll-*a* density		Integrated water sample	
	Phytoplankton (cyanobacteria)		Integrated water sample	
	Atrazine pesticide screen		Integrated water sample	
	Algal toxins (microcystins and cylindrospermopsin)		Integrated water sample	
Other Indicators (desktop, some field observations)	Lake area, basin morphometry, and characteristics of watershed	Using GIS		
	Sediment contaminants, TOC, and grain size		Sediment core	
	Dissolved gases (3 types)		Grab sample	
	Fish eDNA		Grab sample	

2.0 LOGISTICS

2.1 Roles and Contact Information

Effective communication between field crews, USEPA coordinators, and NLA 2017 contractor support staff is essential for the survey to proceed with maximum efficiency and to ensure collection of high quality data. This section provides:

- A general description of the roles of key NLA 2017 personnel in providing logistical and technical support to the field crews;
- Flow of communication between Field Crews and these individuals (i.e., *who to call for specific types of questions or support needs*); and
- Contact information.

The **EPA Headquarters (HQ) Project Management Team** consists of the Project Leader, Alternate Project Leaders, and Project QA Lead. The Team is responsible for overseeing all aspects of the project and ensuring technical and QA requirements are properly carried out. The Team is the final authority on all decisions regarding field sampling, site evaluation, site replacement, and laboratory analysis.

The **EPA Regional Coordinators** are the primary USEPA point of contact for Field Crews operating in their Region. Field Crews should direct all technical and logistical questions to their EPA Regional Coordinator, who will work with the EPA HQ Team to resolve the issue. Field Crews should also work with their EPA Regional Coordinator to schedule an **Assistance Visit** to occur within the first two weeks of field sampling. An Assistance Visit is part of the QA component of the NLA 2017 QAPP. To meet the requirements of the QAPP, each Field Crew will allow an EPA employee or contractor to observe that crew sampling for one day. The Assistance Visit is used to confirm the protocols are implemented as intended and to suggest corrective actions, if needed, to the Field Crew's sampling approach.

The **Information Management (IM) Coordinator** provides the Field Crews with packets of forms and labels for each site scheduled to be sampled. Crews will request these packets through a fillable PDF request form. The IM Team also tracks the transition of each NLA 2017 sample from the field to the laboratory.

The Contract **Field Logistics Coordinator (FLC)** is responsible for tracking the Field Crew's sampling activities and overall progress throughout the field season, ensuring that requests for supplies and equipment are filled, and assisting Field Crews with questions concerning field logistics, equipment, and supplies as they arise during the field season. The FLC will also review submitted status and tracking forms to ensure that the correct samples have been taken and that those samples are being sent to the laboratories in an appropriate timeframe.

Table 2.1 Personnel to call for specific types of questions and support needs.

Personnel	Call
EPA Regional Coordinators	First, to ask any questions about NLA, including questions on field protocols Grant questions Schedule Field Assistance Visit
EPA HQ Project Management Team	Ask questions about site access, site evaluation, and site replacement Ask questions about shipping locations and sample

	handling procedures Ask questions about Field Methods Ask questions about Survey Design Ask questions about QA procedures Ask questions about Laboratory Methods If you can't reach Regional Coordinator, IM Coordinator, or Field Logistics Coordinator If you are unsure who to call
Personnel	**ONLY Call**
Information Management (IM) Coordinator	Order field forms or site kits Submit a status report Notify EPA about change in sampling schedule Ask questions about submitting data packet If EPA Regional Coordinator directs you to them
Contract Logistics Coordinator	Order replacement items for site kits, base kits, or miscellaneous supplies Ask questions about shipping contract, or to order more shipping forms If EPA Coordinator directs you to them If you can't reach an EPA HQ or Regional Coordinator and it is an urgent question

Table 2.2 Contact information

Title	Name	Contact Information
EPA HQ Project Lead	Amina Pollard, OW	pollard.amina@epa.gov 202-566-2360
EPA HQ Project QA Lead	Sarah Lehmann, OW	lehmann.sarah@epa.gov 202-566-1379
HQ Logistics Lead	Brian Hasty, OW	hasty.brian@epa.gov 202-564-2236
Contract Field Logistics Coordinator	Chris Turner, Great Lakes Environmental Center, Inc.	cturner@glec.com 715-829-3737
Information Management (IM) Coordinator	Marlys Cappaert, SRA International Inc.	Cappaert.marlys@epa.gov 541-754-4467
Regional EPA Coordinators	Hilary Snook, Region 1	snook.hilary@epa.gov 617-918-8670
	Jim Kurtenbach, Region 2	kurtenbach.james@epa.gov 732-321-6695
	Frank Borsuk, Region 3 William Richardson, Region 3	borsuk.frank@epa.gov 304-234-0241 richardson.william@epa.gov 215-814-5675
	Chris McArthur, Region 4	mcarthur.christopher@epa.gov 404-562-9391
	Mari Nord, Region 5	nord.mari@epa.gov 312-886-3017
	Rob Cook, Region 6	cook.robert@epa.gov

		214-665-7141
	Gary Welker, Region 7	welker.gary@epa.gov 913-551-7177
	Kris Jensen, Region 8 Jeff McPherson, Region 8	jensen.kris@epa.gov 303-312-6237 mcpherson.jeffery@epa.gov 303-312-7752
	Janet Hashimoto, Region 9 Matthew Bolt, Region 9	hashimoto.janet@epa.gov 415-972-3106 Bolt.matthew@epa.gov 415-972-3578
	Lil Herger, Region 10	herger.lillian@epa.gov 206-553-1074

2.2 Key Information and Materials

2.2.1 Site Maps

Geospatial files in the form of Google Earth (i.e., .kmz) and geographic information system (GIS) shapefiles have been provided to assist in the site evaluation process. From these files, crews should generate their own site maps with relevant information displayed. If preferred, crews can request hard copy site maps from EPA. These maps will include: an aerial image, topographic map, and road map. The site maps will be helpful in the planning and preparation for visiting and sampling a particular NLA 2017 site. These maps will become part of your site packet. See more information on the site packet in Section 4.1.

2.2.2 Forms (Paper or Electronic)

Forms are the key to data collection and tracking for the NLA 2017. For NLA 2017 we have developed electronic forms as well as paper forms. These electronic forms should streamline data collection. Field crews will have the option of using paper or electronic forms.

2.2.2.1 Field Forms

Field forms are the primary documents where crews will record measures, observations, and collection information during the course of the field day. Additional information regarding specifics of data entry is contained in Section 3.2.

- **Paper Field Forms:** Crews that have elected to use paper field data collection will request a paper field form packet for each site which will be provided by the NARS (National Aquatic Resource Surveys) IM Coordinator. Crews will need to add these forms to the Site packet (see Section 4.1) prior to going in the field. After a site is sampled, the completed NLA 2017 paper field forms are checked for completeness and organized sequentially into a Data Packet. The Data Packets from several sites are batched together and sent every one to two weeks to the NARS IM Center and are accompanied by a Tracking - Packets form (see Section 2.2.2.2) to track which data packets have been shipped. Extra paper field forms will be provided to field crews to serve as backup copies in case of lost forms or problems with electronic field forms (see below). In extraordinary circumstances, paper field forms may be provided as fillable PDFs.

- **Electronic Field Forms:** This form of data collection can be collected through an Apple iOS platform portable electronic device (e.g., tablet, phone, etc.). This data collection method will

require a field crew to download or install the developed Application (or "App") onto the device. The electronic field forms will be optimized for tablet devices. Once downloaded and the App launched, each of the field forms are separated into their own sections for easier data entry. It is important for a field crew to familiarize themselves with the App prior to field sampling. In addition, field crews should note that all data must be submitted through one device (even if multiple devices are used in the field). While a data or Wi-Fi connection is required to submit the data, no data connection is required for the data collection process.

2.2.2.2 Tracking Forms

Tracking forms describe the status and location of all samples collected during NLA 2017. Field crew leaders will transmit these forms electronically to the NARS IM Center at specified times and will place hard copies of the pertinent forms in the shipping containers with the samples. See APPENDIX B: SHIPPING GUIDELINES for more information.

- **Site and Sample Status/Water Chemistry Lab Tracking Form**: Transmitted within 24 hours of sampling or visiting a site with the intent to sample to report on the status of the site (e.g., sampleable or not), to record the Sample ID numbers, and to indicate the status of all samples collected at the site (immediate shipment and batched shipments). This also serves as the tracking form for samples shipped to the water chemistry laboratory.
- **Tracking – Daily Shipped Samples Tracking Form:** Accompany chilled daily samples that are shipped within 24 hours of sampling. This form indicates which samples are being shipped and lists the Sample ID numbers for all samples packed in the shipping container. It is included in the shipping package and is also transmitted electronically to the NARS IM Center.
- **Tracking – Batched Samples:** Accompany samples that are batched together from multiple sites and shipped every one or two weeks. Whenever batched samples are shipped to their designated laboratory for analysis, the appropriate tracking form(s), which list the Sample ID numbers for all samples packed in a shipping container, is included in the shipping package and is also transmitted electronically to the NARS IM Center.
- **Tracking – Packets:** Accompany packets that are batched together from multiple sites and shipped every one or two weeks. These packets are sent to the NARS IM Center.

2.2.3 Equipment and Supplies

2.2.3.1 Request Form

Field Crews will submit requests for field forms, labels, and site kits via an electronic Request Form. This form will be submitted to the NARS IM Coordinator who will ensure that the request reaches the appropriate entity. Crews must submit basic sampling information (i.e., tentative start date and number of sites crews are planning to sample) to the FLC at or before the time of submitting request forms. Crews should submit the Request Form at least two weeks prior to their desired sampling date.

The Request Form will be in fillable PDF format. Users must enter the required information, save a copy of the form to their computer or device and attach the updated copy to an email to sampletracking@epa.gov. The IM Team will send email notification that the request has been received.

2.2.3.2 Base Kit

The Base Kit is comprised of the subset of durable equipment and supplies needed for NLA 2017 sampling and is provided by USEPA through the FLC. Typically one Base Kit is provided to each Field

Crew and contains some of the equipment that is used throughout the field season. SeeAPPENDIX A: EQUIPMENT & SUPPLIES for a list of the items provided by USEPA in the Base Kit. USEPA anticipates that Base Kitequipment will be available for use in future NLA efforts.

2.2.3.3 Site Kit

A Site Kit contains the subset of consumable supplies (i.e., items used up during sampling or requiring replacement after use) provided by USEPA through the FLC. The site kit will contain all the sample bottles necessary for sampling a single lake. A new Site Kit should be requested for each site sampled, and crews should consider having at least one additional site kit available as a spare should any supplies be lost See APPENDIX A: EQUIPMENT & SUPPLIES for the consumable items that will be provided by USEPA.

2.2.3.4 Field Crew Supplied Items

The field crew will also supply particular items for the field sampling day. These items might include supplies from the NLA 2007 or NLA 2012, typical field equipment (like a global position system (GPS) receiver or multi-paramter probe), or boat equipment. See APPENDIX A: EQUIPMENT & SUPPLIES for the items that the field crew will need to provide.

2.2.4 Other Resources

The complete documentation of overall project management, design, methods and standards, and QA/QC measures is contained in this document and companion documents (listed in NOTICE and described below). The NLA 2017 participants must agree to follow the QAPP, including the protocols and design, and the associated documents – the NLA 2017 FOM, LOM, and SEG.

2.2.4.1 Quick Reference Guide

Field crews will receive a NLA 2017 QRG containing tables and figures summarizing field activities and protocols from the NLA 2017 FOM. The QRG is meant to be used in the field to give NLA 2017 Field Crews a list of the required sampling protocols. While comprehensive, the steps contained in the QRG are not as detailed as the descriptions found within the NLA 2017 FOM. The user is assumed to have attended Field Training and completely read and understood the FOM before using this QRG at a field site. This waterproof handbook will be a field reference used by field crews after completing a required field training session. The field crews are also required to keep the FOM available in the field for reference and for possible protocol clarification.

2.2.4.2 Site Evaluation Guidelines

The NLA 2017 SEG (EPA 841-B-16-001) outlines the process to compile the final list of candidate lakes for sampling. The process includes locating a candidate lake, evaluating the lake to determine if it meets the criteria for inclusion in the target population and is accessible for sampling, and if not, replacing it with an alternate candidate lake.

2.2.4.3 Quality Assurance Project Plan

Large-scale and/or long-term monitoring programs such as those envisioned for national surveys and assessments require a rigorous QA program that can be implemented consistently by all participants throughout the duration of the monitoring period. QA is a required element of all EPA-sponsored studies that involve the collection of environmental data (USEPA 2000a, 2000b). Field crews will be provided a copy of the NLA 2017 QAPP (EPA 841-B-16-003) and the field crew leader is required to sign the QAPP signature page prior to beginning field sampling activities. The QAPP contains more detailed

information regarding QA/QC activities and procedures associated with general field operations, sample collection, measurement data collection for specific indicators, and data reporting activities. For more information on the project level QA procedures, refer to the NLA 2017 QAPP.

2.2.4.4 Laboratory Operations Manual

The methods used for the laboratory sample analysis is available in the NLA 2017 Laboratory Operations Manual (LOM) (EPA 841-B-16-004).

3.0 DAILY FIELD ACTIVITIES SUMMARY

This section presents a general overview of the activities that a field crew conducts during a typical 1-day sampling visit to a lake. The following sections include general guidelines for health and safety, recording data, and using standardized field data forms and sample labels.

3.1 Health and Safety

Collection and analysis of samples can involve risks to personal safety and health, and **the safety of the field crew must always be the primary consideration during sampling**. This section describes general safety considerations, some safety equipment, and safety guidelines for field operations.

This section does not substitute for an official Health and Safety Plan. The crew MUST ALWAYS carefully follow the protocols in their Health and Safety Plan for the NLA field work that was approved by the state, tribe, or other organization with which the field crew is affiliated. The crew should carry a copy of this approved Health and Safety plan in the field.

3.1.1 General Considerations

It is the responsibility of the group safety officer or project leader to ensure that the necessary safety courses are taken by all field personnel and that all safety policies and procedures are followed. Each state, tribe, or other organization must have a specific safety plan for the sampling the NLA sites, including a communications plan that addresses safety and emergency situations. The plan should have a daily check-in procedure for field personnel, and emergency contacts for police, ambulance, fire departments, hospitals, and search and rescue personnel. Important considerations related to field safety are listed below. It is the responsibility of the group safety officer or project leader to ensure that the necessary safety courses are taken by all field personnel and that all safety policies and procedures are followed. Sources of information regarding health and safety considerations and/or safety-related training include the American Red Cross (http://www.redcross.org/m/phssmrd/take-a-class), the National Institute for Occupational Safety and Health (1981) (see the most recent revisions at https://www.cdc.gov/niosh/docs/81-123/, and the U.S. Coast Guard (http://www.uscgboating.org/recreational-boaters/boating-safety-courses.php).

3.1.1.1 Recommended Training

- First aid;
- Cardiopulmonary resuscitation (CPR);
- Vehicle safety (e.g., operation of 4-wheel drive vehicles, trailer towing and maneuvering);
- Boating and water safety;
- Field safety (weather, personal safety, orienteering, site reconnaissance prior to sampling);
- Equipment design, operation, and maintenance; and
- Handling of chemicals and other hazardous materials.

3.1.1.2 Communications

A communications plan to address safety and emergency situations is essential. All field personnel need to be fully aware of all lines of communication. Field personnel should have a daily check-in procedure with their supervisor for safety. An emergency communications plan should include contacts for police, ambulance, fire departments, hospitals, and search and rescue personnel. Below are some items to

address:

- Check-in schedule;
- Sampling itinerary (vehicle used & description, time of departure & return, and travel route);
- Contacts for police, ambulance, hospitals, fire departments, and search and rescue personnel;
- Emergency services available near each sampling site and base location; and
- Cell (or satellite) phone number, if possible.

3.1.1.3 Personal Safety

Proper field clothing should be worn to prevent hypothermia, heat exhaustion, sunstroke, drowning, or other dangers. Field personnel should be able to swim, and a personal flotation device (PFD) must be used. Chest waders made of rubberized or neoprene material and suitable footwear must always be worn with a belt to prevent them from filling with water in case of a fall. Below are some personal safety items to address:

- Field clothing and other protective gear including lifejackets for all crew members;
- Medical and personal information (allergies, personal health conditions, and required medications);
- Personal contacts (family, telephone numbers, etc.); and
- Physical exams and immunizations.

Many hazards lie out of sight in the bottoms of lakes, rivers, and streams. Broken glass or sharp pieces of metal embedded in the substrate can cause serious injury if care is not exercised when walking or working with the hands in such environments. Infectious agents and toxic substances that can be absorbed through the skin or inhaled may also be present in the water or sediment. Personnel who may be exposed to water known or suspected to contain human or animal wastes that carry causative agents or pathogens must be immunized against tetanus, hepatitis, typhoid fever, and polio. Biological wastes can also be a threat in the form of viruses, bacteria, rickettsia, fungi, or parasites.

Lakes and surrounding landscapes can be home to dangerous organisms. Field crews should take care to minimize contact with biting insects, bees, poisonous snakes and dangerous animals. Insect repellent and protective clothing will help to limit exposure. At the end of each field day, workers should inspect their bodies for ticks. Any person allergic to bee stings, other insect bites, or plants (i.e., poison ivy, poison oak, poison sumac, etc.) should take proper precautions and have any needed medications on hand. In addition, field crew members should always be aware of their surroundings to protect themselves from dangerous animals, such as alligators, mountain lions, bears, and wolves.

3.1.1.4 Sampling Equipment

Field crew members should be familiar with hazards associated with the use of sampling equipment and establish appropriate safety practices prior to their use. They must ensure that all equipment is in safe working condition.

Because boats are used to access NLA sampling sites, personnel must be trained in operating the type of boat in use incouding appropriate state or other certifications. Personnel must consider and prepare for hazards associated with the operation of motor vehicles, boats, winches, tools, and other incidental equipment. Boat operators should be familiar with U.S. Coast Guard rules and regulations for safe boating contained in a pamphlet, *Federal Requirements for Recreational Boats*, available from a local U.S. Coast Guard Director or Auxiliary or State Boating Official and online (U.S. Coast Guard,

https://www.uscgboating.org/images/420.PDF). All boats with motors must have fire extinguishers, boat horns, life jackets or flotation cushions, and flares or communication devices.

3.1.2 Safety Equipment

Appropriate safety apparel such as life jackets, waders, gloves, safety glasses, etc. must be available and used when necessary. First aid kits, fire extinguishers, and blankets must be readily available in the field. Cellular or satellite telephones and/or portable radios should be provided to field crews working in remote areas for use in case of an emergency. Supplies such as anti-bacterial soap and an adequate supply of clean water or ethyl alcohol must be available for cleaning exposed body parts that may have been contaminated by pollutants in the water or sediments.

3.1.2.1 Safety Guidelines for Field Operations

General safety guidelines for field operations are presented below.

Personnel participating in field activities on a regular or infrequent basis should be in sound physical condition and have a physical examination annually or in accordance with Regional, State, or organizational requirements.

All surface waters and sediments should be considered potential health hazards due to potential toxic substances or pathogens. Persons must become familiar with the health hazards associated with using chemical fixing and/or preserving agents. Chemical wastes can be hazardous due to flammability, explosiveness, toxicity, causticity, or chemical reactivity. All chemical wastes must be discarded according to standardized health and hazards procedures (e.g., National Institute for Occupational Safety and Health [1981]; for the most recent revisions see https://www.cdc.gov/niosh/docs/81-123/;.)

During the course of field research activities, field crews may observe violations of environmental regulations, may discover improperly disposed hazardous materials, or may observe or be involved with an accidental spill or release of hazardous materials. In such cases it is important that the proper actions be taken and that field personnel do not expose themselves to something harmful. The following guidelines should be applied:

1. First and foremost, protect the health and safety of all personnel. Take any necessary steps to avoid injury or exposure to hazardous materials. If you have been trained to take action such as cleaning up a minor fuel spill during fueling of a boat, do it. However, you should always err on the side of personal safety.
2. Field personnel should never disturb or retrieve improperly disposed hazardous materials from the field to bring back to a facility for "disposal." To do so may worsen the impact, may incur personal liability or liability for the crew members and their respective organizations, may cause personal injury, or may cause unbudgeted expenditure of time and money for proper treatment and disposal of the material. However, it is important not to ignore environmental incidents. Notify the proper local, state, and/or federal authorities of any incident of this type so that they may take the necessary actions to properly respond to the incident.
3. For most environmental incidents, the following emergency telephone numbers should be provided to all field crews: State or Tribal department of environmental quality or protection, U.S. Coast Guard, and the U.S. EPA regional office. In the event of a major environmental incident, the National Response Center may need to be notified at 1-800-424-8802.

Specific Safety Guidelines are below:

- Two persons must be present during all sample collection activities, and no one should be left alone while in the field.

- Minimize exposure to lake water and sediments as much as possible. Use gloves when necessary, and clean exposed body parts as soon as possible after contact.
- All electrical equipment must bear the approval seal of Underwriters Laboratories (UL) and must be properly grounded to protect against electric shock.
- Use heavy gloves when hands are used to agitate the substrate during collection of benthic macroinvertebrate samples.
- Use appropriate protective equipment (e.g., gloves, safety glasses, specialized garments, etc.) when handling and using hazardous chemicals.
- Persons working in areas where venomous snakes may be encountered must check with the local Drug and Poison Control Center for recommendations on what should be done in case of a bite from a venomous snake.
- Any person allergic to bee stings, other insect bites, or plants (i.e., poison ivy, oak, sumac, etc.) must take proper precautions and have any needed medications handy (e.g., an "Epi-Pen").
- Protect yourself against the bite of deer or wood ticks because of the potential risk of acquiring pathogens that can cause Rocky Mountain spotted fever, Lyme disease, and other diseases.
- Be familiar with the symptoms of hypothermia and know what to do in case symptoms occur. Hypothermia can kill a person at temperatures much above freezing (up to 10°C or 50°F) if he or she is exposed to wind or becomes wet.
- Be familiar with the symptoms of heat/sun stroke and be prepared to move a suffering individual into cooler surroundings and hydrate immediately.
- Handle and dispose of chemical wastes properly. Do not dispose of any chemicals in the field.

3.2 Recording Data and Other Information

All samples need to be identified and tracked, and associated information for each sample must be recorded. It is imperative that field and sample information be recorded accurately, consistently, and legibly. The cost of a sampling visit coupled with the short index period severely limits the ability to resample a lake if the initial information recorded was inaccurate or illegible. As mentioned in Section 2.2.2, there are two forms for collecting sample data: paper field forms and electronic field forms. Whichever format your field crew chooses to utilize, see below for important information pertaining to data entry for each of these forms.

3.2.1 Paper Field Forms

The NLA 2017 data and tracking forms are formatted so that the data you record can be scanned into a data entry system. It is important that field data and sample information are recorded accurately, consistently, and legibly. General guidelines for recording field measurements are presented in Table 3.1. More detailed instructions for filling out specific forms are provided in each protocol chapter of this manual.

- **Official Data Forms** – The NARS IM Center will provide all forms for use in the NLA 2017. These forms will be provided in the **Field Form Packet** for each site. It is important to use only the forms provided by the NARS IM Team and not photocopies or other printouts, because they are formatted to be read by the digital data scanners. **Data not recorded on the official NLA 2017 Field Forms are unusable**.
- **Site Number, Date, and Page Numbers:** Field forms will arrive without any site information completed, and each field crew must complete the header area of each page with the

appropriate information (e.g., site ID, Date, Crew ID, etc.). If any of this information is incorrect or omitted, it may be impossible to connect data or samples to a particular site, resulting in lost data.

- The Site ID is NOT preprinted on the forms or on the labels and tags.
- The Sample ID numbers ARE preprinted on sample labels and tags as well as tracking forms provided by the IM Team. Thus, it is vital to ensure that you correctly enter the Sample ID numbers in the correct areas on the field forms. It is also essential that you correctly enter the Site ID and Date on the labels and tags, along with any other required information for the specific sample.
- Record the date sampling is initiated wherever it is requested.

- **Form Instructions** – Carefully follow all instructions on each data form. If you have questions not answered by the form instructions, consult the appropriate protocol chapter for more detailed information about how to record data for a particular form.
- **Confirmation Bubbles** – Most NLA 2017 forms have confirmation bubbles to indicate the meaning of blank data fields or unfilled data bubbles. Read these statements carefully and fill in the bubbles as requested to confirm exactly what empty data fields or unfilled data bubbles on a particular form mean. Completing the confirmation bubbles is critical to note that a data element was not observed at the site, rather than overlooked by the Field Crew.
- **Data Flags and Comments** – There is space on all forms to flag data for which additional information or explanation may be needed.

Table 3.1 Guidelines for recording field measurements and tracking information.

ACTIVITY	GUIDELINES
Field Measurements	
Data Recording	• Record observations and measurement values only on official NLA paper field forms (water-resistant) or electronic field forms. • Use a writing instrument that leaves clear, dark text (e.g., a No. 2 pencil for field forms or a water and smear proof fine-point indelible marker for labels, as appropriate) to record information. • If you make an error when recording data and changes are required, it is best to cross out the error with a single horizontal line and rewrite the correct information. Use a flag if there isn't enough room in the data field and write the correct information in the comments section. • Complete all header information and record all data and sample ID information requested on each form. • Use the correct Crew ID that was assigned during field training. • Use the formats specified. • Print legibly (and as large as possible). Clearly distinguish letters from numbers (e.g., 0 versus O, 2 versus Z, 7 versus T or F, etc.), but do not use slashes (i.e., lines drawn through the character). Printing in capital letters enhances legibility. • For data that is recorded by filling in a data bubble, be certain to keep markings inside the circle while completely filling the bubble. • When recording comments, print **legibly**. Make notations in comments field only; avoid marginal notes. Be concise, but avoid using abbreviations or "shorthand" notations. If you run out of space, attach a sheet of paper with the additional information, rather than trying to squeeze everything into the space provided on the form. • Do not doodle on the forms, including the margins.

ACTIVITY	GUIDELINES
Data Qualifiers (Flags)	• Use only defined flag codes and record on data form in appropriate field. K = No measurement or observation made. U = Suspect measurement; re-measurement not possible. F_n = Miscellaneous flags (*n*=1, 2, etc.) assigned by a field crew during a particular sampling visit. • Explain reason for using K or U flags and define F_n flag in the comments section of the data form. Ensure the F_n numbers are unique on the data form and matched to the flag definition. F_n flags and definitions are not linked from one form to the next, so definitions need to be rewritten on each sheet whenever necessary.
Sample Collection	
Sample Labels and Tags	• Use a writing instrument that leaves clear, dark text to record information (e.g., a No. 2 pencil on paper tags or a water or smear proof fine-point indelible marker on adhesive labels). • Use the sample-type appropriate adhesive labels with preprinted Sample ID numbers for each sample. Be sure to fill in any requested information about the sample on the sample label and affix it to the outside of the sample container. Cover completed labels with clear tape. • Place a waterproof paper tag inside each benthic macroinvertebrate collection jar with the required information written with a No. 2 lead pencil.
Sample Collection Information	• Record that each sample has been collected on the appropriate data form. Be sure to record the Sample ID number from labels and tags in the appropriate fields on the data forms using the format requested on each data form.
QA and Tracking	
Before Leaving Site: Review of Data Forms and Comparison of Sample Labels and Data Forms	• Review all data forms for accuracy, completeness, and legibility. • Review all sample labels for accuracy, completeness, and legibility. • Verify that the information recorded on the sample labels and tags is consistent with all Sample IDs listed on all data forms and on the tracking form. • Confirm that the forms have been reviewed by recording your initials in the "Reviewed by" field in the upper right corner of each form.
Before Shipping Data Packets and Samples: Review of Data Packets, Sample Labels and Tracking Forms	• The Field Crew Leader must review the completed Data Packet before its transfer to the NARS IM Center to ensure it is complete and all data forms are consistent, correct, and legible. • Complete all tracking forms required for all samples being shipped. Review tracking forms for consistency, correctness, and legibility. • Compare labels and tags on samples with the Sample IDs recorded on the tracking form for accuracy, completeness, and legibility before shipping samples and transmitting the tracking forms to the NARS IM Center.

3.2.2 Electronic Field Forms

Many of the above guidelines will be followed for Electronic field forms. Additional data checks are built into the App which will not allow particular data to be entered if certain conditions are not met. Despite those checks, it is important to note that the field crew is responsible for the data entered and it must be careful to make sure the data entered is accurate for the site.

3.3 Sampling Scenario

Field methods for the NLA 2017 are designed to be completed in one field day for most lakes. Depending on the time needed for both the sampling and traveling for that day, an additional day may

be needed for pre-departure and post-sampling activities (e.g., cleaning equipment, repairing gear, shipping samples, and traveling to the next lake). Remote lakes with lengthy or difficult approaches may require more time to gain access to the lake, and field crews will need to plan accordingly.

A field crew typically will consist of at least two people. Two people are always required in the boat together to execute the sampling activities and to ensure safety. Any additional crew members may either remain on shore to provide logistical support or be deployed in a second boat to assist in data collection. Figure 3.1 and Figure 3.2 present a daily field sampling scenario showing how the work load may be split between crew members. Each field crew should define roles and responsibilities for each crew member to organize field activities efficiently. Minor modifications to the sampling scenario may be made by crews; however, the sequence of sampling events presented in Figure 3.1 cannot be changed and is based on the need to protect some types of samples from potential contamination and to minimize the possibility of holding time exceedance once samples are collected. The following sections further define the sampling sequence and the protocols for sampling activities.

***NOTE**: When sampling large lakes (lakes > 10,000 hectares), field crews may omit the physical habitat and benthic macroinvertebrate sampling efforts altogether.*

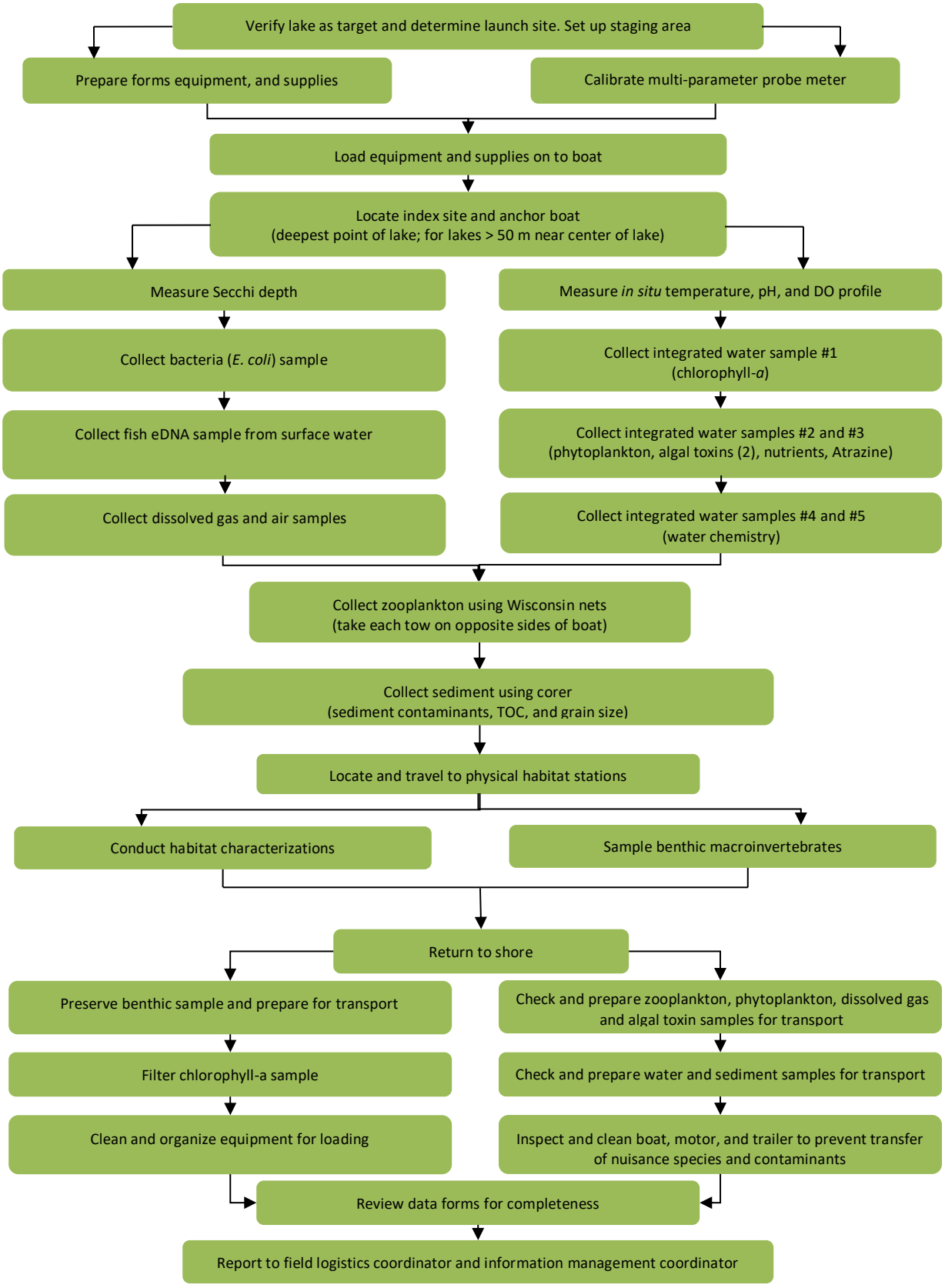

Figure 3.1 Daily operations summary

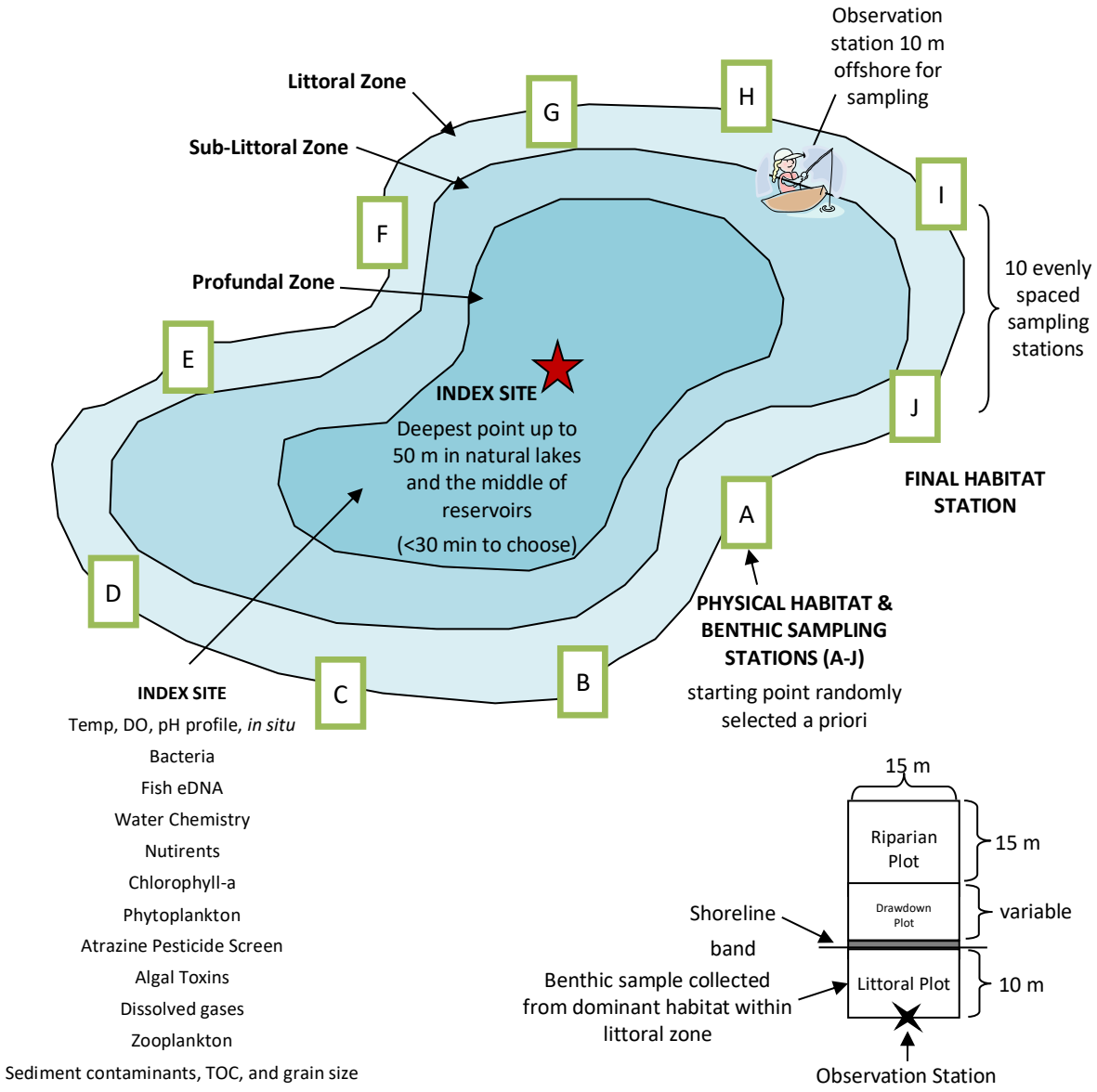

Figure 3.2 Location of sample collection points and physical habitat (PHab) stations.

The field crew arrives at the lake in the early morning to complete the sampling in a single day. The sampling sequence is to:

1. verify lake, calibrate equipment, locate and travel to the index site;
2. conduct depth profile measurements of DO, temperature, and pH;
3. take Secchi disk transparency depth measurement;
4. collect bacteria sample;
5. collect fish eDNA sample;
6. use the integrated sampler to collect water chemistry, chlorophyll-*a*, Atrazine pesticide screen, algal toxin, nutrient, and phytoplankton samples;
7. collect dissolved gas samples;

8. collect zooplankton samples;
9. collect sediment core sample for sediment contaminants, TOC, and grain size;
10. conduct physical habitat characterization around the margin of the lake at ten littoral zone stations (A,B,C,D,E,F,G,H,I,J);
11. collect benthic samples at ten littoral zone stations (A,B,C,D,E,F,G,H,I,J) concurrent with physical habitat characterization;
12. filter chlorophyll-*a* sample;
13. preserve and prepare all samples for shipment;
14. review field forms (electronic or paper);
15. report sampling event; and
16. ship time-sensitive samples (water chemistry, nutrients, chlorophyll-*a*, and bacteria samples).

4.0 BASE SITE ACTIVITIES

Field crews are to conduct a number of activities at their base site (i.e., office or laboratory, camping site, or motel). These include tasks that must be completed both before departure to the lake site and after return from the field (Figure 4.1). Close attention to these activities is required to ensure that the field crews know:

- where they are going;
- that access is permissible and possible;
- that equipment and supplies are available and in good working order to complete the sampling effort; and
- that samples are packed and shipped appropriately.

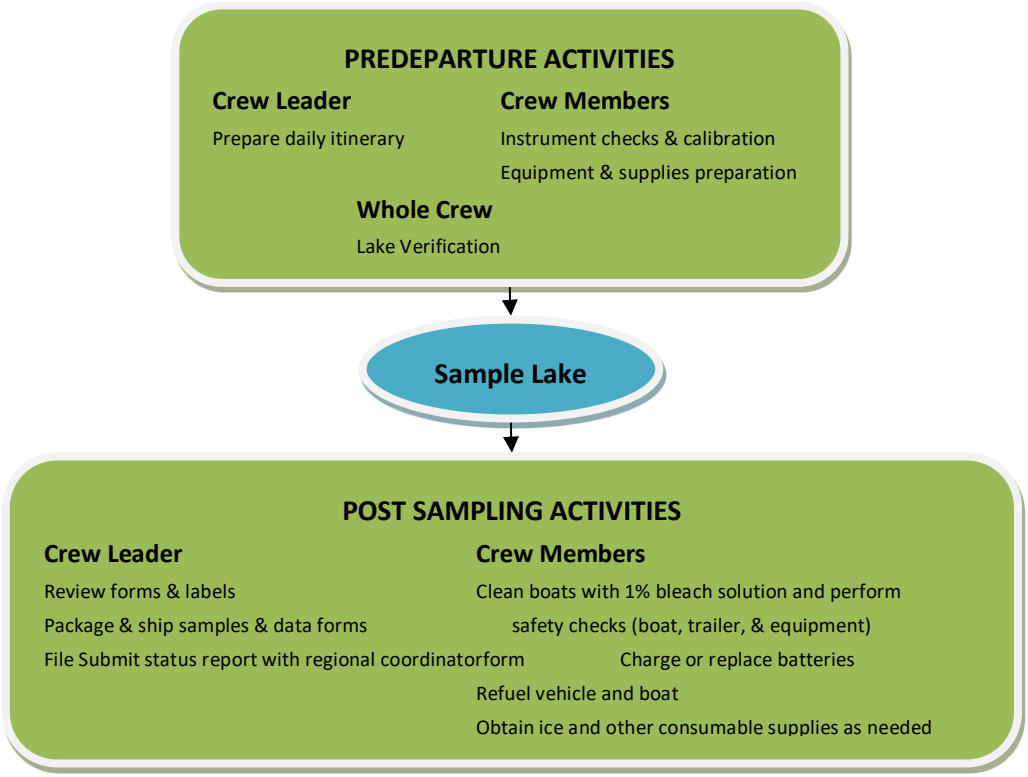

Figure 4.1 Overview of base site activities

4.1 Pre-departure Activities

Pre-departure activities include developing daily itineraries, checking and calibrating instruments, and preparing equipment and supplies. Procedures for these activities, which will take place at your office or laboratory, camping site, or motel, are described in the following sections.

4.1.1 Daily Itineraries and Site Packets

The Field Crew Leader is responsible for developing daily itineraries and a site packet. A site packet contains information key to the planning and preparation for visiting and sampling a particular NLA site. Development of site packets should have been initiated during site evaluation and reconnaissance (See

NLA 2017 Site Evaluation Guidelines); however, the field crew may need to gather additional information for the site packet during preparation for the sampling visit. Also, it is the responsibility of the field crew to obtain access permissions and any needed permits as part of developing the site packet.

Prior to a field crew traveling to a NLA site for sampling, the information for the site packet must be gathered and reviewed. Site packet development entails compiling maps, contact information, copies of permission letters, and access instructions. The Field Crew Leader must be sure to lay out the physical habitat (PHab) stations on a site map before the sampling day (see Section 6.1.3). Additional activities include confirming the best access routes, calling the landowners or local contacts, and confirming lodging plans. The site packet may include the following documents:

- **Field forms:** Paper or electronic with paper back up
- **Site maps**: Generated by crew or provided by USEPA HQ Team, see section on Site Maps.
- **Other Maps, Imagery, or GIS Data:** Any other maps, aerial photos, GIS data, or sources of information compiled by Field Crews and/or their partners that could be helpful to sampling the NLA sites.
- **Land Ownership Status, Requirements and Permissions for Access:**
    - Landowner identity and contact information.
    - Results of communication with landowners.
    - Documentation of permission to access private land.
    - Permissions for crossing private lands to reach sites located on public lands.
    - For public land, response of relevant agency to notification that you will be accessing a site, and, if needed, permissions to do so.
- **Permits:** Any permits or documentation required for site access, or for data collection activities or sample/specimen collection.
- **Information for Accessing the Site:**
    - Contact information for landowners.
    - Notes about whether landowner(s) want to be informed when Field Crew is on site.
    - Contact information for individuals who must be available to open gates or allow entry to a site, and the time and location for meeting them.
    - Notes on locked gates, pets, livestock, or other things that could impede access.
    - Notes about active hunting, farming, mining, or other activities on or near the site.
    - Current conditions that could prevent access (e.g., high water, forest fires, road closures, etc.).
- **Site Evaluation Notes:**
    - Site Evaluation notes, annotated aerial photos, sketch map, and completed site evaluation form that can aid with planning for accessing or sampling a site.
- **Driving and Hiking Routes to the Site:**
    - Detailed driving directions may be obtained from the NLA Google Earth files.
    - Results from the Site Evaluation may include driving directions and notations about site access or logistically challenging conditions on the site, which can be useful in relocating the site or helpful in anticipating special circumstances.

- **Preliminary Plan for Establishing Physical Habitat Stations:** As part of the base location activities to prepare for field work, review aerial photos and maps of the site and make a plan for laying out the PHab stations. This plan should be included in the site packet.
- **Any other site specific information (e.g., road construction and road closings) useful to the Field Crew.**

4.1.2 Instrument Checks and Calibration

Test and calibrate instruments prior to sampling. You can calibrate instruments and probes prior to departure for the lake site or at the lake, with the exception of the DO probe. Because of the potential influence of altitude, calibrate the DO probe at the lake site (NOTE: some newer instruments may allow for calibration independent of altitude). Field instruments include a multi-parameter probe unit for measuring temperature, DO, and pH and a GPS receiver. Field crews should have access to backup instruments if any instruments fail the manufacturer performance tests or calibrations.

4.1.2.1 Depth sounding equipment

Crews are responsible for checking the accuracy of their devices against a sounding line at the beginning of the field season to verify that the device is providing accurate depth information.

4.1.2.2 Multi-parameter probe Meter Performance Test

Test and pre-calibrate the multi-parameter probe meter prior to departure from the base site, following either the Standard Operating Procedure (SOP) developed for the instrument or the manufacturer's calibration and maintenance procedures. Field crews should perform a QC check of the pH meter calibration (and conductivity meter calibration, if this optional measurement is taken) at regular intervals designated by the manufacturer. Field crews will be responsible for preparing or purchasing their own QC solution.

4.1.2.3 Global Positioning System Use and Battery Check

A GPS unit is used to locate the launch, index site and each of the physical habitat stations. Therefore, it is imperative that the Field Crew understands how to operate their GPS unit.

The Global Positioning System (GPS) uses signals sent from multiple orbiting satellites to a ground-based sensor in order to fix a position on the earth. GPS uses signals sent from multiple orbiting satellites to a ground-based sensor in order to fix a position on the earth. Position accuracy depends on the Position Dilution of Precision (PDOP) which is a measure of the geometry of the satellite spread over the location of the observer. Low PDOP values are typically conveyed to the user as a measure of accuracy or precision and represent more advantageous satellite geometry and therefore less locational error. For NLA, crews should regularly monitor the accuracy reading on their GPS and should record coordinates only after achieving the lowest amount of error possible.

GPS uses many alternative mathematical models to describe the spherical shape of the earth and each is a separate datum. Commonly used datums include NAD27 CONUS, NAD83, and WGS84. Each represents a different interpretation of the shape of the earth. The NARS standard is **NAD83**. Thus, all GPS units should be switched to this standard - prior to completing any field activities. Crews should also confirm that the NAD83 datum is being used when the GPS is turned on prior to data collection. If the GPS is not set for NAD83 and the unit cannot be changed readily, note the datum used on the data forms for later conversion.

GPS devices use a variety of units for position designation based on an imaginary latitude and longitude

coordinate grid system laid across the earth (degrees, minutes, seconds, or degrees and decimal minutes, and UTM (a metric system). The NARS standard is **decimal degrees** for reporting all GPS positions.

Refer to the GPS user's manual to provide specific instructions on setting the Datum, coordinate system, and units to NLA standards. Turn on the GPS receiver and check the batteries prior to departure. Replace batteries immediately if a battery warning is displayed.

4.1.2.4 Electronic Data Capture Device Battery Check (if applicable)

Turn on the electronic device and check the batteries prior to departure. Charge immediately if a battery warning is displayed and charge fully to ensure enough battery for a full field day. External battery packs are often available for these devices if battery life is a concern.

Table 4.1 Instrument checks and calibration

Equipment	Preparation
GPS Unit	Check the batteries prior to departure Ensure map datum is set to NAD83 Ensure locational units are set to decimal degrees (hddd.dddd) Perform manufacturer checks as necessary to ensure accuracy
Multi-parameter Probe	Calibrate per manufacturer guidelines (DO to be calibrated at lake) Check the batteries prior to departure Perform QC Check as directed by manufacturer and/or laboratory protocols (field crews will supply QC check solution)
Electronic Data Capture Device (Optional)	Check the batteries prior to departure Ensure NLA Data collection App is installed and functioning

4.1.3 Equipment and Supply Preparation

Check your inventory of supplies and equipment prior to departure using the equipment and supplies checklists provided in the Appendix; use of the lists is strongly recommended. Pack meters, probes, and sampling gear in such a way as to minimize physical shock and vibration during transport. If necessary, prepare stock preservation solutions as described in Table 4.2. Follow the regulations of the Occupational Safety and Health Administration (OSHA).

Table 4.2 Stock solutions, uses, and methods for preparation.

Solution	Use	Preparation
Bleach (1%)	Clean nets, other gear, and inside of boat.	Add 40 mL bleach to 4,000 mL distilled water.
Lugol's	Preservative for phytoplankton samples.	Lugol's will be supplied with base kit. If preparation is needed: Dissolve 100 g KI in 1 L of distilled water. Dissolve 50 g iodine (crystalline) in 100 mL glacial acetic acid. Mix these two solutions. Remove any precipitates. Store in the dark.
95% Ethanol	Preservative for benthic invertebrate samples and zooplankton samples.	No preparation needed (use stock solution as is).

Refuel vehicle(s) and conduct maintenance activities the night before a sampling trip. Check trailer lights, turn signals, and brake lights before departure. In addition, inspect your vehicles, boats, and trailers every morning before departure. Pay particular attention to the trailer hitch, electrical connections, tie downs, tire pressure, and the overall condition of the boats.

Label and package as many of the sample containers as possible in the site kit prior to departure. Container labels should not be covered with clear tape until all information is completed during sampling at the lake. Store an extra kit of sampling supplies (Cubitainers®, bottles, glass fiber filters, foil, gloves, forms, pencils, permanent markers, and labels) in the vehicles. Inventory these extra supply kits prior to each lake visit. Be sure to order field sampling site kits well in advance (two week minimum) by submitting the electronic **Request Form**.

4.1.4 General Equipment and Supplies for all Activities

Table 4.3 indicates equipment and supplies that will be used for all activities.

Table 4.3 Equipment and supplies – all activities.

Type	Item	Quantity
Forms	NLA 2017 Verification	1
	NLA 2017 Index Profile (front & back)	1
	NLA 2017 Index Sample Collection (pages 1-3)	1
	NLA 2017 Physical Habitat (front & back)	10+
	NLA 2017 Littoral Sample Collection	1
	NLA 2017 Assessment (front & back)	1
	NLA 2017 Site and Sample Status/Water Chemistry Lab Tracking	1
	NLA 2017 Daily Shipped Samples Tracking	1
	NLA 2017 Tracking – Batched Samples	1
	NLA 2017 Tracking – Packets	1
Reference	NLA 2017 Field Operations Manual (FOM)	1
	NLA 2017 Quick Reference Guide (QRG)	1
	NLA 2017 Quality Assurance Project Plan (QAPP)	1
	NLA 2017 Site Evaluation Guidelines (SEG)	1
	NLA 2017 Fact Sheets	10
Documentation	Clipboard	1
	Pencils (#2, for data forms)	1
	Permanent markers (fine tip, for most labels)	1
	Labels	
	Field Notebook (optional)	1
	Clear tape strips (to cover sample labels)	As needed
Collection	Access permission documents/permit (if required)	1

4.2 Lake Verification

4.2.1 Equipment and Supply List

Table 4.4 is the checklist for equipment and supplies required to conduct protocols described in this section. It is similar to, but may be somewhat different from, the checklist that is used at a base site to assure that all equipment and supplies are taken to, and available at, the lake. Field crews should use the checklist presented in this section to ensure that the equipment and supplies are organized and available on the boat in order to conduct protocols correctly and efficiently.

Table 4.4 Equipment and Supplies – lake verification.

Type	Item	Quantity
Form	NLA 2017 Verification Form	1
Collection	Depth Finder (hand-held or boat mounted sonar)	1

	GPS unit (with manual, reference card, extra battery)	1
	Anchor (with 75 m line or sufficient to anchor in 50 m depth)	1-2

4.2.2 Lake Verification at the Launch Site

Before sampling activities begin, you must verify that you are at the correct lake and whether it meets the criteria for sampling. Confirming that you are at the correct lake is based on map coordinates, locational data from the GPS when possible, and any other evidence such as signs or conversations with local residents. Record locational coordinates for the lake on the **Verification** form. If GPS coordinates are obtained, fill in the bubble to indicate GPS and record the latitude, longitude, and record the type of GPS fix (2D or 3D) displayed on the unit for the launch site. If the GPS unit being used does not display the GPS fix in terms of 2D or 3D, determine the number of satellites being used by the GPS and assume that 3 or fewer satellites is 2D and 4 or more satellites is 3D. All coordinates will be recorded in the NAD83 datum. Compare the map coordinates given on the lake spreadsheet for the lake with the GPS coordinates displayed for the launch site, and verify that you are at the correct lake. [Note: The map coordinates in the spreadsheet represent the "labeling point" in NHD and may not be near either the index site or the launch site]. Verification that you are at the target lake can be confirmed via other methods (e.g., map, landowner confirmation). If GPS coordinates are not available, do not record any information, but try to obtain the information at a later time during the visit. A GPS location may be taken at any time during a lake visit and recorded by flagging the launch site coordinates and providing a comment.

Record directions to the lake and a description of the launch site on the **Verification** form regardless of whether the site is sampled or not. This information is very important and will be used in the future if the lake is revisited by another sampling crew. Provide information about signs, road numbers, gates, landmarks, and any additional information you feel will be useful to another sampling crew in locating this lake in the future. It is also helpful to describe the distance traveled (miles) between turns. Also describe the launch site. For example: Can the boat be launched with a trailer? Are there fees? Is the launch paved or does it consist of soft sand? What landmarks are at the launch? Due to privacy concerns, do not record landowner contact information (e.g., name, address, phone, email address) on the Verification form.

In addition to, or in the absence of, an accurate GPS reading, use as many of the following methods as possible to verify the site:

- Obtain confirmation from a local person familiar with the area.
- Identify confirming roads and signs.
- Compare the lake shape to that shown on a topographic map (United States Geological Survey (USGS) 7.5-minute map or equivalent).
- Determine lake position relative to identifiable topographic features shown on the map.

If the lake shape on the USGS topographic map does not correspond with the actual lake shape from your site map, and you cannot verify the lake by any other means, check "Not Verified" and provide comments on the **Verification** form. At each lake, evaluate whether or not the lake meets the NLA operational definition of a "lake":

- ≥ one ha in total surface area;
- ≥ 1,000 square meters of open water;

- ≥ one meter in depth;
- Not saline (due to salt water intrusion or tidal influence); and
- Not used for aquaculture, disposal-tailings, mine-tailings, sewage treatment, evaporation, or other unspecified disposal use.

If the lake does not meet this definition, fill in the "non-target" bubble in the lake sampled section on the **Verification** form and provide an explanation for not sampling the lake. Add any additional explanation as required. (For complete details on the Site Evaluation process, refer to the companion document *Site Evaluation Guidelines* [EPA 841-B-06-003]).

Record the names of each crew member on the **Verification** form.

Regardless of whether the lake is sampled or not, the field crew must fill out and submit a **Verification** form for every lake that is visited with the intent to sample.

4.2.3 Locating Index Site

When determining lake origin, i.e., lake vs. reservoir in the field, a body of water that was a stream or river and subsequently dammed to create a lake is considered a reservoir. A lake which has had its level raised because of a dam is an “enhanced” lake and will be considered a natural lake for NLA 2017.

Go to the deepest point in the lake to locate the index site (or middle of the lake for reservoirs). If the deepest point exceeds 50 m in depth, do not establish the index site at this location; instead, choose a point as close to the middle of the lake as you can without exceeding 50 m in depth. The procedure below outlines sonar operation and procedures for finding the index site. For reservoirs, the index site is located near the mid-point of the reservoir rather than at the deepest point, which may be near the dam. If this would result in an index site that is very shallow or otherwise non-representative of the reservoir as a whole, choose a point near the center of a main basin, where depths and the water column will be more representative of the reservoir.

Once in the general area, use the sonar unit to locate the deepest point (≤ 50 m). When an acceptable site is located, anchor the boat. Lower the anchor slowly to minimize disturbance to the water column and sediment. Determine the coordinates of the index site by GPS (if satellite coverage is available) and record on the **Index Profile** form. In addition, indicate the type of GPS fix (2D or 3D). If satellite coverage is not available at that time, try again before leaving the index site. The following is the procedure to be used to locate the index site:

1. Operate sonar unit according to manufacturer's specific operating procedures. If possible, depth readings should be made and recorded in metric units (be sure to specify units on the **Index Profile** form).
2. Use the sonar in the area expected to be the deepest. Spend no more than 30 minutes searching for the deepest point; the maximum depth for the index site is 50 meters.
3. Anchor the boat.
4. Determine the coordinates using GPS. Record GPS coordinates on the **Index Profile** form.

4.3 Post Sampling Activities

Upon return to the launch site after sampling, review all labels and completed data forms for accuracy, completeness, and legibility and make a final inspection of samples. If information is missing from the forms or labels, the Field Crew Leader gathers and records the missing information. The Field Crew Leader initials all paper field forms after review. If using electronic forms, the Field Crew Leader will

need to confirm that they have reviewed forms prior to submission. Other post sampling activities include: sample filtering, inspection and cleaning of sampling equipment, inventory and sample preparation, sample shipment, and communications.

4.3.1 Equipment Cleanup and Check

You must inspect all equipment, including nets, boat, and trailer, and clean off any plant and animal material. This effort ensures that introductions of nuisance species such as Eurasian watermilfoil (*Myriophyllum spicatum*) and zebra mussels (*Dreissena polymorpha*) do not occur between lakes. Prior to leaving a lake, drain all bilge water or live wells in the boat. Inspect, clean, and handpick plant and animal remains from vehicle, boat, motor, and trailer that contact lake water. Inspect and remove any remnants of vegetation or animal life. Before moving to the next lake, if a commercial car wash facility is available, thoroughly clean vehicle, boat, and trailer (hot water pressurized rinse – no soap). Rinse equipment and boat with 1% bleach solution to prevent spread of exotics. Procedures are below.

1. Clean for biological contaminants (e.g., Eurasian watermilfoil, zebra mussels, and alewife):
    a. Prior to departing from a lake, drain all bilge water from the boat.
    b. At the lake, inspect motors, boat, and the trailer for evidence of plant fragments, especially in or near the propeller and water intakes. Remove all plant fragments.
    c. At the lake or base site, dry out and inspect nets and buckets and remove any remnant vegetation or animal life. Disinfect gear with 1% bleach solution.
    d. If a commercial car wash facility is available, thoroughly clean vehicle and boat (hot water pressurized rinse--no soap).
2. Clean and dry other equipment prior to storage:
    a. Rinse chlorophyll-*a* collection bottles three times with DI water after each use.
    b. Rinse graduated cylinders, bulk water sampling containers, and other sampling devices three times with distilled water after each use.
    c. Briefly soak zooplankton nets in a 1% bleach solution and dry after each use. Do not dry in sunlight because the mesh is photosensitive.
    d. Clean core sampler, sectioning apparatus, mixing bowl and spoons and siphon thoroughly with tap water, Alconox or other phosphate-free detergent, and bottle brush at the base site.
    e. Rinse coolers with water to clean off any dirt or debris on the outside and inside.
    f. Rinse boots and waders with water to clean off any dirt, debris, or biological contaminants on the outside and inside.
3. Inventory equipment and supply needs and request supplies via the electronic **Request Form** (forms or site kits) or from the FLC (other items or urgent requests).
4. Remove multi-parameter probe meter and GPS from carrying cases and set up for pre-departure checks and calibration. If present (i.e., not using optical DO sonde), examine the oxygen membranes for cracks, wrinkles, or bubbles. Replace if necessary.
5. Recharge/replace batteries as necessary.
6. Recheck field forms from the day's sampling activities. Make corrections and completions where possible, and initial each form after review.

4.3.2 Shipment of Samples and Forms

You must ship or deliver time-sensitive samples (i.e., all chilled samples) to the appropriate analytical laboratories as soon as possible after collection and no later than the day after collection. These samples will be shipped in two groups for next day delivery. Other non-chilled samples may be shipped or delivered in batches provided they can be adequately stored. Report all sample shipments to the NARS

IM Coordinator (by transmitting the appropriate tracking forms) as soon as possible so that the analytical laboratories can be notified to receive samples and they can be tracked if they do not arrive when expected.

Field crews are to fill out one sample tracking form (either **Site and Sample Status/Water Chemistry Lab Tracking, Daily Shipped Samples Tracking,** or **Batched Samples Tracking** form) for each sample shipment. On each sample tracking form, the following information must be recorded:

- Airbill or package tracking number
- Date sample(s) were sent
- Site ID where each sample was collected
- Sample type code:
  - BACT – Bacteria (*E. coli*)
  - BENT – Benthic macroinvertebrates
  - CHEM – Water Chemistry
  - CHLX – Chlorophyll-*a*
  - FDNA – fish eDNA
  - GSCA and GSCB – Gas concentration
  - GSIA and GSIB – Gas isotopes
  - GSAA and GSAB – Air samples
  - MICX – Algal toxin (microcystins and cylindrospermopsin)
  - MICZ – Algal toxin (for bottle comparison study)
  - NUTS – Nutrient
  - PHYX – Phytoplankton
  - SEDO – Sediment contaminants
  - SEDG – Sediment grain size
  - SEDC – Sediment TOC
  - TRIA – Atrazine Pesticide Screen
  - ZOCN – Zooplankton coarse (150-micron mesh)
  - ZOFN – Zooplankton fine (50-micron mesh)
- Date when the sample(s) was collected
- Site visit number (e.g., 1 for first visit, 2 for second sampling visit to revisit site)
- Sample ID number from preprinted sample label
- Number of containers for each sample
- Any additional comments

See APPENDIX B: SHIPPING GUIDELINES for further information.

4.3.3 Communications

The Field Crew Leader must review all data forms for consistency, correctness, and legibility before transferring them to the NARS IM Center. Each field crew leader must submit a Site and Sample Status/WRS Tracking to the NARS IM Center (typically via email) after each site visit (whether the site is sampled or not). General communications information, including contact information for the NARS IM Center, is outlined in Section 2.1.

5.0 INDEX SITE ACTIVITIES

Field crews will collect several different measurements and indicators at the index site (as described in Table 1.1): a temperature, DO, and pH depth profile, Secchi transparency, bacteria (*E. coli*), fish eDNA, chlorophyll-*a*, phytoplankton, algal toxins, water chemistry, nutrients, dissolved gases, atrazine, zooplankton samples, and a sediment core. A detailed description of the individual elements is provided below.

5.1 Temperature, DO, and pH profile

5.1.1 Summary of Method

Use a multi-parameter water quality meter (or sonde) to measure temperature, DO, and pH at predefined depth intervals. Measurement intervals for the profile are based on the site depth (see Section 5.1.3). Once the profile is completed, make another DO measurement at the surface and compare it to the initial reading to see if the probe is functioning correctly and holding calibration. If the lake is thermally stratified, note the top and bottom of the metalimnion based on the temperature readings (observed as a change of ≥1 °C per meter of depth).

The meters and probes are delicate; take care to avoid putting the probe into contact with sediments which could foul the probes. An accurate measure of the site depth will help prevent contact between the sediment and the probes.

5.1.2 Equipment and Supplies

Table 5.1 Equipment and supplies – temperature, pH, and DO profiles.

Type	Item	Quantity
Form	NLA 2017 Index Profile	1
Collection: **Water column depth**	Depth Finder (hand-held or boat mounted sonar) Sounding line (50 m, calibrated, marked in 0.5 m intervals) with clip OR Sounding rod (calibrated) for very shallow lakes	1 1
Collection: **Profile measurements & calibration**	Multi-parameter water quality meter (with temperature, pH, and DO probes) Sounding line or 50-meter tape Sounding weight Squirt bottle (1 L Nalgene) – De-ionized (DI) Squirt bottle (1 L Nalgene) – lake water Calibration cups and standards QC check solution Barometer or elevation chart to use for calibration	1 1 1 1 1

5.1.2.1 Multi-parameter probe Sonde

The multi-parameter probe sonde must be heavy enough to minimize sway and wobbling as it is lowered and raised in the water column. The instrument must be stabilized prior to taking a reading. Experiment with the sonde prior to sampling and add weight to the cable if needed.

5.1.2.2 Temperature Meter Calibration

Check the accuracy of the sensor against a thermometer (a non-mercury type is recommended) that is

traceable to the National Institute of Standards and Technology (NIST) at least once per sampling season. The entire temperature range encountered in the NLA 2017 should be incorporated in the testing procedure and a record of test results kept on file.

5.1.2.3 DO Probe Calibration

Calibrate the DO probe preferably at the lake prior to each sampling event (Note: some newer instruments and probes may not require calibration as frequently). It is recommended you calibrate the probe in the field against an atmospheric standard (ambient air saturated with water, or water saturated with air for optical probes) prior to launching the boat. In addition, manufacturers typically recommend periodic comparisons with a DO chemical analysis procedure (e.g., Winkler titration) to check accuracy and linearity. Small "mini-Winkler" titration kits are suitable for this check and can be taken into the field.

5.1.2.4 pH Meter Calibration

Calibrate the pH electrode prior to each sampling event in accordance with the manufacturer's instructions and your organization's existing SOP. Conduct a QC check with a different standard to verify the calibration and periodically evaluate instrument precision (see Section 2). Ideally, a quality control solution (QCS) should be used that is similar in ionic strength to the lake water samples you will be measuring. Standard buffer solutions used to calibrate electrodes may not be representative of typical lake waters.

5.1.2.5 Conductivity Calibration

A field conductivity measurement is optional for the NLA 2017. If the Field Crew opts to take conductivity measurements, the conductivity meter must be calibrated prior to each sampling event. Calibrate the meter in accordance with the manufacturer's instructions. Ensure that the conductivity meter is temperature corrected to 25 °C.

5.1.3 Depth Profile Procedure

Below are the step-by-step procedures for measuring temperature, pH, and DO profiles at the index site.

1. Calibrate Instrument
    a. Check meter and probes and calibrate according to manufacturer's specifications.
    b. Enter calibration information on the **Index Profile** form.
2. Record Site Conditions:
    a. Observe site conditions and fill out the "Site Conditions" portion of the **Index Profile** form. Conditions to be reported include:
        i. Precipitation ("None", "Light," or "Heavy")
        ii. Surface conditions ("Flat," "Ripples," "Choppy," or "Whitecaps")
    b. Presence or absence of odor or scum. (Choice of "Yes" or "No" plus space to describe the odor or scum if present)
3. Determine Site Depth:
    a. Accurately measure the depth using a sounding line with a weight or other means and record on the **Index Profile** form.
    b. Indicate method used.
4. Determine Measurement Intervals:
    a. The number of readings and the depth intervals taken depends on the site depth. Below is a list of rules for determining the intervals:

i. The profile will always begin with a measurement just below the surface (e.g., approximately 10 cm or the minimum depth required to keep all probes submerged).
ii. The last (deepest) measurements will always be at 0.5 m above the bottom.
iii. If the site is < 3.0 m deep, record measurements beginning just below the surface and at 0.5 m intervals, until 0.5 m above the bottom.
iv. If the depth is between 3.0-20 m (inclusive), record measurements beginning just below the surface and then at 1.0 m intervals until reaching 0.5 m above the bottom.
v. If the depth exceeds 20 m, record measurements beginning just below the surface, then at 1.0 m intervals until you reach 20 m, then at 2.0 m intervals until 0.5 m above the bottom.
vi. If the metalimnion is encountered (observed as a change of ≥1 °C per meter of depth), take measurements at least every meter within the metalimnion.

5. Measure Temperature, DO, and pH:
    a. Lower the sonde in the water and measure the vertical profile of temperature, DO and pH at the predetermined depth intervals. Be careful not to let the probe touch the bottom.
    b. Record the intervals and measurements on the **Index Profile** form.
    c. Flag any measurements that the crew feels needs further comment or when a measurement cannot be made.
    d. Use the flag codes on the form and the comment box found on the second page.
6. Duplicate Surface DO Measurement
    a. When the profile is completed, take another DO measurement at the surface, record it, and compare it to the initial surface DO reading.
    b. Mark 'Yes' or 'No' on the form if the second DO reading is within 0.5 mg/L of the initial surface reading. This provides information regarding measurement precision and possible calibration drift during the profile.
        i. If measurement is not within 0.5 mg/L, verify your calibration.
        ii. If DO is found to be out of calibration, re-calibrate and re-record DO measurements on a backup form.
7. Determine the Metalimnion:
    a. If the lake is thermally stratified, note the top and bottom of the metalimnion in the Metalimnion column.
    b. For standardization purposes, the metalimnion has been defined in the protocol as an area where water temperature changes at least 1 degree Celsius per meter.
    c. If you suspect that the metalimnion exists but does not change at the specified rate, estimate the top and bottom of the metalimnion to the best of your ability, flag the data, and explain.
    d. In deep sites where measurement intervals are 2.0 meters apart, decrease the interval to 1.0 meters while taking measurements within the metalimnion.

5.2 Secchi Disk Transparency

5.2.1 Summary of Method

A Secchi disk is a black and white patterned disk used to measure a lake's clarity (see Figure 5.1). Take the reading on the shady side of the boat, without sunglasses, hat, or other viewing aids. Record the depths where the disk disappears when descending and reappears when retrieving.

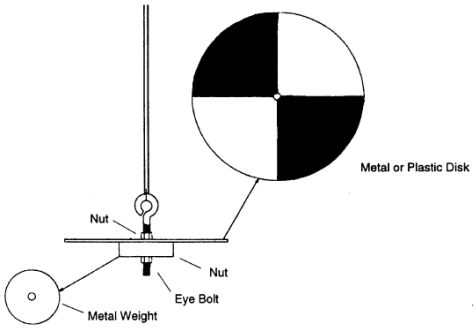

Figure 5.1 Secchi disk diagram (EPA, 1991).

5.2.2 Equipment and Supplies

Table 5.2 Equipment and supplies – Secchi disk transparency.

Type	Item	Quantity
Form	NLA 2017 Index Sample Collection	1
Collection	Metric tape measure Secchi Disk (20 cm diameter) Sounding line (50 m, calibrated, marked in 0.5 m intervals) with clip	1 1 1

5.2.3 Procedure for Determining Secchi Transparency

Because different people measuring Secchi transparency at the same site may obtain different results (due to differences in vision and interpreting disk disappearance and reappearance), it is recommended that one crew member conduct Secchi disk measurements at all lakes.

If the lake is shallow and the water clear, the Secchi disk might reach the bottom and still be visible. If this is the case, it is important to not stir up the bottom sediments while anchoring the boat. Move the boat away from the anchor before taking the reading. If the disk is visible at the bottom of the lake, indicate this by filling in the "clear to bottom" bubble on the **Index Sample Collection** form and record the water depth in both the disappearance and reappearance fields.

States that wish to take additional measurements for comparisons using a view scope are encouraged to do so after completing the Secchi disk measurements are completed following the NLA protocols.

The following procedure is to be followed when collecting NLA Secchi disk measurements:

1. Confirm that the lowering line is firmly attached to the Secchi disk.
2. Remove sunglasses and hat. Also, do not use view scopes or other visual aids. If wearing prescription sunglasses, temporarily replace them with regular clear lens prescription glasses.
3. Lower the Secchi disk over the shaded side of the boat until it disappears.
4. Read the depth indicated on the lowering line. If the disappearance depth is <1.0 meter, determine the depth to the nearest 0.05 meter by marking the line at the nearest depth marker and measuring the remaining length with a tape measure or meter stick. Otherwise, record the disappearance depth to the nearest 0.1 meter. Record the disappearance depth on the **Index Sample Collection** form.

5. Lower the disk a bit farther and then slowly raise the disk until it reappears and record the reappearance depth, using the same level of precision as before.
6. Calculate the euphotic zone depth by multiplying the depth where the Secchi disk reappears by two. Use this calculation to determine the depth at which water samples will be taken with the integrated water sampler:
    - 6.1 If euphotic zone is less than 2 meters, water samples will be collected only within the euphotic zone.
    - 6.2 If euphotic zone is greater than 2 meters, water samples will be taken from the top 2 meters of the water column.
    - 6.3 If the Secchi is clear to the bottom and the lake is less than 2.5 m deep, water samples will be collected 0.5 m from the depth at the index site.
7. Record the depth that will be targeted for the integrated water samples.
8. Note any conditions that might affect the accuracy of the measurement in the comments field.

5.3 Bacteria Sample Collection

5.3.1 Summary of Method

Collect water samples for bacteria with a grab sample from about 0.3 m below the water surface. This sample should be collected from the boat.

5.3.2 Equipment and Supplies

Table 5.3 provides the equipment and supplies needed for field operations to collect bacteria samples at the index site.

Table 5.3 Equipment and supplies - bacteria samples.

Type	Item	Quantity
Form	NLA 2017 Index Sample Collection	1
Documentation	Label: Bacteria Clear tape strips (to cover sample labels)	1 As needed
Collection	Sterile IDEXX Bottle (290 mL) Surgical gloves (latex/nitrile, non-powdered)	1 1 pair
Storing and preserving	Wet ice Cooler Plastic electrical tape	As needed 1 As needed

5.3.3 Sampling Procedure

While anchored at the index site, collect a grab sample from 0.3 m below the water surface using the sterile IDEXX bottle.

1. Make sure all necessary data has been written on the sample label and the label is completely covered with clear tape.
2. Put on surgical gloves (non-powdered). Do not handle any food, drink, sunscreen, or insect repellant until after the water sample has been collected, or implement measures to reduce contamination by such chemicals, if applied, such as washing, wearing long gloves, etc.

3. Remove the tamper seal from the bottle lid. Remove the cap from the bottle.
4. Do not rinse the bottle and avoid touching the inside of the bottle or the inside of the cap.
5. Carefully invert the bottle. Dip the sample container to a depth of 0.3 m avoiding surface scum and other obvious debris. At that depth, push the mouth of the sample container away from the boat. Then lift the bottle from the water.
6. Pour off excess water until the bottle is filled just above the 200 mL line, which will leave headspace for air and shaking. Filling to just above the 200 mL line is important for the laboratory to implement the analytical procedure correctly.
7. Carefully replace the cap. Seal the cap with plastic electrical tape.
8. Immediately after sample is collected, place in a cooler with ice to minimize exposure to light and begin chilling the sample.
9. The sample must remain well chilled until it arrives at the laboratory.

5.4 Fish eDNA Sample Collection

5.4.1 Summary of Method

Collect water samples for fish eDNA with a grab sample collected at the water surface from the boat.

5.4.2 Equipment and Supplies

Table 5.4 provides the equipment and supplies needed for field operations to collect fish eDNA samples at the index site.

Table 5.4 Equipment and supplies - fish eDNA samples.

Type	Item	Quantity
Form	NLA 2017 Index Sample Collection	1
Documentation	Label: fish eDNA Clear tape strips (to cover sample labels)	1 As needed
Collection	Sterile PETG Bottle (1 L) Gloves (latex/nitrile, non-powdered)	1 1 pair
Storing and preserving	Wet ice Cooler Plastic electrical tape	As needed 1 As needed

5.4.3 Sampling Procedure

While anchored at the index site, collect a grab sample from the water surface using the sterile 1 liter PETG bottle.

1. Make sure all necessary data has been written on the sample label, the label is placed on the outside of the bottle, and the label is completely covered with clear tape.
2. Put on surgical gloves (non-powdered). Do not handle any food, drink, sunscreen, or insect repellant until after the water samples have been collected, or implement measures to reduce contamination by such chemicals, if applied, such as washing, wearing long gloves, etc.
3. Remove the cap from the bottle.

4. Do not rinse the bottle and avoid touching the inside of the bottle or the inside of the cap.
5. Dip the sample container in the surface water (do not fully submerge mouth of bottle). At the surface of the water, angle the mouth of the sample container away from the boat. Collect any surface scums, if present, at the site.
6. Fill the 1 L bottle to the 1000 mL mark, leaving headspace for air. This headspace is important should the samples be frozen at the laboratory.
7. If the bottle is filled above the 1000 mL mark, discard excess water.
8. Carefully replace the cap. Seal the cap with plastic electrical tape.
9. Immediately after sample is collected, place in a cooler with ice to minimize exposure to light and begin chilling the sample.

5.5 Water Sample Collection and Preservation

5.5.1 Summary of Method

Collect water samples using an "integrated sampler", which is based on a design by the Minnesota Pollution Control Agency (MPCA) (see Figure 5.2). The device is a PVC tube 6.6 feet (2 meters) long with an inside diameter of 1.24 inches (3.2 centimeters) fitted with a stopper plug on one end and a valve on the other. The device allows collection of water from the upper two meters of the water column (e.g., within the euphotic zone). If the euphotic zone is < 2.0 m deep (as calculated in the Secchi Disk Transparency section of the form), lower the integrated sampler only to the depth of the euphotic zone, and take additional grab samples as necessary to collect the total volume needed for the samples.

5.5.2 Equipment and Supplies

Table 5.5 provides the equipment and supplies needed to collect water samples at the index site.

Table 5.5 Equipment and supplies – water samples.

Type	Item	Quantity
Form	NLA 2017 Index Sample Collection	1
Documentation	Labels: water chemistry, nutrients, algal toxins, phytoplankton, atrazine, and chlorophyll-*a*	1 per sample
	Clear tape strips (to cover sample labels)	As needed
Collection	Integrated sampler device (MPCA design)	1
	Funnel	1
	Gloves (latex/nitrile, non-powdered)	1
Storing & Preservation	Cubitainer® (4L) – water chemistry	1
	HDPE bottle (60 mL, white, wide-mouth) – atrazine	1
	HDPE bottle (250 mL, brown, wide-mouth) – nutrients	1
	PETG bottle (500 mL, clear, square) – algal toxins (MICX)	1
	HDPE bottle (500 mL white, round) – algal toxins (MICZ)	1
	HDPE bottle (1 L, white, narrow-mouth) – phytoplankton	1
	Poly bottle (2 L, brown) – chlorophyll-*a*	1
	H_2SO_4 ampoules – nutrients	1-2
	pH paper – nutrients	1
	Wet ice	As needed

	Lugol's solution (250 mL bottle)	5-10 mL
	Cooler	1
	Plastic electrical tape	As needed

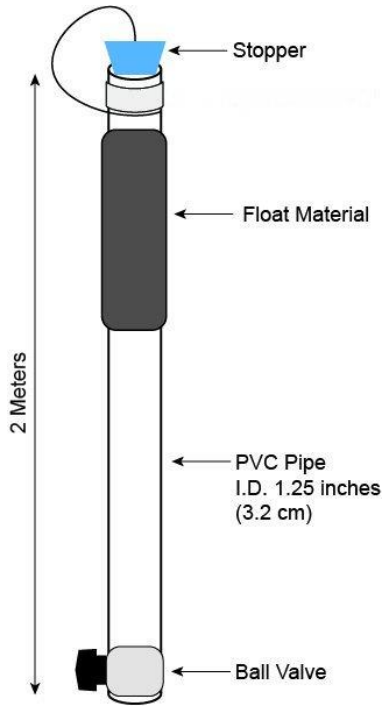

Figure 5.2 Integrated water sampler device (MPCA).

5.5.3 Sampling Procedure

Assuming the euphotic zone is ≥ 2 meters, collect five integrated water samples (Figure 5.3). Sample #1 is emptied into a 2 L sample bottle for ***chlorophyll-a*** filtering. Samples #2 and #3 are to be transferred from the sampler to the 4 L Cubitainer®, mixed thoroughly, and, poured off into one 1 L sample bottle for ***phytoplankton*** processing, two 500 mL bottles for the ***algal toxins*** samples, one 250 mL sample bottle for ***nutrients***, and one 60 mL bottle for the ***atrazine*** pesticide sample. Samples #4 and #5 are to be transferred from the sampler to the 4 L Cubitainer® for the ***water chemistry*** sample. If the euphotic zone is <2 meters, only collect water from the euphotic zone and increase the number of grab samples accordingly.

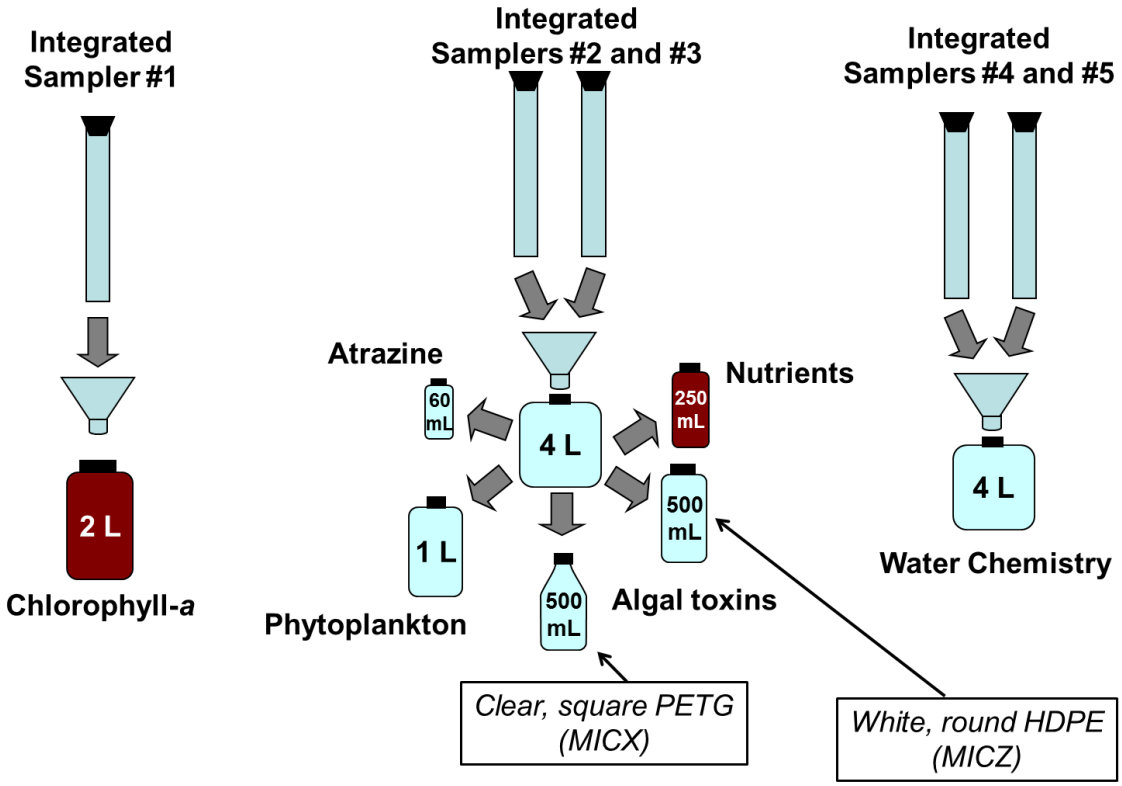

Figure 5.3 Procedure for using the integrated sampler device to collect depth integrated samples.

5.5.3.1 Sample Collection

1. Make sure all necessary data has been written on the sample labels and labels are completely covered with clear tape.
2. Put on surgical gloves (non-powdered). Do not handle any food, drink, sunscreen, or insect repellant until after samples have been collected, or implement measures to reduce contamination by such chemicals, if applied, such as washing, wearing long gloves, etc.
3. Rinse each water sample collection container and lid with surface water three times.
4. Remove the rubber stopper cap and open the valve on the bottom end of the integrated sampler. Rinse by submerging it three times in the lake and draining after each rinse. Complete rinsing on the opposite side of the boat from which you plan to sample. Do not take samples near the motor or other sources of contamination.
5. Slowly lower the sampler into the lake as vertically as possible. For a 2 m sample, stop lowering the device when the upper end is just above the surface. If the euphotic zone is < 2.0 m deep (as calculated in the Secchi Disk Transparency section of the form), the integrated sampler will be lowered only to the depth of the euphotic zone; additional samples will be taken to collect the volume needed for the samples (8 L total).
6. Cap the upper end with the rubber stopper firmly and slowly raise the sampler.
7. When the bottom of the sampler is near the surface, reach underwater and close the valve on the bottom end.

8. Lift the sampler into the boat, keeping it as vertical as possible. When possible, move the containers to a shaded area of the boat to avoid exposing the sample to direct sunlight when dispensed.
9. Carefully open the valve and dispense the contents of sample #1 (or more, as necessary) into the 2 L brown bottle. This is the chlorophyll-*a* sample, which will be filtered on shore (see Section 7.2.2). Place the bottle on ice until filtration can be initiated.
10. Repeat the collection process in steps 5-8 and dispense the contents of sample #2 and sample #3 (or more, as necessary) into the 4 L Cubitainer® and mix well.
11. Fill the 1 L phytoplankton bottle from the 4 L Cubitainer®, allowing enough headspace to add at least 5 mL of Lugol's preservative.
12. Fill the two 500 mL bottles from the 4 L Cubitainer® to the 500 mL mark (or just below the shoulder of the bottle), leaving headspace so that the bottles don't burst when frozen at the laboratory. These are the algal toxin samples. Seal the caps with plastic electrical tape. Place the bottles in the cooler with ice.
13. Fill the 250 mL bottle from the 4 L Cubitainer®, leaving headspace to add acid. This is the nutrient sample.
14. Fill the 60 mL bottle from the 4 L Cubitainer® to just below the shoulder, leaving headspace so that the bottle does not burst when frozen at the laboratory. This is the atrazine sample. Seal the cap with plastic electrical tape. Place the bottle in the cooler with ice.
15. Pour the contents of sample #4 and sample #5 (or more, as necessary) from the integrated sampler into the 4 L Cubitainer®, removing as much air from the Cubitainer® as possible. This is the water chemistry sample. Seal the cap with plastic electrical tape. Place the Cubitainer® in the cooler with ice.

5.5.3.2 Sample Preservation

1. For the phytoplankton sample, add 5 mL of Lugol's solution to the 1 L phytoplankton bottle. (Fill the provided plastic transfer pipette up to the bulb with Lugol's three times to get 5 mL). Cap the bottle and invert until well mixed. The sample should resemble the color of weak tea. If needed, add additional Lugol's 2-3 mL at a time up to a maximum of 10 mL.
2. For the nutrients sample, add H_2SO_4 from an ampoule to the water to stabilize the sample. Test the acidity level of the stabilized sample with pH paper by pouring a small amount of water over the pH test strip. The water must have a pH <2. If the pH is ≥2, add one more ampoule of acid. In most cases one ampoule will be sufficient, but add no more than two ampoules to the sample. Dispose of ampoule(s) properly.
3. Seal the caps with plastic electrical tape. Place all samples in the cooler with ice.

5.6 Dissolved Gases Sample Collection

5.6.1 Summary of Method

Field crews will collect four water samples from near the surface of the lake: two for the analysis of dissolved carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) concentration (GASC), and two for the analysis of carbon isotopic composition of CO_2 and CH_4 (GASI). Two air samples will also be collected for gas concentration measurements (GASA). The results will be used to estimate the

magnitude of CO_2, CH_4, and N_2O emissions from lakes and reservoirs across the nation.

5.6.2 Equipment and Supplies

Table 5.6 provides the equipment and supplies needed to collect dissolved gas and air samples at the index site

Table 5.6 Equipment and supplies – dissolved gases.

Type	Item	Quantity
Form	NLA 2017 Index Sample Collection	1
Documentation	Labels: gas concentration, gas isotopes, and air samples Clear tape strips (to cover sample labels)	1 per sample As Needed
Collection	Pre-evacuated glass vials Tray for glass vials Poly syringe (140mL) with attached 3-way stopcock Poly syringe (30mL) with attached 2-way stopcock Poly syringe (30mL) ('transfer syringe') 27 gauge needles 50 mL centrifuge tube Alcohol thermometer Bucket, tub or cooler	6 1 4 2 1 20 1 1 1

5.6.3 Sampling Procedure

Four dissolved gas and two air samples will be collected from the index site. The samples will be transferred from syringes to pre-evacuated vials using a small needle. If lake conditions are too hazardous (e.g., windy, breaking waves) to safely work with a needle at the index site, complete steps 9 – 18 in less turbulent waters (littoral stations) or at the shoreline. It is important that steps 9 – 16 are conducted as soon as possible to reduce the likelihood of the sample becoming contaminated or altered by microbial activity.

1. There are six pre-evacuated sample vials per site: four are for dissolved gas samples and two are for air samples. All six vials are identical and it doesn't matter which are used for the dissolved gas and air samples.
2. Make sure that the EPA NLA 2017 tracking labels are affixed properly to the 6 sample vials. Do not cover the labels with clear tape or they will not fit in the sample trays at the laboratory.
3. Ensure that a 3-way stopcock is attached to a 140 mL syringe (Figure 5.4). With the 3-way stopcock pointed toward the side port (3 o'clock position) and the syringe pointed downwards (i.e., stopcock pointed towards bottom of boat), flush the syringe three times with air drawn from approximately 1.5m above the water surface (i.e. syringe at eye-level when standing in boat). This will remove residual gas and water from the syringe. Care should be taken to ensure that the air pulled

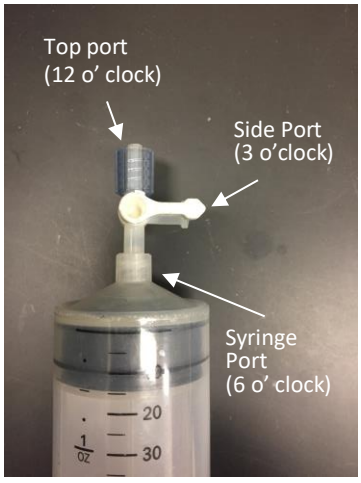

Figure 5.4 Port identities on 3-way stopcock.

into the syringe is not contaminated with exhaust gas from the boat motor. The air should be collected upwind of the boat motor while the motor is not operating.

4. Pull 35 mL of air into the syringe from approximately 1.5m above the water surface (i.e. syringe at eye-level when standing in boat), while avoid airborne contaminants such as motor exhaust.
5. Place the tip of the stopcock ~ 5 cm under water and pull in 105 mL of lake water (i.e., plunger will be at 140mL graduation). Keep the tip of the syringe under water and close the syringe port on the stopcock (handle pointed toward the syringe port [6 o'clock position]), then remove the syringe from the lake. Place the syringe in a bucket, tub, or cooler of lake water to maintain the temperature of the water in the syringe.
6. Repeat steps 3-5 for the remaining 3 dissolved gas samples.
7. Collect two 30 mL air samples from ~1.5 m above the water surface (i.e. syringe at eye-level when standing in boat) using 30 mL syringes and 2-way stopcocks. Flush the syringes with air three times to remove any residual gas before collecting the samples. Exhaust gas from the boat motor poses a contamination risk and the samples should be collected upwind of the boat motor while the motor is not operating. Seal the samples in the syringe by closing the stopcock (handle perpendicular to syringe).
8. If conditions preclude using a needle at the index site (i.e. rough water), keep the 140 mL syringes submerged in the bucket, tub, or cooler of lake water and finish the procedure (steps 9-16) after reaching calmer waters or the shore. If a needle can be used at index site, continue with the procedure at the index site.
9. Attach a 27-gauge needle to the 30 mL syringes containing the air samples, open the stopcock (handle in line with syringe), and pierce the septa of a pre-evacuated sample vial that has been labelled with an air sample label. The vacuum in the vial should be sufficiently strong to cause the syringe plunger to move ~2 cm toward the bottom of the syringe. Depress the syringe plunger until it hits the bottom of the syringe barrel. While firmly holding the plunger in place, withdraw the needle from the sample vial. Repeat for the second air sample.
10. To reduce the possibility of losing track of which vials contain a sample and which are empty, place empty vials in the vial tray at the beginning of the operation and place filled vials in the shipping bag as soon as the transfer is complete.
11. Gently shake each of the 140 mL syringes for five minutes to allow the dissolved gases to partition between the aqueous and gas phases (all four syringes can be shaken at the same time).
12. Attach a 27-guage needle with the safety cap to the 140 mL syringe. Attach the 30 mL transfer syringe (without a stopcock attached) to the side port of the 3-way stopcock on the 140 mL syringe (Figure 5.5 A).
13. Hold the 140 mL syringe in an upright position and close the top port on the 3-way stopcock (handle pointed toward the top port [12 o' clock position]). Push the 35 mL of gas from the 140 mL syringe into the 30 mL transfer syringe (Figure 5.5 B). This will cause the plunger in the transfer syringe to extend past the

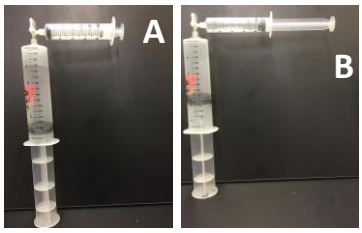

Figure 5.5 Pictures of 30 mL transfer syringe connected to 140 mL syringe

A) 140 mL syringe containing 35 mL of gas and 105 mL of water.

B) 35 mL of gas transferred to 30 mL syringe.

30mL graduation mark on the syringe barrel, which is OK. You may need to gently pull the plunger on the 30 mL transfer syringe to assist the gas transfer. Take care to minimize the amount of water that gets transferred to the 30 mL syringe.

14. Close the syringe port on the 3-way stopcock (handle pointed toward 140 mL syringe [6 o' clock position]) and expel all but 30 mL of gas from the transfer syringe. Use the needle to pierce the septa on a pre-evacuated glass vial that has been labelled with a gas sample label and depress the plunger on the transfer syringe until it hits the bottom of the syringe barrel (Figure 5.6). While firmly holding the plunger in place, withdraw the glass vial from the needle. Replace the safety cap and remove the needle from the 140 mL syringe.
15. Immediately transfer the water from the 140 mL syringe into a 50 mL centrifuge tube (secured in the vial tray) and record the temperature on the **Index Sample Collection** form. Water can be discarded after temperature has been recorded.
16. Repeat steps 12 – 15 for the remaining three dissolved gas samples.

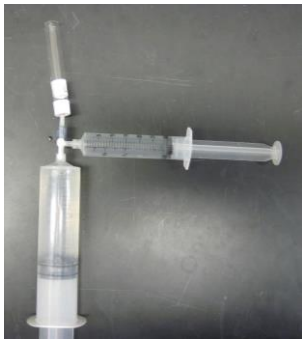

Figure 5.6 Glass vial connected to 3-way stopcock

Depress plunger on transfer syringe to transfer samples to glass vial.

17. Complete the data form and repack the sampling equipment. Needles, syringes, centrifuge tube, and thermometer can be reused without cleaning.
18. Prepare samples for shipping.

5.7 Zooplankton Collection

5.7.1 Summary of Method

Collect two vertical samples using a fine mesh (50 μm) and coarse mesh (150 μm) Wisconsin nets with collection bucket attached at the cod end. Each net is slowly lowered over the side of the boat into the water. The net is retrieved back to the surface at a slow, steady rate. Lift the net out of the water; rinse it from the outside to free organisms from the side of the net and to concentrate them in the collection bucket. Transfer the sample from the collection bucket to a 125 mL sample container. Narcotize the organisms with an effervescent sodium bicarbonate tablet (e.g., Alka-Seltzer® tablet) and preserve each sample with 95% ethanol. You will repeat the procedure with the other net on the opposite side (or end) of the boat. The cumulative tow length for each net is 5 m. In shallow lakes, multiple tows with each net are required to achieve the cumulative tow length. The objective is to sample a sufficient volume of water to obtain at least 300 organisms per sample from all but the most oligotrophic lakes.

5.7.2 Equipment and Supplies

Table 5.7 provides the equipment and supplies needed to collect a zooplankton sample. Figure 5.7 is an illustration of the zooplankton nets and collection buckets.

Table 5.7 Equipment and supplies – zooplankton collection.

Type	Item	Quantity
Form	NLA 2017 Index Sample Collection	1
Documentation	Labels: zooplankton course and zooplankton fine	1 per sample
	Clear tape strips (to cover sample labels)	As needed
Collection	Plankton net (50 µm) and collection bucket	1
	Plankton net (150 µm) and collection bucket	1
	Sounding line (50 m, calibrated, marked in 0.5 m intervals) with clip	1
	Funnel	1
	Squirt bottle (1 L Nalgene) – de-ionized (DI)	1
	Squirt bottle (1 L Nalgene) – lake water	1
	Effervescent sodium bicarbonate (Alka seltzer) tablets	1
	Pail (narcotization and concentration chambers)	1
		2
Storing & Preservation	HDPE bottle (125 mL, white, wide-mouth)	2
	Ethanol (95%)	1
	Plastic electrical tape	As needed

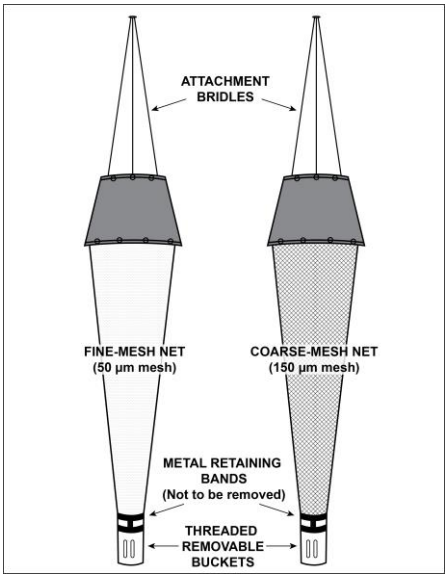

Figure 5.7 Wisconsin net and collection bucket diagram.

5.7.3 Sampling Procedure

The procedures for collecting and processing zooplankton samples are presented below.

5.7.3.1 Sample Collection

1. Determine and record the number of tows required to achieve the standard cumulative 5 m tow on the **Index Sample Collection** form.
    a. For lakes deeper than 7 m, you will take a single 5 m tow with each net.
    b. For lakes with a depth less than 7 m, you will determine and record the number of tows that will be required to achieve a standard cumulative 5 m tow for each net (Table 5.8). For example, if the lake is 6 meters deep, you will take two 2.5 m tows with each net. All

lakes less than 7 m deep require at least two tows because the collection bucket should never touch the sediment due to potential fouling.

Table 5.8 Lengths and numbers of zooplankton tows based on Index Site depth

Depth of lake (m)	Length of Tow	Number of Tows
7 or more	5 m	1
4 - 6	2.5 m	2
2 - 3	1 m	5
less than 2	0.5 m	10

c. The zooplankton collection methods vary slightly depending on the number of tows required to achieve a standard cumulative 5 m tow.
   i. If the number of tows = 1: follow steps 2 through 13 described below.
   ii. If the number of tows ≥ 2, follow steps 2 through 12 described below. After step 12, pour the contents of the collection bucket into a clean (i.e., DI rinsed) 1-gallon pail. Rinse the collection bucket with DI. While taking care not to tip the zooplankton sample in the pail, repeat steps 2 through 12 for the second tow. Add the contents of the collection bucket from the second tow to the pail. Continue to take zooplankton tows and add samples from the collection bucket into the pail until the target number of tows (2, 5, or 10) is reached. On the last tow, pour the contents of the pail into the collection bucket to filter the excess water. Rinse the bucket with DI water and pour the contents of this rinse into the collection bucket with the zooplankton sample. Once the zooplankton sample has been filtered down to an appropriate volume in the collection bucket, continue on to step 13.

2. Prior to each use, carefully clean and thoroughly rinse the interior of the plankton nets and buckets with DI water.
3. Carefully inspect the nets and buckets for holes or tears.
4. Attach the collection buckets to the “cod” end of the nets and secure. Make sure the correct bucket is attached to the correct net (i.e., the mesh sizes match).
5. Attach the bridled end of the plankton net to a 0.25” calibrated line with markings every 0.5 m (use the same line as was used with the Secchi disk).
6. Carefully and slowly lower the first net in a constant upright position over the side of the boat.
7. Continue lowering the net to the correct depth (remember to account for the length of the bridle). If more than one tow is needed, be sure to take additional tows from different locations around the boat.
8. Retrieve the net by pulling back to the surface at a steady rate (0.3 m or 1 ft/s) without stopping.
9. Once at the surface, slowly dip the net up and down in the water without submersing the net mouth to rinse contents into the collection bucket.
10. Complete the rinsing of the net contents by spraying lake water against the outside of the net with a squirt bottle or similar tool. Be careful not to splash or squirt lake water into the net mouth, or additional organisms may be collected.
11. If additional rinsing is needed on the interior of the net, use a squirt bottle with DI water only to avoid introducing additional organisms.

12. Once all organisms have been rinsed into the collection bucket, hold the collection bucket in a vertical position, and carefully remove the bucket from the net.
13. Repeat steps 5-12 with the second net on the opposite side (or end) of the boat.

5.7.3.2 Sample Processing

1. Set the collection bucket in a pail filled half full of lake water to which 2 CO_2 (e.g., Alka-Seltzer) tablets have been added outside of the collection buckets. Wait 30 to 60 seconds for the CO_2 tablets to dissolve before placing the collection buckets into the water. Ensure that the organisms in the collection bucket are submerged in the water, but be careful not to submerge the top of the collection bucket, or sample loss will occur. The CO_2 narcotizes the zooplankton to relax their external structure prior to preservation in 95% ethanol. This facilitates taxonomic identification. Wait until zooplankton movement has stopped (usually about one minute). Raising and lowering the collection buckets in the pail can help water exchange within the bucket.
2. Check the sample label on the bottle to verify which sample has been collected (coarse or fine mesh). Record the sample ID number on the **Index Sample Collection** form.
3. Use small volumes of DI water from a squirt bottle to rinse the contents of the mesh net collection bucket into the 125 mL polyethylene bottle. Rinse the collection bucket with DI water three to four times or until the majority of zooplankton have been removed without allowing the bottle to fill more than half full (~60-70 mL of sample and rinse water combined). After the zooplankton have been transferred and the sample bottle is half full with sample and rinsate, fill the bottle to the shoulder with 95% ethanol. Use a funnel, if necessary, when transferring the sample, rinsate or ethanol to the 125 mL sample bottle
4. In some cases, the volume of zooplankton collected in the collection bucket may exceed 125 mL. Under this scenario do not try to force the entire sample into a single bottle, or the preservative will not function properly and the sample may be lost. In such cases, fill the first bottle half full, and then use a second bottle to preserve the additional amount of sample. Use an “extra jar” label (i.e., one with no sample ID pre-printed on it) for identification purposes. Complete the label, and print in the sample number assigned to the first container on the label of the second container. On the form, record a “2” in the “No. Jars” field.
5. On the **Index Sample Collection** form, fill in the bubble to indicate that the sample is preserved.
6. Verify that all information on the labels and the form is complete and correctly recorded.
7. Repeat steps 1-6 for the second sample collected.

5.8 Sediment Contaminants, TOC, and Grain Size

5.8.1 Summary of Method

Field crews will collect surficial sediment samples (top 5cm) at the index site using the supplied gravity corer. A corer is used to collect two intact sediment core samples at the index site, crews will slice off the top 5 cm from these two sediment cores and composite them for analysis. The laboratory will analyze this composite sample for sediment contaminants, TOC, and grain size. The results will be used to assess the current condition of a variety of metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and pesticides in lakes across the country.

5.8.2 Equipment and Supplies

Table 5.8 provides the equipment and supplies needed to collect a sediment core sample. Figure 5.8 is an illustration of the gravity corer, 68 mm diameter / 60 cm long core tube, and sectioning apparatus.

Table 5.8 Equipment and supplies – sediment core sample.

Type	Item	Quantity
Form	NLA 2017 Index Sample Collection	1
Documentation	Labels: Sediment TOC, sediment grain size and sediment contaminants	1 per sample
	Clear tape strips (to cover sample labels)	As needed
	Thin permanent marker (to complete labels)	1
Collection	Gravity Corer head (gravity, with 20m cable and messenger)	1
	Extra-long cable (for 20m and deeper lakes)	1
	Core tube	1
	Sectioning tube (6.35 cm ID, line marked 5 cm from bottom of tube)	1
	Sectioning stage	1
	Extruder rod	1
	Syringe (60 mL) with tubing siphon overlying water	1
	Core plug	1
	Screwdriver	1
	Gloves (latex/nitrile, non-powdered)	1
	Stainless steel bowl	1
	Stainless steel spoonulette	1
	Large tub or bucket	1
	Metric tape measure	1
	Scrub brush	1
	Alconox or other phosphate-free detergent	As needed
	Aluminum foil	As needed
	KimWipes or paper towels	As needed
Storage & Preservation	Glass jar (60 mL, amber) [SEDC]	1
	Glass jar (120 mL, amber) [SEDO]	1
	Zip top bag (1 quart) [SEDG]	2
	Wet Ice	As needed
	Cooler	
	Plastic electrical tape	

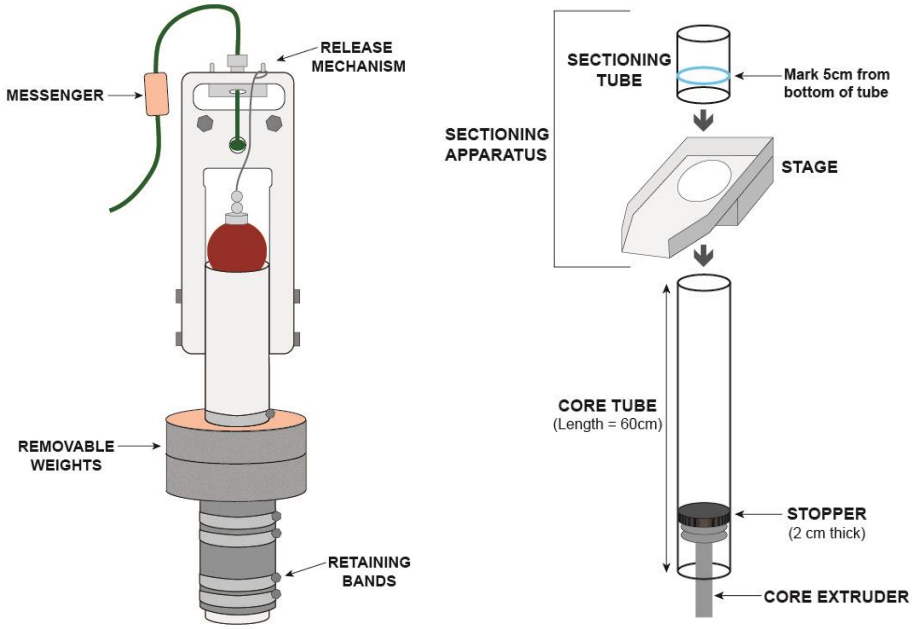

Figure 5.8 Illustration of the gravity corer, core tube, and sectioning apparatus.

5.8.3 Sampling Procedure

Collect two sediment cores that are at least 6 cm long from undisturbed sediments at or near the index site and section off 5 cm of sediment from the top of the cores for analysis.

The composition and texture of the bottom will vary from lake to lake and, in some lakes, it will be impossible to obtain a 6 cm core because the bottom is too rocky, the sediments are too dense, or, if it is a shallow lake, there are macrophytes covering the bottom. It is okay to move from the index site to attempt to collect sediment samples. Use best professional judgement and a minimum of three attempts to try to collect sediment samples in the lake.

If you collect a core less than 6 cm long on your first try, move to another location near the index site to find an area with a softer bottom. In addition, you can experiment with getting improved penetration by adding additional weight to the corer, and/or by releasing the corer further above the sediments. If a 6 cm core sample cannot be collected at one location, you may combine multiple cores with a combined depth that equals 10 cm (i.e., obtain the necessary volume of sediment). In this case, note the total number of cores and approximate depth intervals collected (but with no single core exceeding 5 cm) in the comments section of the **Index Sample Collection** form. The procedures for collecting and processing sediment cores are presented below. Figure 5.9 provides an overview of the sediment sampling process and Figure 5.10 provides a detailed diagram of the processing of a single sediment core.

5.8.3.1 Sediment Core Sample Collection

1. Wear surgical gloves at all times during sample collection to protect yourself from any potential contaminants in the sediments, and to prevent contamination of the sample from trace contaminants on the skin of the sampling crew.
2. If the bottom has been disturbed during the initial depth determination or for any other reason, move at least 5 m to take the core. It is critical that the corer strikes undisturbed surface sediments.

3. Insert the core tube into the sampling housing apparatus and tighten the hose clamp screws to secure the tube. Note that the hose clamps are labelled as yes/ no, only loosen and tighten those labelled yes. If the lake depth is greater than 20 m, change the sediment corer cable to the supplied 55 m rope. Be sure to use a very secure knot. Ensure the messenger is attached to the sampler line. Set the release mechanism.

4. Slowly lower the corer through the water column until the bottom of the core tube is just touching the sediment surface. Raise the corer 0.5 to 1 m and, while maintaining a slight tension on the line, lower the corer allowing it to settle into the bottom substrate. Immediately after the corer drops into the sediments, maintain line tension to prevent the corer from tilting and disturbing the core sample. Keep in mind that the goal is to obtain a core at least 6 cm in length. If this core length is not obtained the first time, the operation might need to be repeated at a new location using additional weights on the corer and/or a greater release height in order to improve penetration and obtain a longer core. If the core length exceeds the length of the core tube, the operation might need to be repeated at a new location using less weight on the corer and/or a shorter release height.

5. Trip the corer by releasing the messenger weight so that it slides down the line. Keeping the line vertical and keeping tension on the line will help ensure that the messenger reaches the sampler and trips the mechanism.

6. Slowly raise the corer back to the surface, keeping the bottom of the core tube under the water.

7. While keeping the bottom of the core tube under water, reach under the surface with a gloved hand and plug the bottom of the corer with a corer tube plug. To do this without disturbing the water-sediment interface, you cannot tilt the corer more than 45 degrees. (Note: core tube plugs are easily lost; ensure that spares are available at all times.)

8. Keeping your hand under the corer tube plug, raise the corer into the boat in a vertical position. Stand the corer in a large tub or bucket to prevent contaminating the boat with sediment material.

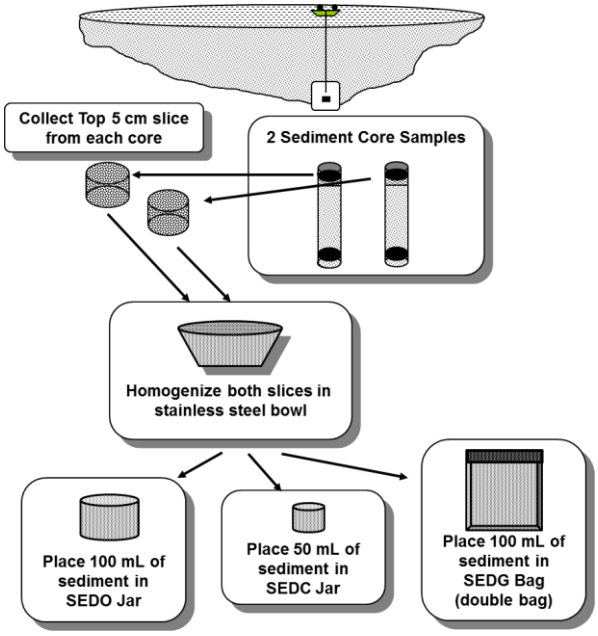

Figure 5.9 Sediment core sample summary.

5.8.3.2 Sediment Core Processing

1. Record the sample IDs for each of the samples on the **Index Sample Collection** form.
2. Put on clean gloves. Record the Site ID, date, and visit number on sediment core sample labels. Prepare containers and attach the labels to one 120 mL glass container (SEDO for sediment contaminants), one 60 mL glass container (SEDC for sediment TOC), and a one-quart plastic bag (SEDG for sediment grain size). IMPORTANT: only handle containers with clean gloved hands, and keep the sample jars in the provided plastic bags whenever possible.
3. Detach the core tube from the corer. One crew member should hold the sampler in a vertical position while the second person dismantles the unit. Note: only loosen the hose clamps labelled 'yes'.
4. Position the extruder under the corer tube plug at the base of the coring tube. Supporting both the core tube and the extruder in a vertical position, **slowly** lower the coring tube onto the extruder until the sediment is approximately 1 cm below the top of the tube. This operation is best done with the core tube standing in a bucket or tub to catch the discarded water.
5. Remove the remaining water above the sediment core by using a syringe with tube (or turkey baster) so that the surface sediments are not disturbed. Wait a few minutes for flocculent matter to settle, when possible. Decant surface water from the core to minimize dilution of the sediment sample.
6. Secure the sectioning stage onto the top of the coring tube. Place the Plexiglas sectioning tube (marked with a line 5 cm from the bottom) on the stage directly over the coring tube. Slowly extrude the sediment core into the sectioning tube until the top of the sediment reaches the 5 cm line on the sectioning tube. Slide the sectioning tube onto the flat part of the stage and use the spoonulette to scrape the top 5 cm section of sediment into a clean stainless steel bowl.
7. Rinse core tube, sectioning tube, and stage with lake water to remove particles and repeat sample collection and sediment core processing through step 6 until a total of 10 cm of sediment core have been sectioned into the bowl.
8. Store the first sample in the bowl covered with aluminum foil and on a bag of ice or in an unused cooler out of direct sunlight while collecting the second core.
9. Remove any twigs, stones or trash from sample then mix using a clean stainless steel or Teflon spoon that has been rinsed with ambient site water. Stir the sediments to homogenize the sample for one minute or until the sample is completely visibly blended.
10. Divide the composite into the three sample types listed below.
    a. **Contaminants [SEDO]:**
        i. Using a clean stainless steel spoonulette, carefully place approximately 100 mL of sediment into the 120 mL jar. Be sure that ½ inch headspace is available to avoid breakage due to possible sample expansion from freezing in the laboratory. Clean kimwipes may be used to wipe the outer thread so that the cap screws on tightly.
        ii. Replace the lid and seal tightly with plastic electrical tape, wrap the jar in the provided foam sleeve to protect it from breakage, replace sleeve back in the bag, and place the sample in a cooler with wet ice.
    b. **Total organic carbon [SEDC]:**

i. Place approximately 50 mL of sediment into the 60 mL jar. Leave ½ inch headspace to avoid breakage due to possible sample expansion from freezing in the laboratory.

ii. Replace the lid and seal tightly with plastic electrical tape, wrap the jar in the provided foam sleeve to protect it from breakage, and place the sample in a cooler with wet ice.

c. Sediment grain size [SEDG]:

i. Place approximately 100 mL of sediment into the pre-labeled bag. If needed, wipe any sediment off the zipper with a Kimwipe to close the bag. Double bag the sample into a second quart sized plastic bag, ensuring that the tops of both bags are sealed tightly and that the label is clearly visible.

ii. Place the sample on wet ice. The sample must be stored at 4°C, and MUST NOT BE FROZEN. This sample will not be frozen during later handling, so precautions regarding head space are not necessary."

11. Sediment equipment cleanup

a. After sample collection is complete, triple rinse all equipment with site water. Use a scrub brush as needed to remove particles.

b. At shore, spray core tube, sectioning tools, bowls, and mixing spoons with Alconox or other phophate-free detergent, scrub to remove particles if needed, and rinse with tap water followed by a final rinse with DI water.

c. Once the core tube and staging equipment is cleaned, store them in the carrier.

d. Once the bowl and spoons are decontaminated, wrap with aluminum foil in preparation for the next site.

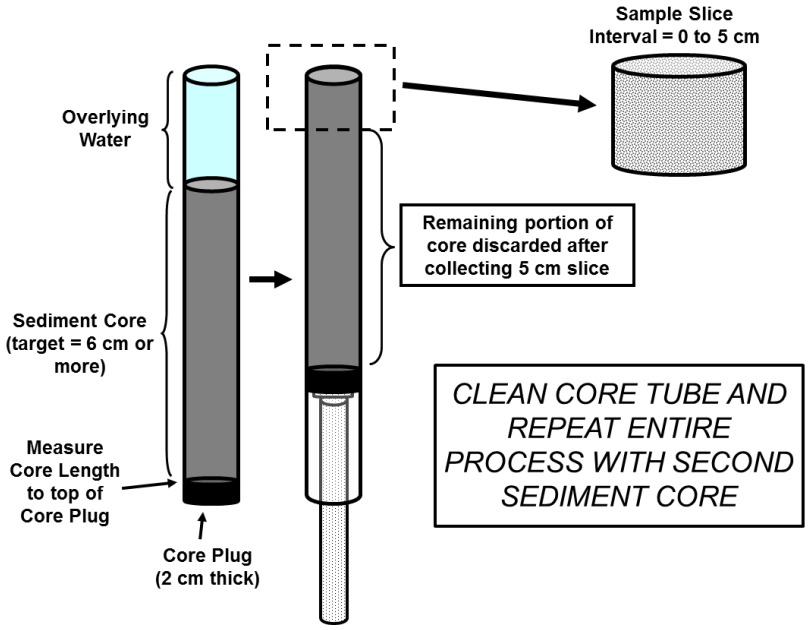

Figure 5.10 Sediment core sample processing - detail.

6.0 LITTORAL AND SHORELINE ACTIVITIES

To better understand near-shore habitats and their condition, travel to 10 evenly spaced physical habitat (PHab) stations around the lake and document conditions and characteristics observed within defined plot areas. The full set of measurements, observations, and sampling described in this chapter includes:

- measures or observations of physical habitat cover and structure in the littoral, shoreline, draw-down, and riparian zone plots at the 10 PHab stations and
- sampling of benthic macroinvertebrates at each of the 10 stations

For lakes with a surface area of greater than 10,000 ha (defined as large lakes), crews are **not** required to travel to the 10 PHab stations to make physical habitat measurements and collect benthic macroinvertebrate samples. The requirement was waived on large lakes because of the increased level of effort required to travel around the shorelines of these large lakes. Nevertheless, we encourage crews to complete the physical habitat characterizations and macroinvertebrate collections on large lakes, just as they are done on smaller lakes, so that large lake physical habitat information can be included in the national assessment.

Note: When large islands are present more than 10 PHab sites will be identified and assessed for a lake.

6.1 Physical Habitat Characterization

6.1.1 Summary of Method

Prior to the sampling visit, determine the approximate locations of the 10 PHab stations and mark them on a Site Map (see Chapter 3), if applicable. Figure 3.2 shows example placement and distribution of PHab stations around a lake. At each of the 10 PHab stations, you will set up a plot as shown in Figure 6.1 based on visually estimated dimensions. The plot measures 15 m wide, and includes a littoral plot extending 10 m out from the shoreline, a drawdown zone plot extending inland from the shoreline to the normal high-water level, a 1 m shoreline zone band at the shore just above the present water line, and a 15 m wide riparian plot that begins at the normal high water mark and extends 15 m landward. The drawdown zone plot extends a variable distance inland depending on the degree of drawdown and, if the distance from the present shoreline to the normal high water mark is negligible (i.e., <1m), there will be no drawdown zone plot.

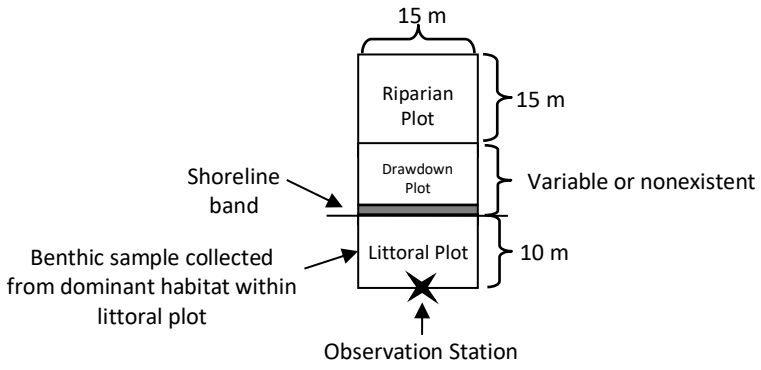

Figure 6.1 Dimensions and layout of a physical habitat station.

As described below, you will record observations from each of the zones on the **Physical Habitat** form.

6.1.2 Equipment and Supplies

Table 6.1 lists the equipment and supplies needed to locate the PHab stations and conduct the physical habitat characterization.

Table 6.1 Equipment and supplies – physical habitat assessment.

Type	Item	Quantity
Form	NLA 2017 Verification	1
	NLA 2017 Physical Habitat	10
Collection	Depth Sounder (hand-held or boat mounted sonar)	1
	Sounding rod (3 m, marked in 0.1 m increments, calibrated, PVC)	1
	GPS unit (with manual, reference card, extra battery)	1
	Rangefinder (for estimating horizontal drawdown)	1 (optional)
	Clinometer (for use as a level to measure vertical drawdown)	1 (optional)
	Surveyors rod (for measuring vertical drawdown)	1 (optional)
	50-meter tape (measurements as needed)	1
	Binoculars (for making observations of distant riparian)	1
	Map wheel or string (for measuring shoreline distances on site map)	1
	Anchor (with 75 m line or sufficient to anchor in 50 m depth)	1
	Buoy (for marking observation point)	1

6.1.3 Locating the Physical Habitat Stations and Defining the Shoreline Boundary

6.1.3.1 Base Site Activities

It is important that you set up PHab stations in the office to minimize bias in site selection and to ensure efficient location of stations once at the lake.

1. Using a lake map, select a random starting point on the lake outline. Any reasonable method may be used select the starting point (e.g., toss a pencil randomly on the map, letting the sharp end point to the nearest shoreline location). This random starting point is your “A” station.
2. It is important that the remaining nine stations be located at equal distances around the lake (see Figure 3.2). These will be your “B” through “J” stations. Field crews can do this manually (by either using a string to trace the perimeter of the lake, which can then be straightened and marked in equal intervals, or by using a map wheel) or electronically (with GIS or other digital mapping tools) to measure the perimeter of the lake and divide by 10.
3. Using a GIS or other digital mapping tool application to locate the coordinates of the 10 stations that can then be entered as GPS waypoints greatly facilitates correctly locating PHab stations by boat in the field, especially on large lakes.
4. Mark the physical habitat stations on a site map, if applicable.

Note: In revisit lakes (see Section 8.1 for more information), crews will re-randomize and relocate the PHab stations. The stations are re-randomized because the revisit data is used to examine variability of the entire lake assessment.

6.1.3.2 Littoral and Shoreline Activities

Using the site maps and GPS, proceed by boat around the lake, locate, and stop at each of the 10 PHab stations. Position the boat at a distance of 10 m from shore, anchor if necessary, and make the

measurements and semi-quantitative observations specified on the **Physical Habitat** form. Complete a separate **Physical Habitat** form for each station.

Make every reasonable attempt to record physical habitat observations and measurements for all 10 PHab stations. Where physical habitat observation and measurements are impossible, record flags as specified in Table 3.1. Remember that numbered "F" flags pertain to the front and back side of each individual form (e.g., you can assign an F1 flag to mean something different at different habitat stations). Similarly, "F" flags do not carry over from one form to the next, so all "F" flags entered on a form must be defined on that same form.

6.1.3.3 Shoreline and Station Location Adjustments

Once in the field, you may encounter situations that require you to modify the shoreline and/or station location(s) from the intended locations marked on the site map. If this occurs, make the corrections and adjustments on the **Physical Habitat** form and note the reasons in the comments section of the form. The general guidelines for locating or modifying the location of the littoral and shoreline stations are summarized below.

1. Locate station using maps, aerial photos, or GPS units.
2. Define shore as either the current waterline OR the boundary between open water and the edge of dense vegetation (terrestrial, wetland, or emergent vegetation) or extensive very shallow water (shoreline defined by limit for navigating your boat).
3. If the shoreline observed in the field differs from the mapped shoreline, mark "Station Relocated" and enter a comment on the **Physical Habitat** form stating the apparent reason (e.g., drought, lake drawdown, flooding, dredging, limited boat access, etc.).
4. If a PHab station is inaccessible because of shoreline changes, mark "Station Relocated" at the top of the **Physical Habitat** form, and position one or more new stations at approximately equal intervals.
5. If a station is eliminated, fill in the "Dropped" bubble at the top of the form.
6. If the shoreline observed in the field differs radically from the site map and you are sure you are at the correct lake, you can sketch a map of the lake. Use a string to measure the new outline, divide it into 10 equal parts, and lay out the 10 station locations.

6.1.3.3.1 Islands

Islands may be an additional source of shoreline habitat on a lake and those will be accounted for by adding island physical habitat stations. Island stations are in addition to the 10 stations (A-J). The guidelines for adding island stations follow:

- If the combined shoreline of all islands make up 10-20% of the lake's total shoreline, add one PHab station (stations will now be labeled A through K)
- If the combined shoreline of all islands make up more than 20% of the lake's total shoreline, add two PHab stations (stations will now be labeled stations A through L)
- Island stations are designated by marking the "Is it an island?" bubble on the form, by a new station letter (K or L), and by marking the island location and station on a site map.

Island stations, i.e., K and L, should be selected at random (e.g., toss a pencil randomly on a map, letting the sharp end point to the nearest island location, if needed, repeat to identi.fy station L).

6.1.3.3.2 Ambiguous Shorelines

The shoreline is defined as the interface between "lake-like" conditions and riparian or wetland conditions. In most cases, the shoreline will be easily identified as the current waterline. In some instances, however, the shoreline might not be obvious. Listed below are some general situations and rules that should be applied to ambiguous shorelines.

- If there has been a significant drop in lake level due to drought, purposeful drawdown, dam repair, or other reasons, shallow areas may be exposed that are usually covered with water. In this case, consider the current waterline as shoreline for the purposes of this survey, not the normal waterline.
- If there are extensive very shallow areas or shoals, consider the shoreline to be the boundary between the shallow area and deeper open water, as defined by ease of access by a small sampling boat.
- If access to the true shoreline is prevented by an area of dense aquatic or terrestrial vegetation, consider the shoreline to be the boundary between the vegetation and deeper open water. Again, define the operational shoreline by ease of access by a small sampling boat. This may result in a riparian zone that can be more of a wetland than an upland vegetation plot.
- If a river or stream enters a lake, the shoreline begins where no flow is visible.
- If there is flooding, try to find the position of the normal high water mark. The normal high water mark may be evident by the extent of flooded trees or other terrestrial vegetation.

6.1.3.3.3 Actual shoreline is different than appears on the map

The goal of the physical habitat survey is to characterize the lakeshore based on observations of conditions at 10 evenly spaced PHab stations around the lake. Adjustments to station locations might be needed if the field crew runs into unusual conditions or problems. Below are some rules concerning modifications to the station location(s).

- If only a small portion of the shoreline differs and it does not affect, or only slightly affects, a PHab site location, sketch the lake shoreline on the site map and reposition the station (if needed).
- If the difference causes a contraction of the shoreline and a PHab station location is lost, sketch the lake shoreline on the site map and make a decision to either: (a) keep the station, relocate it on the revised shoreline map and adjust some or all other stations in order to keep stations evenly spaced around the lake (i.e., keep 10 stations), or (b) eliminate the station altogether (i.e., reduce the number of stations).
- If the Site Map does not in any way match the lake shoreline, draw a new sketch map approximating the shoreline, and re-establish the 10 PHab stations. A quick way to locate 10 evenly-spaced PHab stations is to: (a) lay a piece of string on the lake perimeter, (b) pick up the string, measure it, and mark out 10 equal parts, and (c) lay the string back on the perimeter and use the marks to locate the 10 sites on the map.

6.1.3.3.4 PHab Station is inaccessible

- If a PHab station is inaccessible, you must make a decision to either: (a) relocate the station and adjust some or all other stations so that they are evenly spaced around the lake (i.e., keep 10 stations), or (b) eliminate the station altogether (i.e., reduce the number of stations). The size of the lake will help drive this decision.

- Draw all adjustments to the shoreline based on field observations directly on the Site Map and explain the adjustments in the comments section of the **Physical Habitat** form.

6.1.3.3.5 Identifying Relocated and New Stations on the Form

Use the following notations when recording station location modifications.

- If you relocate a station, note the new location on the Site Map and fill in the bubble corresponding to the original station letter (e.g., "C") on the **Physical Habitat** form. In addition, fill in the "Station Relocated" bubble on the form to indicate that the station has been moved from its originally intended location.
- If a station is lost and cannot be replaced, cross out the original station location on the Site Map, fill in the bubble corresponding to the original station letter, and fill in the "Dropped" bubble on the **Physical Habitat** form.

6.1.4 Establishing the Physical Habitat Plots at each station

Establish a plot for physical habitat characterization at each PHab station by visually estimating the plot dimensions. Most littoral, shore, and near-shore observations and measurements can be made from the boat at the observation point 10 m off-shore (estimated by eye). Limit observations at each station to the area that is within the defined plot dimensions (additional observations of human activities are made adjacent to or behind the defined plots). After setting plot dimensions, you may need to move around within the littoral plot to see or probe the bottom, or even go onto shore to make observations.

6.1.4.1 Physical Habitat Plot Dimensions

You will identify up to four distinct zones within each physical habitat plot (Figure 6.1), where you estimate the zone dimensions by eye or with the aid of a rangefinder.

6.1.4.1.1 Littoral

This within-lake zone is a fixed size that is 15 m wide along the shoreline and extending 10 m offshore into the lake.

6.1.4.1.2 Shoreline

The shoreline band is a fixed 15 m wide strip along the shore from the present water line to 1 m inland. If the horizontal drawdown distance is <1 m, the shoreline band is within the riparian plot; if the horizontal drawdown is >1 m, the shoreline band is within the drawdown plot. The shoreline boundary is defined as the approximate interface between "lake-like" conditions and riparian or wetland conditions. In cases where the lake shoreline is not obvious (e.g., where there is evidence of large seasonal change in lake level), define the shoreline as the current waterline. In cases where the lake shoreline is not visible, define the lake shoreline as the approximate boundary between open water and swamp or marsh conditions into which your boat could not easily move.

6.1.4.1.3 Drawdown

Under all circumstances, vertical height and horizontal distance of drawdown or water level fluctuations are measured or estimated. When horizontal drawdown is greater than 1 m, establish a drawdown zone plot with a fixed width (15 m) but with a variable extent inland. The inland extent of the drawdown plot is equal to the horizontal extent of drawdown and may differ among the 10 PHab stations, depending on the topography at each station. It is determined by your judgment and measurement of the horizontal drawdown distance from the shore to the normal high water mark at each station.

The vertical height can be visually estimated or measured using a clinometer as a level in combination

with a survey rod or metric tape measure. Similarly, the horizontal distance up the bank between the current lake level and the evidence of the normal high water level can be estimated visually, or measured with with a laser range finder, survey rod or metric tape measure when distances are greater than approximately 15m.

The intent of the drawdown assessment is to capture and track changes in the magnitude of lake level fluctuation or progressive decline or drawdown of lake levels relative to the normal high water mark. The normal high water mark is best indicated by the terrestrial vegetation and substrate present. In most regions of the US, summertime lake levels will be below the high water mark, so the NLA assessment may register a small amount of drawdown for most lakes. Negligible drawdown depends, to some extent, on the size of the lake. For NLA, an assessment of the vegetation and potential fish cover in the drawdown plot is not required when horizontal exposure of the littoral bottom is <1m. The vertical and horizontal drawdown must still be measured and recorded in order to establish the range of natural variability in lake level fluctuations.

6.1.4.1.4 Riparian

At stations that have less than 1 m horizontal distance of lake-level fluctuations or anthropogenic drawdown, there will be no drawdown zone and you will therefore define the riparian plot as a 15 m x 15 m square located at the water's edge, as defined in section 6.1.4.1.2, extending 15 m inland. When a drawdown plot is defined, the riparian zone plot is a 15m x 15m square just inland of the drawdown plot (i.e., a fixed size 15 m wide along the shoreline and extending 15m inland from the normal high-water mark.

6.1.4.2 Physical Habitat Station Layout Procedures

6.1.4.2.1 Normal High Water

1. Using the present shoreline, place the littoral plots lakeward from the current water's edge or the operational shoreline as defined in previous sections.
2. Draw or sight a straight line inland perpendicular to the shoreline. If this line does not intersect with the normal high water mark, move the littoral plot laterally in either direction until the perpendicular line intersects the normal high water mark. Choose to move the plot left or right based on the direction which results in the least distance moved.
3. Establish a 1 m shoreline band at the current water's edge or the operational shoreline.
4. Establish the riparian plot inland from the current water's edge or the operational shoreline identified above. If using an operational shoreline, the riparian plot may include shallow water and/ or impenetrable wetland.
5. The left and right edges of the riparian plot, the shoreline band, and the littoral plot should all align with one another.

6.1.4.2.2 Below Normal High Water (Drawdown)

1. Using the present shoreline, place the littoral plots from the current water's edge or the operational shroreline lakeward as defined in previous sections.
2. Draw or sight a straight line inland perpendicular to the shoreline. If this line does not intersect with the normal high water mark, move the littoral plot laterally in either direction until the perpendicular line intersects the normal high water mark. Choose to move the plot left or right based on the direction which results in the least distance moved.

3. Establish a 1 m shoreline band at the current water's edge or the operational shoreline.
4. Establish the riparian plot inland from the normal high water mark identified above.
5. Establish a drawdown plot between the littoral and riparian plots. If the drawdown plot is greater than 1 m, cover estimates will be assessed in this plot.
6. The left and right edges of the riparian plot, the shoreline band, the littoral plot, and the drawdown plot should all align with one another.

6.1.4.2.3 Above Normal High Water (Flooding)

1. Establish the littoral plots lakeward from the normal high water mark, which may be evident by the extent of flooded trees or other terrestrial vegetation, lakeward.
2. Establish the riparian plot inland from the normal high water mark. In flooded situations, the riparian zone might be dry, partly flooded, or completely flooded with lake water.
3. Establish a 1 m shoreline band at the current water's edge. In flooded situations, the shoreline band might be within or inland from the riparian plot.
4. The left and right edges of the riparian plot, shoreline band, and the littoral plot should all align with one another.

6.1.5 General Observations

Begin the physical habitat characterization with general observations.

1. Set up your plots within your physical habitat station.
2. Measure and record the lake depth 10 m from the shore at each PHab station (observation point)
3. Note on the **Physical Habitat** form whether there is shoreline flooding (i.e., observed water level presently above the normal high water mark) by filling in the Yes or No bubble.
    a. If flooding is present, try to find the position of the normal high water mark, which may be evident by the extent of flooded trees or other terrestrial vegetation. Measure the depth at this point; record this as the "depth of flooding." Measure or estimate the distance from this normal high water position landward towards the margin of flooding; this establishes the location of the 1m shoreline band and the horizontal distance of flooding. The riparian plot is located as a 15 x 15 meter square abutting the normal high water mark, regardless of whether it is dry, partly flooded, or completely flooded with lake water.
    b. If the current water level is not above normal high water, enter zeros for height and horizontal distance of flooding.
4. Note on the **Physical Habitat** form whether the horizontal drawdown distance is greater than 1m by filling in the Yes or No bubble. Regardless of the amount of drawdown of lake level fluctuation, measure or estimate it by recording the vertical height and the horizontal distance between the present lake level and the normal high water line. If the horizontal drawdown distance is <1m, drawdown zone cover estimates are left blank in the form, but you still need to measure the vertical height and horizontal distance of drawdown. Note that these

measurements or estimates may be zeros if the water level is at or above the normal high-water mark, but should never be left blank.

5. Record the bank angle description that best reflects the current shoreline that is dominant within your field of vision in the 1 m shoreline band:
    a. Near vertical/undercut (>75 degrees);
    b. steep (>30 to 75 degrees; need hands to climb up);
    c. gradual, (5 to 30 degrees; can walk up); or
    d. flat (< 5 degrees).

    NOTE: Complete this estimate even if there is no drawdown.
6. Record the presence or absence of water surface scums, algal mats, or oil slicks within the littoral zone.

6.1.6 Estimate Substrate Characteristics

You will estimate and assign percentage areal cover for substrate types (e.g. bedrock, boulders, cobble, gravel, sand, silt/clay/muck, woody debris, organic matter, and vegetation) and also for fish habitat cover, aquatic macrophytes, and terrestrial vegetation. The categories are as follows:

- 0 = absent (0% cover)
- 1 = sparse (<10% cover)
- 2 = moderate (10 – 40% cover)
- 3 = heavy (>40 – 75% cover)
- 4 = very heavy (>75% cover)

When estimating cover combinations in the substrate section of the **Physical Habitat** form, combinations consider that the combined cover of the various types of substrate should add up to approximately 100%. Because you are assigning cover percentage categories, look at various combinations of the high and low end of each class. Accordingly, more than one class might be given sparse (1), moderate (2), or heavy (3) ratings. One dominant class with no clear subdominant class might be ranked very heavy (4) with all the remaining classes either sparse (1) or absent (0). Two dominant classes with more than 40 percent cover can both be given a 3.

Estimate the areal cover of bottom substrate types and particle size classes observed within the littoral and the shoreline zones. Cover categories range from absent to very heavy. Record all observations by filling in the appropriate bubbles on the **Physical Habitat** form. In most cases these estimates can be made from the boat or can be made while wading, if preferred. Substrate types not present should be assigned zero cover.

- If the bottom substrate is not visible, you should probe the bottom beneath the boat with the sounding rod (you may have to move closer to shore if you are too deep to use the rod). Soft sediment can be brought to the surface for examination. Hard sediments can be "felt" with the sounding rod. Sandy substrate can be "felt" or "heard" by twisting the sounding rod and detecting grittiness. Estimating cover of various substrate types will typically require multiple probes within the littoral plot. If you have to move into shallow water to use the sounding rod to observe sediment characteristics, flag the observation and record the depth where you observed the sediment.
- If the bottom is covered with materials other than mineral substrates, choose “Woody Debris”, “Organic (leaf pack, detritus)”, or “Vegetation/Other”.

- If the substrate is concealed and remote sampling is not possible, use "Not observed" flag (K).

Record the color of sediment within the littoral plot. Select "None" or "Other" if the sediment does not match one of the color categories options on the **Physical Habitat** form.

Record sediment odor within the littoral zone. For sediment odor, the choices are "H_2S" (sulfurous, rotten egg), "Anoxic" (sewage odor), "Chemical" (strong odor like turpentine, paint, etc.), "Oil", or "Other" (including musty, organic, and fishy odors). If "Other" is indicated, explain the observation in the comment section of the form.

6.1.7 Estimate Aquatic Macrophyte Cover

Note and record whether macrophytes extend lake-ward from the observation point (e.g., further than 10 meters from the shoreline).

Estimate the areal cover of submerged, emergent (i.e., has erect portions above the water surface), floating/floating leafed (either rooted or non-rooted vegetation), and total macrophytes within the littoral zone. Cover categories range from absent to very heavy, as described in 6.1.6. Each of the three types of aquatic macrophyte (submergent, emergent, floating) can have cover ranging from 0 – 100%. They are evaluated independently, so the sum of their separate covers is theoretically 0% to 100% times the number of types. So, in contrast to substrate cover percentages, the combined cover of submergent, floating, and emergent aquatic macrophytes could theoretically add up to 300% due to the overlap of plant types within the water column. The fourth question on the form about aquatic macrophyte cover asks how much areal cover is there if you ignore the types and just estimate how much of the littoral plot has aquatic macrophyte cover of any type, where this value is constrained to 0-100%.

As with substrate, estimating aquatic macrophyte cover may require multiple probes within the littoral plot. Record all observations by filling in the appropriate bubbles on the **Physical Habitat** form. These estimates can be made from the boat or while wading.

- If you cannot see or probe the bottom, move closer to shore, flag your observation and note your new location and depth in the comments field on the form.

6.1.8 Estimate Fish Habitat Cover

Estimate the areal cover of potential fish habitat observed within the littoral plot and, when present, in the separate drawdown zone plot. Littoral fish habitat cover features are within or partially within the water and conceal fish from aquatic and terrestrial predators such as large fish, otters, kingfishers, and osprey. When evaluating cover of potential fish habitat in the drawdown zone, however, estimate the percentage cover that would be present at normal high water conditions, when these features would be inundated. Cover categories range from absent to very heavy, as specified in Section 6.1.6. Record all observations by filling in the appropriate bubbles on the **Physical Habitat** form. In most cases these estimates can be made from the boat. Estimating fish habitat cover may require multiple probes within the littoral plot. If certain cover types are not present, mark absent (0% cover) as your entry. In contrast to the cover of substrate types, the sum of the various types of fish habitat features can exceed 100%, sometimes summing to several hundred percent. Each type of fish cover can have cover ranging from 0 to 100%. They are evaluated independently, so the sum of their covers is theoretically 0 to 100% times the number of cover types. Estimate and record cover for the following fish habitat types:

- Aquatic macrophytes and Inundated Herbaceous Vegetation: Submerged, floating, or emergent live aquatic or non-woody herbaceous plants
- Woody Debris/Snags: Inundated or partially inundated dead trees, branches, or rootwads

with diameter >0.3 m (1 ft)

- Woody brush/woody debris: Inundated dead or living woody vegetation <0.3 m diameter.
- Inundated Live Trees: Inundated portions of trees >0.3 m in diameter
- Overhanging Vegetation: <1 m from the water surface (do not include higher overhanging vegetation, which might provide perches for birds such as kingfishers)
- Ledges or Sharp Drop-offs: Overhanging banks, submerged rock shelves, and steep sloping rock walls
- Boulders: Larger than basketball size
- Human Structures: Docks, barges, houseboats, swimming platforms, tires, car bodies, and habitat enhancement structures (e.g., log rafts)

Note: In the drawdown zone you will estimate the potential fish cover. The potential fish cover estimates are made only if there is a visible drawdown zone extending >1 m from the shoreline. For these observations, the question is "What cover would there be if the drawdown zone were inundated – i.e., it were to become part of the littoral zone? " Then, for example, a bunch of dried aquatic macrophtes would be "Aquatic and Inundated Herbaceous Vegetation", as would newly-grown terrestrial grasses. Cyprus trees left "high and dry" would qualify as "Inundated Live Trees >0.3m diameter" and overhanging vegetation rooted above the drawdown zone could be "Overhanging Vegetation within 1m of the Surface".

6.1.9 Estimate the Cover and Type of Riparian and Drawdown Zone Vegetation

Three independent layers of riparian vegetation will be examined, each of which can range in cover from 0 to 100%. The ground layer components must add up to 100% because this layer includes bare ground and water. The mid-layer and upper layer do not include ground or water, so vegetation within these layers do not have to add up to 100%.

The areal cover of different types of vegetation will be estimated in the riparian plot and, when present, in the drawdown zone. Vegetation cover is divided into three layers, which are described below. Remember that individual plants can contribute cover to more than one layer and cumulatively the three layers can potentially add up to 300% cover (up to 100% per layer). Also note that some things other than vegetation are possible entries for the "Ground Cover" layer (e.g., water or barren ground). Within each layer, the summed cover cannot exceed 100%, but, the ground layer covers must add up to 100%. As with the other visual cover estimates, you are assigning cover percentage categories, so the summed cover is estimated by looking at various combinations of the high and low end of each percentage cover class.

6.1.9.1 Canopy Vegetation (greater than 5 m high)

Record the type of vegetation in the canopy as deciduous, coniferous (needle-leafed evergreen), broadleaf evergreen, or mixed, where mixed is defined as a segment wherechosen if there is more than one of these types of vegetation that has at least 10% areal coverage. If no canopy exists in the plot, do not mark any of the bubbles.

Estimate the areal cover of big (trunk >0.3 m diameter at breast height [dbh]) and small (trunk <0.3 m dbh) trees. Cover categories range from absent to very heavy, as described in Section 6.1.6. Record all observations by filling in the appropriate bubbles on the **Physical Habitat** form.

Total cover in the canopy layer can range anywhere from 0% to 100% depending on conditions present.

6.1.9.2 Understory Vegetation (5m to 0.5m high)

Record the type of vegetation in the understory as deciduous, coniferous, broadleaf evergreen, or mixed, where mixed is defined as above. If no understory exists in the plot, do not mark any of the bubbles.

Estimate the areal cover of all woody vegetation (which includes both the trunks and branches of trees and shrubs, woody stems of perennial plants, etc.) and tall herbs, grasses, and forbs. Cover categories range from absent to very heavy, as specified in Section 6.1.6. Record all observations by filling in the appropriate bubbles on the **Physical Habitat** form.

Total cover in the understory layer can range anywhere from 0% to 100% depending on conditions present.

6.1.9.3 Ground Cover (lower than 0.5m high)

Estimate the areal cover of woody vegetation; tall herbs, grasses, and forbs; standing water or inundated vegetation; and barren, bare dirt, or buildings. Areas of exposed rock or bedrock should be considered 'barren'. Cover categories range from absent to very heavy, as specified in Section 6.1.6. Record all observations by filling in the appropriate bubbles on the **Physical Habitat** form.

Ground cover is the only layer in which cover estimates should add up to roughly 100% (as opposed to ranging from 0-100% in the other two layers). Certain ground cover types should be considered mutually exclusive, i.e., if ground layer vegetation overlays barren ground, record the vegetation cover even though there is barren ground beneath it.

6.1.9.4 Considerations for Drawdown conditions

Drawdown Zone vegetation entries are located to the right of Riparian Zone Vegetation on the **Physical Habitat** form. They are filled out only if there is a drawdown zone extending >1 m from shore. Unlike the case with potential fish cover, record these vegetation estimates just as you see them --- i.e., do not in this case imagine that the drawdown zone is under water. For example: there must be water on the ground (e.g., puddles) to have an entry for "standing water or inundated vegetation" in the drawdown zone. Large trees rooted above the drawdown zone can contribute cover over the drawdown zone. Dried aquatic macrophyte vegetation cover is entered under "Herbs, Grasses, & Forbs" with comment that it is dried aquatic macrophytes. There may also be a lot of zeros for vegetation in the drawdown zone (especially if the drawdown is fairly recent).

6.1.10 Record Evidence of Human influence

You will record any observations of human influences within the littoral, riparian, and, when present, drawdown zone plots. Human influences within the littoral plot will be recorded in different locations on the form depending on whether or not there is a drawdown plot.

- When drawdown or lake level fluctuations are minimal, i.e., <1 m horizontal distance, there is no drawdown zone plot, and the drawdown zone human influence field will be left blank. Human influences in the littoral plot are recorded along with the influences in riparian zone plot and recorded in the riparian zone portion of the form.
- When a drawdown zone extending >1 m from shore is present, a drawdown plot is defined and human influences within both the drawdown and littoral zone are recorded in the drawdown portion of the **Physical Habitat** form.

Within each zone, observations are recorded as not present (0), present outside and/or adjacent to (P), or contained within (C) the plot area (Figure 6.2). Record all observations by filling in the appropriate bubbles on the **Physical Habitat** form. The proximity "P" zones have a defined width of 15 m as shown in Figure 6.2. If there is no drawdown zone plot, all C and P human disturbances in the littoral plot are recorded on the riparian part of the form along with human influences present in the riparian zone, and the drawdown portion of the human influences section will be left blank. In contast, if there is a drawdown zone, all C and P human disturbances in the littoral plot are recorded in the drawdown part of the form along with human influences present in the drawdown zone.

For each zone and influence, indicate the presence only of the influence 'closest' to the plot itself. Do not mark "P" for a particular influence type if it is already marked "C" in that zone (use the more influential proximity code). Human Disturbances absent (0) and within-plot (C) are straightforward. For 'Present but outside or adjacent to the plots' (P), use these guidelines:

- A disturbance is marked "P" if the disturbance is seen entirely outside of any of the plots, but is adjacent to (i.e., within 15 meters left or right hand side of the entire Littoral-Drawdown-Riparian plot), or behind, the riparian plot within the defined areas.
- A disturbance is also marked "P" if the disturbance is seen behind, but entirely outside of all of the plots but is visible looking on-shore through the three plot zones (littoral, drawdown, and riparian)
- As a result, a single disturbance that is adjacent to both plots would be marked "P" in both the Riparian-Littoral and the Drawdown zones.
- If there is a drawdown plot, the presence of a human influence item WITHIN THE LITTORAL PLOT is recorded as "C" in the DRAWDOWN portion of the form (e.g., consider the littoral and drawdown zones to be a single plot when drawdown is present).
- If there is NO DRAWDOWN PLOT, i.e., the riparian plot abuts the shoreline, then human disturbances in the littoral plot are recorded by entering "C" in the Riparian portion of the form (e.g., consider the littoral and riparian zones to be a single plot when no drawdown is present).

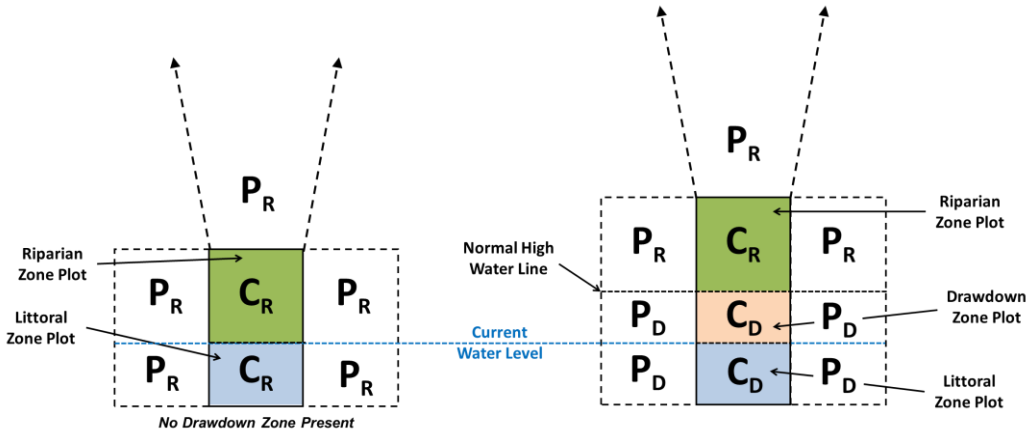

Figure 6.2 Human disturbance and proximity determinations
C_R = Marked on form as contained within riparian plot
P_R = Marked on form as adjacent to riparian plot
C_D = Marked on form as contained within drawdown zone
P_D = Marked on form as adjacent to drawdown zone

6.2 Benthic Macroinvertebrate Sampling

6.2.1 Summary of Method

Benthic macroinvertebrates are collected using a semi-quantitative sampling of multiple habitats in the littoral zone of lakes using a 500 µm mesh D-frame dip net (Figure 6.3). Sample collection is stratified on the following specific habitat types: rocky/cobble/large woody debris; macrophyte beds; fines (including mud, sand, or silt); and leaf packs.

Figure 6.3 D-frame net (500 µm mesh) used for collecting benthic macroinvertebrates.

6.2.2 Equipment and Supplies

Table 6.2 provides the equipment and supplies needed for field operations to collect benthic macroinvertebrates.

Table 6.2 Equipment and supplies – benthic macroinvertebrate collection.

Type	Item	Quantity
Form	NLA 2017 Littoral Sample Collection	1
Documentation	Inner and outer labels: Benthic samples	1
	Labels: Benthic extra jar	As needed
	Scissors	1
	Clear tape strips (to cover sample labels)	As needed
Collection	Kick net (500 µm D-shaped, modified) with 4-foot handle	1
	Spare net(s) and/or spare bucket assembly for end of net	As needed
	Bucket (5-gallon capacity, plastic)	1
	Sieve bucket (500 µm)	1
	Watchmakers' forceps	1
	Squirt bottle (1 L Nalgene) – lake water	1
	Spoon (stainless steel)	1
	Funnel	1
	HDPE bottle (1 L, white, wide-mouth)	1 or more
	Ethanol (95%)	2 gal
	Gloves (latex/nitrile, non-powdered, box)	2 pair
Storing and preserving	Cooler	1
	Plastic electrical tape	As needed

6.2.3 Sampling Procedure

6.2.3.1 Site Selection and Sample Collection

The process for selecting the PHab stations is described in Section 6.1. All benthic samples should be collected from the dominant habitat type within the 10 m x 15 m littoral zone component of each of the PHab stations (Figure 3.2). The sampling process is described below.

6.2.3.2 Sample Processing in the Field

Use a 500-µm mesh sieve bucket placed inside a larger bucket full of lake water while sampling to carry the composite sample as you travel around the lake.

6.2.3.2.1 Benthic macroinvertebrate sampling

1. After locating the sample site according to procedures described in the physical habitat section, identify the dominant habitat type within the plot from the classifiers below:
   - Rocky/cobble/large woody debris;
   - Macrophyte beds;
   - Fines (including mud, sand or silt); or
   - Leaf pack.
2. After identifying the dominant habitat type, use the D-frame dip net (equipped with 500-µm mesh) to sweep through one linear meter of the dominant habitat type at a single location within the 10 m x 15 m littoral zone sampling area, making sure to disturb the substrate enough to dislodge organisms.
   - When safe to do so, it is preferable that you exit the boat and disturb the substrate (e.g., overturn rocks, logs) using your feet while sweeping the net through the disturbed area.
   - Because a dip-net is being used for sampling, the maximum depth for sampling will be approximately 1 m (the length of the dip-net handle); therefore, in cases in which the depth of the lake quickly drops off, it may be necessary to sample in the nearest several meters to the shore.
3. After completing the 1 m sweep, remove all organisms and debris from the net and place them in a bucket following sample processing procedures described in the following section.
4. Proceed to the next sampling station and repeat steps 1-3. The organisms and detritus collected at each station on the lake should be combined in a single bucket to create a single composite sample for the lake. After sampling at all PHab stations is complete, process the composite sample in the bucket according to procedures described in the following section. One to five bottles should be sufficient to hold the composited sample from each lake.
   - If there is a large amount of debris (rocks, sticks, etc.) accumulating in the composite sample, remove debris between sampling stations, after the debris is inspected, picked, and/or washed to ensure no organisms are lost.
   - If your first collection at a sampling station results in too much debris, discard it, move the location within the same habitat station, and take another sample.
   - It is recommended that crews carry a sample bottle containing a small amount of ethanol with them to enable them to immediately preserve larger predaceous invertebrates such as hellgrammites and water beetles. Doing so will help reduce the chance that other specimens

will be consumed or damaged prior to the end of the field day. Crews should NEVER, however, attempt to 'field-pick' the samples.

6.2.3.2.2 Preparing composite samples for benthic macroinvertebrates

1. Pour the entire contents of the bucket through a sieve (or into a sieve bucket) with 500 µm mesh size. Remove any large objects and wash off any clinging organisms back into the sieve before discarding.
2. Using a wash bottle filled with clean lake water, rinse all the organisms from the bucket into the sieve. This is the composite sample for the lake.
3. Estimate the total volume of the sample in the sieve and determine how many jars (1 L jars, each no more than half-full with sample) will be required.
4. Fill in a sample label with the Site ID and date of collection. Attach the completed label to the jar and cover it with a strip of clear tape. Record the sample ID number for the composite sample on the **Littoral Sample Collection** form. For each composite sample, make sure the number on the form matches the number on the label.
5. Wash the contents of the sieve to one side by gently agitating the sieve in the water. Wash the sample into a jar using as little water from the wash bottle as possible. Use a large-bore funnel if necessary. If the jar is too full, pour off some water through the sieve until the jar is not more than half full, or use a second jar if necessary. Carefully examine the sieve for any remaining organisms and use watchmakers' forceps to place them into the sample jar.
6. If additional jars are needed, use a pre-printed benthos extra jar label or, if needed, fill in a blank sample label that does not have a pre-printed ID number on it. Record the ID number from the pre-printed label prepared in Step 4 in the "SAMPLE ID" field of the label. Attach the label to the additional jars and cover them with a strip of clear tape. Record the number of jars required for the sample on the Littoral Sample Collection form. **Make sure the number you record matches the actual number of jars used.** Write "Jar *N* of *X*" on each sample label using a waterproof marker ("*N*" is the individual jar number, and "*X*" is the total number of jars for the sample).
7. Place a waterproof label inside each jar with the following information written with a number 2 lead pencil:
   - Site ID;
   - Site name;
   - Date of collection;
   - Sample type;
   - Collector(s) names or initials; and
   - Sample ID.
8. Completely fill the jar with 95% ethanol (no headspace). It is very important that sufficient ethanol be used, or the organisms will not be properly preserved. Existing water in the jar should not dilute the concentration of ethanol below 70%. **NOTE:** Prepared composite samples can be transported back to the vehicle before adding ethanol if necessary. In this case, fill the jar with lake water, which is then drained using the net (or sieve) across the opening to prevent loss of organisms, and replaced with ethanol at the vehicle.
9. Replace the cap on each jar. Slowly tip the jar to a horizontal position, then gently rotate the jar to mix the preservative. Do not invert or shake the jar. After mixing, seal each jar with plastic electrical tape.
10. Store labeled composite samples in a container with absorbent material that is suitable for use

with 70% ethanol until transport or shipment to the laboratory.

7.0 FINAL LAKE ACTIVITIES

Prior to leaving the lake, make a general visual assessment of the lake and its surrounding catchment. This assessment is based on the collective observations of all crew members. The objective of the lake assessment is to record field crew observations of catchment and lake characteristics that are useful for future data interpretation, ecological value assessment, development of associations, and verification of stressor data. While often subjective, these observations and impressions are extremely valuable.

In addition, review all data forms and sample labels for completeness, accuracy, and legibility. Mare sure all samples are labeled, sealed, and properly preserved. Activities described in this section are summarized in Figure 7.1.

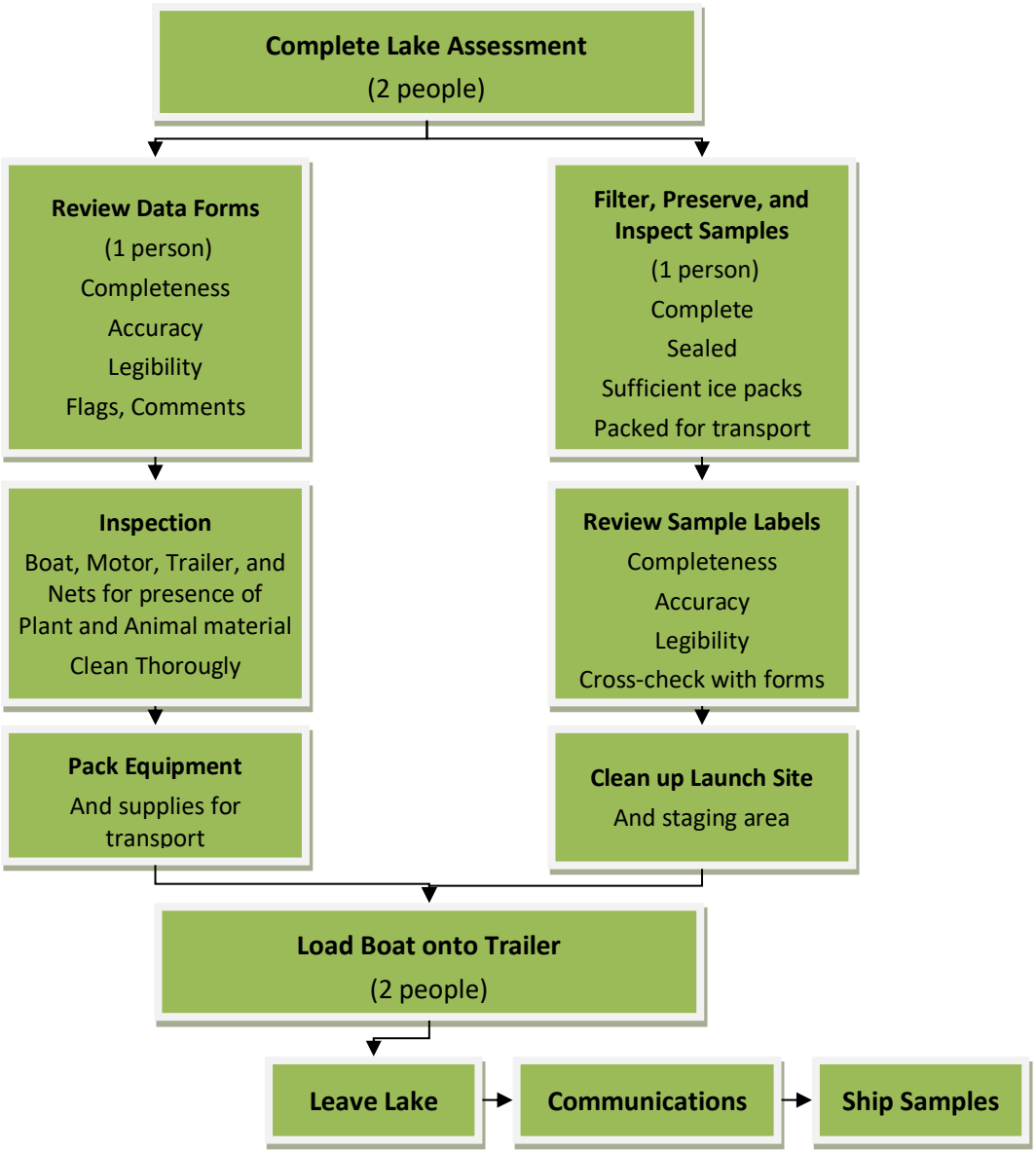

Figure 7.1 Final lake activities summary

7.1 General Lake Assessment

Complete the **Assessment** form at the end of lake sampling, recording all observations from the lake that were noted by all crew members during the course of the visit. This form is designed as a template for recording pertinent field observations. It is by no means comprehensive, and any additional observations should be recorded in the comments section. The form consists of six major sections: 1) Lake/Catchment Site Activities and Disturbances Observed, 2) General Lake Information, 3) Shoreline Characteristics, 4) Qualitative Macrophyte Survey, 5) Waterbody Character, and 6) Qualitative Assessment of Environmental Values.

7.1.1 Lake/Catchment Site Activities and Disturbances Observed

Record any of the sources of potential stressors listed in Table 7.1 on the **Assessment** form. These potential stressors may have been observed while on the lake, while driving or walking through the lake catchment, or while flying over the lake and catchment. For activities and stressors that you observe, rate their abundance or influence as low (L), moderate (M), or heavy (H) by filling in the correct bubble next to each disturbance listed. Leave the line blank for any disturbance not observed. The distinction between low, moderate, and heavy will be subjective. For example, if there are two to three houses on a lake, fill in the "L" bubble for low next to "Houses." If the lake is ringed with houses, rate it as heavy (H). Similarly, a small patch of clear-cut logging on a hill overlooking the lake would rate a low ranking. Logging activity right on the lake shore, however, would get a heavy disturbance ranking. The section for “Lake Site Activities and Disturbances Observed” includes residential, recreational, agricultural, industrial, and lake management categories.

Table 7.1 Site activities and disturbances observed during final lake assessment.

Observe lake activities or disturbances listed and record as L (low), M (moderate), or H (heavy) intensity on the Assessment form (except as noted below):	
Residences	Presence of any houses and residential buildings around the lake.
Maintained Lawns	Presence of any maintained lawns around the lake.
Construction	Presence of any recent construction in the immediate area around the lake or signs of recent sedimentation events (depositional fans).
Pipes/Drain	Presence of any pipes or drains feeding into or out of the lake. If known, record the type of activity with which the pipe is associated (e.g., storm sewer, plant intake) in the "Comments" section of the form.
Dumping	Any evidence of landfill or dumping around the lake, including garbage pits and informal dumping of large amounts of trash or cars and appliances along roads or lakeshore. This does not include small amounts of litter. If informal dumping areas exist, note that they are informal sites in the "Comments" section of the form.
Roads	Presence of any maintained roads in the immediate area around the lake.
Bridges/Causeways	Presence of any bridges or causeways across or in the immediate vicinity of the lake.
Sewage Treatment	Presence of sewage treatment facility.
Hiking Trails	Presence of formal hiking trails around the lake.
Parks, Campgrounds	Presence of organized public or private parks, campgrounds, beaches or other recreational areas around the lake.
Primitive Parks, Camping	Presence of informal or primitive parks, camping areas, beaches or other recreational areas (e.g., swimming holes) around the lake.
Resorts	Level of resort activity; this could include motels, resorts, golf courses, and stores.
Marinas	Presence of any marinas.

Observe lake activities or disturbances listed and record as L (low), M (moderate), or H (heavy) intensity on the Assessment form (except as noted below):	
Trash/Litter	Relative abundance of trash or litter around the lake.
Surface Films, Scum or Slicks	Relative abundance of surface films, scum, or slicks on the lake.
Cropland	Presence of cropland.
Pasture	Presence of pastures.
Livestock Use	Presence of livestock use.
Orchards	Presence of orchards.
Poultry	Presence of poultry operations.
Feedlot	Presence of feedlot or concentrated animal feeding operations.
Water Withdrawal	Any evidence of water withdrawal from the lake.
Industrial Plants	Any industrial activity (e.g., canning, chemical, pulp) around the lake or in the catchment. Describe the type of industry in the "Comments" section of the form.
Mines/Quarries	Any evidence of mining or quarrying activity in the catchment or around the lake.
Oil/Gas Wells	Any evidence of oil or gas wells in the catchment or around the lake.
Power Plants	Presence of any power plants.
Logging	Any evidence of logging or fire removal of trees in the lake area.
Evidence of Fire	Any evidence of forest fires in the lake area.
Odors	Presence of any strong odors.
Commercial	Any commercial activity (e.g., convenient stores, shopping centers, restaurants) around the lake or in the catchment.
Liming	Any evidence of liming activities.
Chemical Treatment	Presence of any chemical treatment facilities.
Angling Pressure	Estimate of the intensity of fishing activity in the lake.
Drinking Water Treatment	Presence of any drinking water treatment facilities.
Macrophyte Control	Any evidence of dredging or other activities to control macrophyte growth; describe these in the "Comments" section of the form.
Water Level Fluctuations	Any evidence of water level fluctuations due to lake management.
Fish Stocking	Any evidence of fish stocking in the lake.
Record any other oddities observed or additional information for any specific activity in the "Comments" section of the form.	

7.1.2 General Lake Information

Observations regarding the general characteristics of the lake are described in Table 7.2. Record these observations on the **Assessment** form. The hydrologic lake type is a very important variable for defining subpopulations for acidic deposition effects. Note if there are any stream outlets from the lake, even if they are not flowing. If no lake outlets are observed, record the lake as a seepage lake. If the lake was created by a manmade dam (not that a dam is present just to raise the water level), record the lake as a reservoir. Otherwise record the lake as a drainage lake. Note any flight hazards that might interfere with either low-altitude fly-overs by aircraft (for future aerial photography or videography) or landing on the lake for sampling purposes (either by float plane or helicopter). When estimating the intensity of motor boat usage, in addition to the actual number of boats observed on the lake during the visit, use other observations such as the presence of boat houses, docks, and idle craft**.** Note your opinion as to the

swimmability of the lake in general. Observe any regular change in the lake levels and estimate the typical elevation change.

Table 7.2 General lake information observed during final lake assessment.

Record general information about the lake as a whole	
Hydrologic Lake Type	Note if there are any stream outlets from the lake, even if they are not flowing. If no lake outlets are observed, record the lake as a seepage lake. If the lake was created by a manmade dam (not that a dam is present just to raise the water level), record the lake as a reservoir. Otherwise record the lake as a drainage lake.
Outlet Dams	Note the presence of any dams (or other flow control structures) on the lake outlet(s). Differentiate between artificial (man-made) structures and natural structures (beaver dams).
Low Elevation Flight Hazards	If there are any hazards (above tree level) that would interfere with low elevation aircraft flights or landing on the lake, check "Yes;" otherwise check "No." Examples include radio towers or power lines.
Motor Boat Density	Record your impression of the density of motor boat usage on this lake (high or low). If there is a restriction on the size of motor boat engines, check "Restricted." If motor boats are banned, check "Banned." Consider the day of the week and weather in your assessment as well as the number of boathouses, idle craft. Count jet skis and any other motorized craft, which could stir up the lake, as motor boats.
Swimmability	Record a subjective impression about the aesthetics of swimming in this lake (swimmability) along the range of "good" to "not swimmable."
Lake Level Changes	Examine the lake shoreline for evidence of lake level changes (e.g., bathtub ring). If there are none, check "zero;" otherwise try to estimate the extent of vertical changes in lake level from the present conditions based on other shoreline signs.

7.1.3 Shoreline Characteristics

Shoreline characteristics of interest during the final lake assessment are described in Table 7.3. Record observations related to this portion of the assessment on the **Assessment** form. To estimate the extent of major vegetation types, limit the assessment to the immediate lake shoreline (i.e., within 20 m of the water). Also estimate the percentage of the immediate shoreline that has been developed or modified by humans.

Table 7.3 Shoreline characteristics observed during final lake assessment.

Record percent of shoreline characteristics (within 20 meters of water):	
Forest	Deciduous, coniferous, or mixed forest, including sapling vegetation.
Grass	Meadows, lawns, or other open vegetation.
Shrub	Shrub vegetation
Wetland	Forested and non-forested wetlands (submerged terrestrial vegetation).
Bare Ground	Non-vegetated areas such as beaches, sandy areas, paved areas, and exposed rock.
Agriculture	Cropland, orchard, feedlot, pastureland, or other horticultural activity.
Shoreline Modifications	Actual shoreline that has been modified by the installation of riprap, revetments, piers, or other human modifications.
Development	Immediate shoreline area developed by human activity; include lawns, houses, stores, malls, marinas, golf courses, or any other human-built land use.

7.1.4 Qualitative Macrophyte Survey

Aquatic macrophytes (aquatic plants large enough to be seen without magnification) are important indicators of lake trophic status. The most important indicator for this survey is the percentage of the entire lake area (not just near the shore) covered with macrophytes, as perceived by observers. For both "emergent/floating" and "submergent" coverage, choose one of the four percentage groupings (<5%, 5-25%, 26-75%, >75%) on the **Assessment** form. In some cases, it will be fairly easy to estimate the percentage from observations made at the PHab stations. In other cases, it will be an educated guess, especially if the water is turbid or the lake is deep. After recording the areal percentage of macrophyte coverage, record the typical density of the plants in the observed macrophyte beds as absent, sparse, moderate, or high. Record your estimates on the **Assessment** form.

7.1.5 Waterbody Character

Rate the ***waterbody character*** which is the physical habitat integrity of the waterbody. Waterbody character is largely a function of riparian and littoral habitat structure, volume change, trash, turbidity, slicks, scums, color, and odor. The NLA 2017 attempts to define waterbody character through two attributes: degree of human disturbance and aesthetics. Rate each of these attributes on a scale of 1 to 5. For development, give the lake a "5" if it is pristine, with no signs of any human disturbance. A "1" would indicate that a lake is highly disturbed; for example, the entire lake is ringed with houses, seawalls, docks, etc. For aesthetics (whether the lake is appealing or not) base the decision on any factors about the lake that disturb you (trash, algal growth, weed abundance, overcrowding). Fill in the bubble next to the number that best describes your opinion about how suitable the lake is for recreation and aesthetic enjoyment today:

1. Enjoyment is nearly impossible;
2. Level of enjoyment is substantially reduced;
3. Enjoyment is slightly impaired;
4. There are very minor aesthetic problems; it is otherwise excellent for swimming, boating, and enjoyment; or
5. It is beautiful and could not be any nicer.

7.1.6 Qualitative Assessment of Environmental Values

The primary goal of this study is to assess three major ecological values with respect to lakes: trophic state, ecological integrity, and human use. Based on your field experience, record your own assessment of these values on the **Assessment** form. Write comments on these values in this section.

- ***Ecological integrity*** is the ability to support and maintain a balanced, integrated, adaptive community with a biological diversity, composition, and functional organization comparable to natural lakes of the region. Record your overall impression of the "health" of the biota in the lake. Note any possible causes of impairment. The presence of higher order consumers (fish-eating birds and mammals) is an indication of a healthy food web and should be noted here. Similarly, the absence of an organism that you might expect to see is an important observation.
- ***Trophic state*** is the rate or amount of phytoplankton and macrophytes produced or present in a lake. Give your visual impression of the trophic status as oligotrophic (little or no biomass in the lake water), mesotrophic (intermediate amounts of biomass in the lake water), eutrophic (large amounts of biomass in the lake water), or hypereutrophic (choked lake, with more biomass than

water). Give your overall impression of algal abundance and general type (e.g., filamentous). List any observed potential nutrient sources to the lake (e.g., septic tanks and agricultural runoff).

- Suitability for ***human use*** is the ability to support recreational uses such as swimming, fishing, and boating. Record your overall impression of the lake as a site for recreation. Note any possible causes of impairment. Note the presence or absence of people using the lake for recreational activities.

Use the comments section on the **Assessment** form to note any other pertinent information about the lake or its catchment. Here the field crew can record any observations that may be useful for future data interpretation, especially data that was not captured during other sampling or data collection activities.

7.2 Processing the Chlorophyll-*a* Samples

7.2.1 Equipment and Supplies

Table 7.4 provides the equipment and supplies needed to process the Chlorophyll-*a* sample

Table 7.4 Equipment and supplies – chlorophyll-*a* processing.

Type	Item	Quantity
Form	NLA 2017 Index Sample Location	1
Documentation	Labels: Chlorophyll-*a* sample label	1
	Chlorophyll-*a* outer bag label	1
	Clear tape strips (to cover sample labels)	As needed
Processing	Poly bottle (2 L, brown)	1
	Centrifuge tube (50 mL, screw top) in zip top bag	1
	Filter forceps (flat blade)	1
	Filtration chamber (with filter holder)	1
	Filtration flask (with silicone stopper and adapter)	1
	Filtration pump (hand vacuum)	1
	Graduated cylinder (250 mL)	1
	Squirt bottle (1 L Nalgene) – de-ionized (DI)	1
	Test tube holder	1
	Whatman 0.7 µm GF/F glass fiber filter	1
Storing and preserving	Cooler	1
	Plastic electrical tape	As needed
	Foil squares	1
	Zip top bag	1
	Wet ice	As needed

7.2.2 Procedures for Processing the Chlorophyll-*a* Samples

The procedure for processing the chlorophyll-*a* sample is presented below. Whenever possible, sample processing should be done in subdued light, out of direct sunlight.

1. Place a glass fiber filter in the filter holder apparatus with the grid side down. Do not handle the filter with bare hands; use clean forceps.
2. Shake the chlorophyll-*a* sample collection bottle to homogenize the sample, measure and pour 250 mL of water into the filter holder using the graduated cylinder, replace the cap of the filter holder, and pump the sample through the filter. Take care not to exceed 7 inches

of Hg (approximately 3.4 psi) in the vacuum gauge on the filtration pump. If 250 mL of lake water will not pass through the filter, discard the filter, rinse the apparatus with DI water, and repeat the procedures using a new filter and 100 mL of lake water. NOTE: If the water is green or turbid, use a smaller volume to start.

3. Observe the filter for visible color. If no visible color is present, repeat step 3 until color is visible on the filter or until a maximum of 2,000 mL have been filtered.
4. Once visible color is present and/or 2,000 mL of lake water has been filtered, record the actual sample volume filtered on the Index Sample Collection form and on the sample label. Rinse the graduated cylinder and upper portion of the filtration apparatus thoroughly with DI water to include any remaining cells adhering to the sides and pump through the filter. Monitor the level of water in the lower chamber to ensure that it does not contact the filter or flow into the pump.
5. Disconnect the upper portion of the filter apparatus from the lower portion. Remove the filter from the holder with clean forceps. Avoid touching the colored portion of the filter. Fold the filter in half, with the colored side folded in on itself.
6. Place the folded filter into a 50 mL screw-top centrifuge tube and replace the cap. Tighten the cap as tightly as possible. The cap will seal tightly after an additional ¼ turn past the point at which initial resistance is met. Failure to tighten the lid completely could allow water to infiltrate into the sample and may compromise its integrity. Seal the cap of the centrifuge tube with plastic electrical tape
7. Record the sample volume filtered on a chlorophyll-*a* label and attach it to the centrifuge tube. Ensure that all written information is complete and legible. Cover with a strip of clear tape. Double check that the "total volume of water filtered" on the **Index Sample Collection** form matches the total volume recorded on the sample label.
8. Wrap the tube in aluminum foil and place in a zip top bag. Place the completed outer label on the outside of the bag. Place this bag on ice in a cooler.
9. Remove the filter holder silicone stopper and adapter from the filtration flask. Pour off water from the bottom chamber.
10. Rinse filter chamber components thoroughly with DI water.
11. Retain the filter chamber, including the graduated cylinder, silicone plug, adapter, and the pad that sits under the filter. Rinse these items with DI water between sites. Thoroughly rinse the graduated cylinder, cups, the brown sample collection bottle and cap with tap water and store for next sample event.

7.3 Preservation of Samples

Preserve the samples as specified in the specific protocol sections. Record the preservation information on the index and littoral sample collection forms.

7.4 Preparation of Samples for Shipping

General information regarding the preparation and shipment of samples is available in Section 4.3.2. General steps that apply to samples are the following:

- Purge the Cubitainer® of any air bubbles, seal the cap tightly and wrap plastic electrical tape clockwise around the cap. Place the Cubitainer® in a cooler with wet ice.
- Seal all pertinent caps tightly.
- Wrap plastic electrical tape clockwise around the caps, and then place the bottles in the cooler with wet ice. Note: do not tape the dissolved gas samples. These samples should not be removed from their respective bags after collection.
- Keep all chilled samples on wet ice until shipment.

7.5 Data Forms and Sample Inspection

After the **Assessment** form is completed, the Field Crew Leader reviews all of the data forms and sample labels for accuracy, completeness, and legibility. This will be done whether you are using electronic field forms or paper forms. The other crew member inspects all sample containers and packages them in preparation for transport, storage, or shipment.

Ensure that all required data forms for the lake have been completed. Confirm that the Site ID, crew ID, and date of visit are correct on all forms. On each form, verify that all information has been recorded accurately, the recorded information is legible, and any flags are explained in the comments sections. Ensure that written comments are legible, with no "shorthand" or abbreviations. After reviewing each form, initial the upper right corner of each page of the form.

Ensure that all samples are labeled, all labels are completely filled in, and each label is covered with clear tape. Make sure that all sample containers are properly sealed.

7.6 Launch Site Cleanup

Load the boat on the trailer and inspect the boat, motor, and trailer for evidence of plant material. Clean the boat, motor, and trailer as completely as possible before leaving the launch site. Inspect all nets for pieces of macrophyte or other organisms and remove as much as possible before packing the nets for transport. Pack all equipment and supplies in the vehicle and trailer for transport; keep them organized as presented in the equipment checklists (APPENDIX A: EQUIPMENT & SUPPLIES). Lastly, be sure to clean up all waste material at the launch site and dispose of or transport it out of the site if a trash can is not available. See Section 4.3 for additional information and follow appropriate state, tribal or other applicable protocols.

8.0 FIELD QUALITY CONTROL

Standardized training and data forms provide the foundation to help assure that data quality standards for field sampling are met. These methods for field sampling and data collection are the primary guidelines for all cooperators and field crews. In addition, repeat sampling and field evaluation and assistance visits will address specific aspects of the data quality standards for the NLA 2017.

8.1 Repeat Sampling

Approximately 10% of the target sites visited will be revisited during the same field season by the same field crew that initially sampled the lake. The primary purpose of this "revisit" set of sites is to provide variance estimates that can be used to evaluate the survey design for its potential to estimate status and detect trends in the target population of lakes. The revisit will include the full set of indicators and associated parameters. The time period between the initial (Visit 1) and repeat visit (Visit 2) to a lake must be at least two weeks and should be as long as possible.

The repeat visit sites were selected by taking the first 2007 resample site for each state (panel ID = "NLA17_07RVT2") and the first 2012 resample site for each state (panel ID = "NLA17_12RVT2"). This method identified 96 lakes (10% of the lakes) from the entire draw of lakes for the survey. If a site selected for repeat sampling is dropped, then the alternate site assigned to replace it should be revisited (see NLA 2017 Site Evaluation Guidelines for further information regarding the replacement of revisit sites).

8.2 Field Evaluation and Assistance Visits

No national program of accreditation for field work currently exists. For this reason, a rigorous program of field evaluation and assistance visits has been developed to support the NLA 2017.

8.2.1 General Information

Evaluation and assistance visits will be conducted with each field crew early in the sampling and data collection process, if possible, and corrective actions will be conducted in real time. These visits provide both a quality check for the uniform evaluation of the data collection methods and an opportunity to conduct procedural reviews, as required, minimizing data loss due to improper technique or interpretation of field procedures and guidance. Through uniform training of field crews and review cycles conducted early in the data collection process, sampling variability associated with specific implementation or interpretation of the protocols will be significantly reduced. The assistance visit also provides the field crews with an opportunity to clarify procedures and offer suggestions for future improvements based on their sampling experience preceding the visit. The field evaluations, while performed by a number of different supporting collaborator agencies and participants, will be based on the uniform training, plans, and checklists. This review and assistance task will be conducted for each unique field crew collecting and contributing data under this program; hence no data will be recorded to the project database that was produced by an 'unaudited' process or individual.

The field evaluations will be based on the evaluation plan and field evaluation checklist. The checklist will be made available to all parties associated with NLA.

One or more designated EPA or Contractor staff members who are qualified (i.e., have completed training) in the procedures of the NLA 2017 field sampling operations will visit trained state, tribal, contractor, and EPA field sampling crews during sampling operations on site. If membership of a field

crew changes, and at least two of the members have not been evaluated previously, the field crew must be evaluated again during sampling operations as soon as possible to ensure that all members of the field crew understand and can perform the procedures.

The purpose of this on-site visit will be to identify and correct deficiencies during field sampling operations. The process will involve the following preparation steps and field day activities.

Additionally, conference calls with crews may be held approximately every two weeks to discuss issues and clarifications as they come up throughout the sampling season.

8.2.2 Preparation Activities

1. Each Field Crew Evaluator will schedule an assistance visit with their designated crews in consultation with the QA Officer, Regional NLA Coordinator, and respective Field Sampling Crew Leader. **Evaluators should be prepared to spend additional time in the field if needed (see below).** Ideally, each Field Crew will be evaluated within the first two weeks of beginning sampling operations, so that procedures can be corrected or additional training provided, if needed.
2. Each Field Crew Evaluator will ensure that field crews are aware of their visit plans and all capacity and safety equipment will be provided for the Field Crew Evaluator.
3. Each Field Crew Evaluator will need to bring along the following in Table 8.1.

Table 8.1 Equipment and supplies – field evaluation and assistance visits.

Type	Item	Quantity
Form	Field Evaluation and Assistance Visit Checklist (sent from EPA)	1
Documentation	NLA 2017 Field Operations Manual NLA 2017 Quality Assurance Project Plan Clipboard Pencils (#2, for data forms)/Pen Field notebook (optional)	1 1 1 1 1
Gear	Field gear (e.g., protective clothing, sunscreen, insect repellent, hat, water, food, backpack, cell phone)	As needed

8.2.3 Field Day Activities

1. The Field Crew Evaluator will review the Field Evaluation and Assistance Visit Checklist with each crew during the field sampling day and establish and plan and schedule for their evaluation activities for the day.
2. The Field Crew Evaluator will view the performance of a field crew through one complete set of sampling activities as detailed on the checklist.
    - Scheduling might necessitate starting the evaluation midway on the list of tasks at a site, instead of at the beginning. In that case, the Field Crew Evaluator will follow the crew to the next site to complete the evaluation of the first activities on the list.
    - If the field crew misses or incorrectly performs a procedure, the Field Crew Evaluator will note this on the checklist and *immediately point this out so the mistake can be corrected on the spot*. The role of the Field Crew Evaluator is to provide additional training and guidance so that the procedures are being performed consistently with the FOM, all data are recorded correctly, and paperwork is properly completed at the site.

3. When the sampling operation has been completed, the Field Crew Evaluator will review the results of the evaluation with the field crew before leaving the site (if practicable), noting positive practices and problems (i.e., weaknesses [might affect data quality]; deficiencies [would adversely affect data quality]). The Field Crew Evaluator will ensure that the field crew understands the findings and will be able to perform the procedures properly in the future.
    - The Field Crew Evaluator will review the list and record responses or concerns from the field crew, if any; on the checklist (this may happen throughout the field day).
    - The Field Crew Leader will sign the checklist after this review.

8.2.4 Post Field Day Activities

1. The Field Crew Evaluator will review the checklist that evening and provide a summary of findings, including lessons learned and concerns.
    - If the Field Crew Evaluator finds major deficiencies in the field crew operations (e.g., less than two members, equipment, or performance problems) the Field Crew Evaluator must contact the EPA NLA 2017 Project Lead. The EPA NLA 2017 Project Lead will contact the EPA NARS QA Project Officer to determine the appropriate course of action.
2. The Field Crew Evaluator will retain a copy of the checklist and submit to the NLA logistics lead.
3. The EPA NLA 2017 Project Lead and EPA NARS QA Project Officer or authorized designee will review the returned Field Evaluation and Assistance Visit Checklist, note any issues, and check off the completion of the evaluation for each field crew.

8.2.5 Summary

Table 8.2 summarizes the plan, checklist, and corrective action procedures.

Table 8.2 Summary of field evaluation and assistance visit information.

Field Evaluation Plan	The Field Crew Evaluator: • Arranges the field evaluation visit in consultation with the QA Officer, Regional NLA Coordinator, and respective Field Sampling Crew Leader, ideally within the first two weeks of sampling • Observes the performance of a crew through one complete set of sampling activities • Takes note of errors the field crew makes on the checklist and immediately point these out to correct the mistake • Reviews the results of the evaluation with the field crew before leaving the site, noting positive practices, lessons learned, and any concerns
Field Evaluation Checklist	The Field Crew Evaluator: • Observes all pre-sampling activities and verifies that equipment is properly calibrated and in good working order, and protocols are followed • Checks the sample containers to verify that they are the correct type and size, and checks the labels to be sure they are correctly and completely filled out • Confirms that the field crew has followed NLA protocols for locating the lake and determining the index site on the lake • Observes the index site sampling, confirming that all protocols are followed • Observes the littoral sampling and habitat characterization, confirming that all protocols are followed • Records responses or concerns, if any, on the Field Evaluation and Assistance Checklist
Corrective Action Procedures	• If the Field Crew Evaluator's findings indicate that the Field Crew is not performing the procedures correctly, safely, or thoroughly, the Evaluator must continue working with this Field Crew until certain of the crew's ability to conduct the sampling properly so that data quality is not

	adversely affected. • If the Field Crew Evaluator finds major deficiencies in the Field Crew operations the Evaluator must contact the EPA NLA 2017 Project Lead.

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APPENDIX A: EQUIPMENT & SUPPLIES

Base Kit

A Base Kit will be provided to the field crews for all sampling sites that they will go to. Some items are sent in the base kit as extra supplies to be used as needed.

Base Kit Item	Quantity	Protocol
Alcohol thermometer	1	Dissolved gases
Bottle brush†	1	Sediment Core
Centrifuge tube (50 mL, screw top)	1	Dissolved gases
Centrifuge tube (50 mL, screw top) - extras	2	Chlorophyll-*a*
Core plug†	2	Sediment Core
Core tube with poly caps†	2	Sediment Core
Corer head (gravity, with cable and messenger) comes with 2 core tubes and core plugs†	1	Sediment Core
Electrical tape*	1	General
Extra lowering line for Gravity Corer (50 m)	1	Sediment Core
Extruder rod (1 ¼ in. PVC, 75 cm long) with cap†	1	Sediment Core
Filter forceps (flat blade)	6	Chlorophyll-*a*
Filtration chamber (with filter holder)	5	Chlorophyll-*a*
Filtration chamber adapter	3	Chlorophyll-*a*
Filtration flask (side arm, 500 mL)	1	Chlorophyll-*a*
Filtration pump (hand vacuum)	1	Chlorophyll-*a*
Foil squares (package of 25)*	1	Chlorophyll-*a*
Funnel	1	Water samples Zooplankton Benthics
Gloves (latex/nitrile, non-powdered, box of 100)	1	General
Graduated cylinder (250 mL)	1	Chlorophyll-*a*
H2SO4 (ampoules) – extras	5	Nutrients
HDPE bottle (1 L, white, wide-mouth) – extras	6	Benthics
HDPE bottle (125 mL, white, wide-mouth) – extras	6	Zooplankton
Integrated water sampler device (MPCA design)†	1	Water Samples
Kick net (500 µm D-shaped, modified) with 4 foot handle†	1	Benthics
Lugol's solution (250 mL bottle)	1	Phytoplankton
Metric tape measure	1	Secchi Physical Habitat
Needle, 27 gauge	20	Dissolved gases
NLA 2017 Quick Reference Guide	1	All
Packing tape (extra rolls)*	2	General
Packing tape (on holder)*	1	General
Pail (narcotizing/concentrating chamber)	2	Zooplankton

Base Kit Item	Quantity	Protocol
pH paper (box)*	1	Nutrients
Plankton net (150 µm)†	1	Zooplankton
Plankton net (50 µm)†	1	Zooplankton
Plastic tranfer pipette	4	Phytoplankton
Poly bottle (2 L, brown)	1	Chlorophyll-*a*
Poly syringe (140 mL) with attached 3-way stopcock	4	Dissolved gases
Poly syringe (30 mL) with attached 2-way stopcock	2	Dissolved gases
Poly syringe (30mL) ('transfer syringe')	1	Dissolved gases
Pre-evacuated vials (spares)	5	Dissolved gases
Rubbermaid action packer	1	General
Secchi disk (20 cm diameter) with weight†	1	Secchi
Sectioning stage†	1	Sediment Core
Sectioning tube (13 cm, 2.5 in ID, line marked 5 cm from bottom of tube)†	1	Sediment Core
Sieve bucket (500 µm)†	1	Benthics
Silicone stopper for filtration flask	2	Chlorophyll-*a*
Small tote with lid	1	General
Sounding line (50 m, calibrated, marked in 0.5 m intervals) with clip	1	Depth Secchi Zooplankton
Sounding rod (3 m , marked in 0.1 m increments, calibrated, PVC, 2-section)†	1	Index Site Depth Physical Habitat
Sounding weight with clip	1	Depth
Spoon (stainless steel)	1	Benthics
Spoonulet (stainless steel)	5	Sediment Core
Squirt bottle (1 L Nalgene) – for de-ionized (DI) water	1	General
Squirt bottle (1 L Nalgene) – for lake water	1	General
Stainless steel bowl	1	Sediment Core
Surveyor's tape (50m)†	1	Depth Physical Habitat
Syringe (60 mL) with tubing to siphon overlying water	1	Sediment Core
Tape strips (3M, pad of 25)*	6	General
Tray for glass vials	1	Dissolved gases
Watchmaker's forceps	1	Benthics
Whatman 0.7 µm GF/F glass fiber filter (box of 100)	1	Chlorophyll-*a*
Zip top bags (1 gal, pack of 25)*	1	General
Zip top bags (1 qt, pack of 25)*	1	General

*Items may need to be replenished by field crews during field season
† Item supplied if needed

Site Kit

A Site Kit will be provided upon request to the field crews for each sampling site. Please submit an electronic **Request Form** well in advance of field sampling to request the Site Kits. Each site kit will also include necessary coolers and shipping supplies for all samples collected.

Site Kit Item	Quantity Per Site Kit	Protocol(s)
Centrifuge tube (50 mL, screw top) in bag	1	Chlorophyll-*a*
CO2 (Alka seltzer) tablets (packet of 2)	1	Zooplankton
Cooler liners (1 per cooler)	2	General
Coolers (Water Chemistry and Daily Shipped Samples)	2	Shipping
Cubitainer® (4L)	1	Water Chemistry
FedEx Envelope	1	Shipping
FedEx Express shipping labels	4	Samples and Data Packs
Glass jar (120 mL, amber) in foam sleeve	1	Sediment Core - Contaminants
Glass jar (60 mL, amber) in foam sleeve	1	Sediment Core - TOC
H2SO4 ampoule	1	Nutrients
PETG bottle (1 L, sterile, clear, narrow-mouth)	1	Fish eDNA
HDPE bottle (1 L, white, narrow mouth)	1	Phytoplankton
HDPE bottle (1 L, white, wide-mouth)	2	Benthics
HDPE bottle (125 mL, white, wide-mouth)	2	Zooplankton
HDPE bottle (250 mL, brown, wide-mouth)	1	Nutrients
PETG bottle (500 mL, clear, narrow-mouth, square)	1	Algal Toxins (MICX)
HDPE bottle (500 mL, white, wide-mouth, round)	1	Algal Toxins (MICZ)
HDPE bottle (60 mL, white, wide-mouth)	1	Atrazine
IDEXX Sterile bottle (290 mL)	1	Bacteria (e.coli)
Pre-evacuated glass vials (12 mL) in bag	6	Dissolved gases
Zip top bag (1 quart)	2	Sediment Core - Grain Size

Forms & Labels

Field forms (paper or electronic) and labels will be supplied by the NARS IM Center upon request.

Item	Quantity	Protocol
Field forms packet (paper): NLA 2017 Verification NLA 2017 Index Profile (front & back) NLA 2017 Index Sample Collection (pages 1-3) NLA 2017 Physical Habitat (front & back) NLA 2017 Littoral Sample Collection (front & back) NLA 2017 Assessment (front & back) Tracking forms: NLA 2017 Site and Sample Status/Water Chemistry Lab Tracking NLA 2017 Daily Shipped Samples Tracking NLA 2017 Tracking – Batched Samples NLA 2017 Tracking – Packets	1	General
Labels packet (for samples)	1	General

Field Crew Supplied Equipment

This equipment will need to be supplied by the field crew. Some items are optional.

Field Crew Supplied Item	Quantity	Protocol(s)
Access instructions	1	Site Evaluation
Access permission documents/permit (if required)	1	Site Evaluation
Alconox or other phosphate-free detergent		Sediment Core
Aluminum foil		Sediment Core
Barometer or elevation chart to use for calibration	1	Calibration
Binoculars	1	Physical Habitat
Bleach (or bleach alternative)	1	General
Buckets (5 gallon capacity, plastic)	2	Benthics Dissolved Gases
Buoy (for marking observation point)	1	Physical Habitat
Calibration cups and standards (for multi-parameter meter)	1	Calibration
Calibration QC check solution (for multi parameter meter, pH and conductivity)	1	Calibration
Clinometer	1	Physical Habitat
Clipboard	1	General
Depth Finder (hand-held or boat mounted sonar)	1	Index Site Profile Physical Habitat
Electronic data capture devices (tablet/phone/computer) with NARS App and extra battery pack (if needed)	1-2 (optional)	General
Ethanol (95%)		Benthics Zooplankton
Field gear (e.g., protective clothing, sunscreen, insect repellent, hat, water, food, backpack, cell phone)		General

Field Crew Supplied Item	Quantity	Protocol(s)
Field notebook - optional	1	General
Field thermometer (not mercury)	1	Calibration
GPS unit (with manual, reference card, extra battery)	1	Site Verification Physical Habitat
Kick net (500 µm D-shaped) with 4 foot handle (spare)	1	Benthics
Laser rangefinder (for estimating drawdown) - optional	1	Physical Habitat
Map wheel or string (for measuring shoreline distances on site map)	1	Physical Habitat
Multi-parameter water quality meter (with temperature, pH, and DO probes)	1	Index Site Profile
Multi-parameter communication cable (50 m)	1	Index Site Profile
Net(s) and/or bucket assembly for end of net (spares)	1	Zooplankton
NLA 2017 Fact Sheets	20	General
Pen	1	General
Pencils (#2, for data forms)	2	General
Permanent marker (fine tip, for labels)	2	General
Scissors	1	General
Screwdriver	1	Sediment Core
Shipping tape	1	Shipping
Surveyors rod - optional	1	Physical Habitat
Tub (shallow) or dish pan	1	Sediment Core
Water (deionized)		General

Boat Equipment List

This is suggested boat equipment.

Item
Anchor (with 75 m line or sufficient to anchor in 50 m depth)
Boat horn
Boat plug (extra)
Bow/stern lights
Emergency tool kit
Fire extinguisher
First aid kit
Gas Can
Hand bilge pump
Life jackets
Motor
Oars or paddles
Second anchor for windy conditions and littoral sampling (w/ 75m line)
Sonar unit
Spare prop shear pin

Type IV PFD (throwable life saving device)

APPENDIX B: SHIPPING GUIDELINES

General Shipping Guidelines

Samples will be shipped according to the chart below. The Field Crew Leader will complete the appropriate tracking form for the samples and will submit tracking via one of the options listed in the tracking forms section above. The Field Crew Leader will place the samples and the tracking form (in a waterproof bag or plastic sleeve) in the shipment cooler, and then the Field Crew Leader will attach the appropriate pre-addressed FedEx label from the site kit marked for the appropriate lab. The field crew will either drop off the cooler for shipment at a local FedEx location or arrange for a pick up at the hotel or other appropriate facility. If the field crew has chosen a pick up, they must follow up with the facility at which it has been left and/or track the package through FedEx tracking tools to ensure that the shipment cooler has actually been picked up by FedEx. Once the package is in the possession of FedEx, the IM Team and Field Logistics Coordinator (FLC) will track the package to its destination and take steps necessary to ensure timely delivery.

A Tracking Form must be filled out to accompany each sample shipment. Be very careful to fill in the information correctly and legibly, especially the Site ID and Sample ID numbers. Use the codes on the bottom of the form to indicate sample type. The Tracking Form is to be placed in a resealable plastic bag/pouch secured to the inside of the cooler lid. Seal the shipping container. Submit the Sample Tracking Form to the NARS IM Center to indicate that samples will be in transit to the laboratory. Tracking forms must be submitted the same day that the samples are shipped.

Before shipping, it is very important to preserve each sample as directed in the sample collection portion of the appropriate chapter in the NLA 2017 FOM. General directions for sample processing, shipping and tracking are found below:

- Preserve the samples as specified for each indicator before shipping.
- Be aware of the holding times for each type of sample.
- Always line the cooler with a heavy duty plastic bag (cooler liner).
- When shipping samples preserved with ethanol, surround the jars with crumpled newspaper or other absorbent material.

When ice is used for shipment:

- Ensure that the ice is fresh before shipment, and use adequate amounts of ice to ensure samples will remain cold for up to 36 hours.
- Place samples and ice inside the cooler liner and seal the cooler liner.
- Secure the cooler with packing tape.

Tracking Forms and Shipment Types

Whenever NLA samples are shipped, one or more tracking forms are completed to relay important shipping information to the IM Team, FLC and the destination lab. These tracking forms are to be submitted both electronically at the time of shipping (typically by emailing a scan of the form) and included as a hard copy in the shipping container with the samples.

Each tracking form has been assigned a “T” number to help crews identify the correct tracking form to use when sending samples. This “T” number is located on the top of each tracking form in the title of the

form (e.g., Form T-1: NLA 2017 Site and Sample Status/Water Chemistry Lab Tracking). Crews will also find reference to the same "T" numbers on the individual sample labels and on the top of the pre-printed FedEx return labels provided in the site kits. The FedEx return labels are pre-paid and allow crews to ship samples to any of the nationally contracted laboratories. States using their own labs for certain samples will need to arrange for shipping on their own.

When crews order sample labels from the IM Team (via the Request Form), a set of tracking forms will accompany each set of labels. Sample IDs for the suite of samples collected at a single site will be pre-populated on both the labels and the tracking forms. It is important to keep the labels and tracking forms organized so the sample IDs will match when shipping occurs. It is a good idea to write the Site ID in the header area of all tracking forms when preparing the Site Packet, even if the form will not be used to ship samples right away. This will help ensure that the sample IDs do not get mis-matched.

NOTE: Crews who are using the NARS App (E-Forms) for data submission will still need to order labels and tracking forms for each site. Data submission will be through the App, while submission of tracking data will be as described below (and will be the same for paper form users).

Crews include copies of all tracking forms in the coolers (placed in the plastic sleeve affixed to the inside of the cooler lid) when they send samples to the labs, and they also must submit an electronic copy to the IM Team. Crews have several different options for electronically submitting sample and tracking information. If a cooler contains samples from more than one site, then multiple forms must be placed in the cooler and submitted to NARS IM.

In order of preference, the options for submitting sample and tracking information are:

1. Hand-enter tracking data on a pre-populated paper tracking form that was provided with the set of sample labels. Scan the form with a handheld device or office scanner. Attach the file (in PDF version) to an email and address to sampletracking@epa.gov. Be sure that the file scanned is clear and legible. If an office scanner is not available at the time of shipping, consider a free application like CamScanner which is widely available for mobile devices and has processing tools that will help to ensure that the scan is clear and legible. After scanning, include the hard copy of the form in the cooler with the samples.
   - Please name the PDF files in the following format: "**SiteID V# T#",** where:
     - 'SiteID' is the NLA17 Site ID as listed in the Site Evaluation Spreadsheet;
     - 'V#' is the visit number (e.g., 1 or 2); and
     - 'T#' is the number of the tracking form.
   - Example: AL-10001 V1 T1
   - The specified naming convention is provided on the bottom of each form as a reminder.
2. Using a handheld device or portable computer, enter data into a fillable PDF tracking form, save and submit it via email. Fillable PDF tracking forms do not have pre-populated sample IDs, so care must be taken to enter all sample IDs *exactly* as they are displayed on the sample labels. The fillable PDFs must be saved as an updated copy to the user's computer or device and then attached to an email to sampletracking@epa.gov. Print the completed PDF and include the hard copy of the form in the cooler with the samples.
   - Please name the PDF files in the following format: "**SiteID V# T#",** where:
     - 'SiteID' is the NLA17 Site ID as listed in the Site Evaluation Spreadsheet;
     - 'V#' is the visit number (e.g., 1 or 2); and
     - 'T#' is the number of the tracking form.
   - Example: AL-10001 V1 T1
   - The specified naming convention is provided on the bottom of each form as a reminder.
3. Hand-enter tracking data on a pre-populated paper tracking form that was provided with the set

of sample labels. After transmitting, include the form in the cooler with the samples.

When a crew visits a site with the intent to sample, they complete and submit a copy of the **T1 – Site & Sample Status/Water Chemistry Lab Tracking Form**. It is very important to submit this form as soon as possible after every attempted sampling event. Prompt status reports allow the FLC to closely track sampling progress. More importantly, it enables NARS IM to track samples that were collected at each successfully sampled site versus those that were not, and to immediately track the shipment of the time-sensitive samples after each sampling event.

Daily Shipments:

T-1 – Site & Sample Status/Water Chemistry Lab Tracking Form

- Complete **Form T-1: NLA 2017 Site and Sample Status/Water Chemistry Lab Tracking Form** as soon as possible after completing a sampling event.
- Complete the header area to indicate site-specific information (see Figure B.1, Section A).
- Complete the Site Status section (Figure B.1, Section B) in all cases to indicate whether the site was sampled or not.
- For sampled sites:
  - Complete the Sample Status – Immediate Shipped Samples section (Figure B.1, Section C) to indicate which of the three samples listed were collected. If not collected, use the comments section to briefly explain why they were not collected.
    - If water chemistry, chlorophyll-*a,* and/or nutrients samples were collected, complete the shipping information for those samples. Write in or verify the sample IDs for the sample(s) being shipped.
    - Indicate to which lab the samples are being shipped (i.e., national or state lab). If shipping to a state lab, provide the laboratory name. These three samples are shipped via FedEx Priority Overnight immediately after sampling a site (i.e., same day or next day).
  - Complete the Sample Status – Daily Shipped Samples section (Figure B.1, Section D) to indicate whether each type of sample was collected. If any of the samples were not collected, use the comments section to briefly explain why they were not collected.
    - Daily shipped samples are shipped via FedEx Priority Overnight immediately after a sampling event (i.e., same day or next day).
  - Complete the Sample Status – Batched Shipped Samples section (Figure B.1, Section E) to indicate whether each type of sample was collected and how many jars of each type were collected (in the case of benthos and zooplankton). If any of the samples were not collected, use the comments section to briefly explain why they were not collected.
    - Batched shipped samples are shipped via FedEx Ground every 1-2 weeks.
  - Complete the Field Data Submission section (Figure B.1, Section F) to indicate how field data will be submitted (Electronic App, Fillable PDF, Paper Forms, or Partial App/Paper)

- Send an electronic copy of this form to NARS IM using one of the options listed above. This serves as the "status report" for that sampling event.
- Ship the water chemistry, chlorophyll-*a,* and nutrients samples to the laboratory in the same cooler with a hard copy of this form.
- Water chemistry, chlorophyll-*a,* and nutrients samples need to be shipped on ample fresh wet ice.
- Water chemistry, chlorophyll-*a,* and nutrients samples should be shipped within 24 hours of collection (i.e., the same day as sampling or the following day).

T-2 – NLA 2017 Daily Shipped Samples Tracking

- Use this form when shipping the remaining chilled samples (bacteria, fish eDNA, algal toxins, phytoplankton, atrazine and sediment samples). These samples should be shipped the same day as sampling, or the following day at the latest.
- In cases where a state lab is processing some, but not all of the samples, one copy of the T-2 form will be filled out for each lab and submitted separately. The T-2 form placed in the cooler should only indicate the sample(s) being shipped in that cooler. Use the 'included in cooler' bubble to indicate which samples are being shipped with the specific form.
- Complete the header area to indicate site-specific information and shipping information.
- Write in or verify the sample IDs for the sample(s) being shipped.
- Indicate to which laboratory the samples are being shipped (i.e., national or state laboratory). If shipping to a state laboratory, provide the laboratory name.
- Send an electronic copy of this form to NARS IM using one of the options listed above.
- Ship the samples to the lab(s) in a cooler with a hard copy of the respective form.
- Daily shipped samples need to be shipped on ample fresh wet ice.
- Daily shipped samples should be shipped within 24 hours of collection (i.e., the same day as sampling or the following day).

Batched Shipments:

- Crews may hold BATCHED samples and ship them within one to two weeks after collection.
- Electronically send the tracking form(s) to NARS IM when the samples are SHIPPED using one of the options listed above.
- Use one tracking form for each site's worth of samples in the cooler (i.e. if you have batched samples from four sites in the cooler, there should be four forms completed).
- Include paper copies of the tracking forms in the cooler.
- All samples in the cooler should be listed on one of the included tracking forms.

T-3 – NLA 2017 Batched Shipped Sample Tracking

- Use this form for shipping batches of non-chilled samples (gas/air samples, benthos and zooplankton).
- These samples are shipped together in a cooler (ordered via the Request form) with a heavy duty liner bag and no ice.

- The number of samples that will fit in a cooler will depend largely on the number of benthos bottles collected from each site. In most cases, samples from three to five site visits will fit in a cooler together. NEVER split samples from one site into more than one cooler.
- In cases where a state laboratory is processing some, but not all of the samples, one copy of the T-3 form will be filled out for each lab and submitted separately. The T-3 form placed in the cooler should only indicate the sample(s) being shipped in that cooler. Use the 'included in cooler' bubble indicate which samples are being shipped with the specific form.
- Complete the header area to indicate site-specific information and shipping information.
- Write in or verify the sample IDs for the sample(s) being shipped.
- Indicate to which laboratory the samples are being shipped (i.e., national or state laboratory). If shipping to a state laboratory, provide the laboratory name.
- Send an electronic copy of this form to NARS IM using one of the options listed above.
- Ship the samples to the lab(s) in a cooler with a hard copy of the respective form.
- Batched shipped samples need to be shipped with absorbent material and NO ice.
- Place 1-liter benthos bottles upright in the lined cooler and use newspaper or cardboard to fill any empty space between benthos bottles to keep them in an upright position during shipping.
- Place bagged zooplankton bottles and bagged dissolved gas and air samples on top of the upright benthos bottles.
- Batched shipped samples should be shipped within two weeks of collection.

T-4 – NLA 2017 Pack Tracking

- If utilizing paper field forms, review and ship all field forms in the envelope provided in the site kit to NARS IM every two weeks.
- Before shipping, make copies or scans for your records and as a backup in the event the forms are lost during shipping.
- Forms should be sequentially ordered by protocol within the Data Packet to aid in determining that all forms are present and to facilitate data entry once the Data Packet reaches the IM Team.
- The T4 form is the only tracking form which has room for multiple sites to be listed. List the site IDs, dates sampled and visit numbers for all data packets being shipped in the envelope or box.
- You do not need to send multiple scans of the same file.
- You can name the file with a composite name such as "AL-10001 V1, AL-10022 V1, AL-10235 V2, AL-11236 V1 T6".

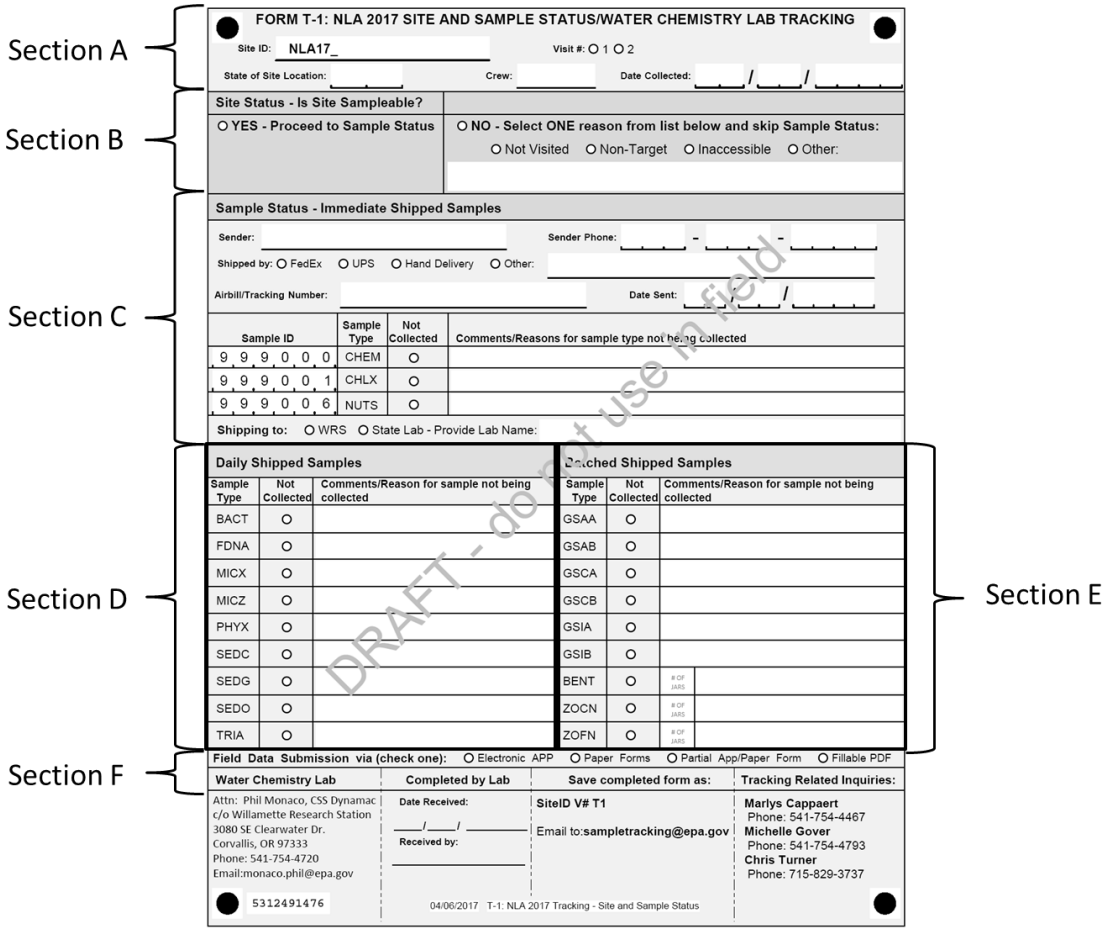

Figure B.1 Example of Tracking Form T-1: Site and Sample Status/Water Chemistry Lab Tracking

Shipping Addresses

USEPA Laboratory, Corvallis, Oregon (Water Chemistry, Chlorophyll-*a*, Nutrients)
Attn: Phil Monaco, CSS
c/o Willamette Research Station
3080 SE Clearwater Dr.
Corvallis, OR 97333

USEPA Data Management Center, Corvallis Oregon (Data Packet)
Attn: Marlys Cappaert, CSRA
c/o U.S. EPA, NHEERL-WED
200 S.W. 35th Street
Corvallis, OR 97333

Great Lakes Environmental Center, Inc. (All other samples)
739 Hastings Street
Traverse City, MI 49686

Figure B.2 Sample preservation, packaging, and holding times

Sample Group & Lab	Sample Type	Sample Code	Location	Sample Target Volume	Container	Preparation/ Preservation	Shipping Time Frame	Packaging for Shipping
Immediate Shipped Samples WRS Laboratory - Corvallis, OR	Water chemistry (raw, unfiltered site water)	CHEM	Index	4 L	Cubitainer (4 L)	Wet ice in field	Immediate (ship within 24 hours of sampling)	**WRS Cooler** with wet ice OVERNIGHT
	Nutrients	NUTS	Index	250 mL	HDPE bottle (250 mL, brown, wide-mouth)	Acid ampoule pH paper check Wet ice in field		
	Chlorophyll-*a*	CHLX	Index Collection	2 L	Poly bottle (2 L, brown)	Wet ice in field		
			Processing	Stain on filter – max 2 L filtration	centrifuge tube (50 mL), in zip-top bag	Wet ice in field (after filtration)		
Daily Shipped Samples GLEC – Traverse City, MI	Bacteria (*E. coli*)	BACT	Index	200 mL	Sterile IDEXX bottle, 290 mL	Wet ice in field	Immediate (ship within 24 hours of sampling)	**Daily Shipped Cooler** with wet ice OVERNIGHT
	Fish eDNA	FDNA	Index	1 L	Sterile PETG bottle (1 L, clear narrow mouth)	Wet ice in field		
	Algal toxins	MICX	Index	500 mL	PETG bottle (500 mL, clear, square)	Wet ice in field		
		MICZ	Index	500 mL	HDPE bottle (500 mL, white, round)	Wet ice in field		
	Phytoplankton	PHYX	Index	1 L	HDPE bottle (1 L, white narrow mouth)	Lugol's added in field Wet ice in field		
	Sediment	SEDO	Index	100 mL	Jar (120 mL)	Wet ice in field		
		SEDC	Index	50 mL	Jar (60 mL)			
		SEDG	Index	100 mL	Double bag			
	Atrazine screen	TRIA	Index	50 mL	HDPE bottle (60 mL, white, wide-mouth)	Wet ice in field		
Batched Samples (non-chilled) GLEC – Traverse City, MI	Dissolved gas and air samples	GSAA GSAB GSCA GSCB GSAA GSAB	Index	30 mL gas or air in each vial	Glass vial with screw top lid and septa (12 mL)	Store at ambient temperature out of direct sunlight. Place vials in provided bubble bag	Batch up to two weeks maximum	**Non-Chilled Batched Cooler** with absorbent material No Ice GROUND
	Benthic invertebrates	BENT	Littoral Stations (10+)	All organisms in grabs	HDPE bottle (1 L, white, wide-mouth)	95% ethanol added in field (at least 500 mL per bottle)		
	Zooplankton (coarse – 150 µm) (fine – 50 µm	ZOCN ZOFN	Index	Vertical tow(s) 5-meter total length	HDPE bottle (125 mL, white, wide-mouth)	95% ethanol added in field		
Data Packs WED, Corvallis,	Data packet	PACK	All completed data forms Checked by crew leader and put in order Copy or scan all forms for your records				Batch up to 2 weeks	Provided envelope

T1 - Water Chemistry Samples

(1) 4 L Cubitainer
- CHEM

(1) 250 mL bottle (Brown)
- NUTS

(1) 50 mL centrifuge tube with filter
- CHLX

Pack 1 site's worth of samples in Lined Cooler with wet ice

ICE

SHIP WITHIN 24 HOURS TO WRS (MONDAY – FRIDAY)

FedEx Express

PRIORITY OVERNIGHT

T2 - Chilled Batched Samples (PER SITE)

(1) 290 mL sterile IDEXX bottle
- BACT

(1) 1 L sterile fish e DNA bottle
- FDNA

(1) 500 mL Clear square bottle
- MICX

(1) 500 mL White round bottle
- MICZ

(1) 60 mL Wide-mouth bottle
- TRIA

(1) 1 L Narrow-mouth bottle
- PHYX

(1) 120 mL Screw top jar
- SEDO

(1) 60 mL Screw top jar
- SEDC

(1) Double bagged zip top
- SEDG

Pack 1 site's worth of samples in Lined Cooler with wet ice

ICE

SHIP WITHIN 24 HOURS TO GLEC (MONDAY – FRIDAY)

FedEx Express

PRIORITY OVERNIGHT

T3 - Non-Chilled Batched Samples (PER SITE)

(2) 125 mL wide-mouth bottles
- ZOCN

(1 or more) 1 L Wide-mouth Bottle(s)
- BENT

(6) Glass serum vials
- GSCA, GSCB, GSIA, GSIB, GSAA, GSAB

Pack 3-5 site's worth of samples in Lined Cooler with NO ICE

NO ICE

SHIP ANYTIME TO GLEC

FedEx Ground

GROUND

T4 - Completed Data Packs

All Data forms from a site, reviewed for completeness, legibility and accuracy

Make copies or scans as backup

Organize in order specified in FOM

Place in envelope or box with tracking form

SHIP TO IM TEAM

FedEx Express