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National Lakes Assessment 2012: Technical Report

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Table of Contents

List of Figures

List of Tables

Chapter 1: Project Overview

1.1 Overview

This document, the *National Lakes Assessment 2012: Technical Report*, accompanies the *National Lakes Assessment 2012: A Collaborative Survey of Lakes in the United States* and related on-line materials. The National Lakes Assessment (NLA) is a collaboration among the U.S. Environmental Protection Agency (EPA), states, tribes, and other partners. It is part of the National Aquatic Resource Surveys (NARS) program design to conduct national scale assessments of aquatic resources. The NLA 2012 provides the second assessment at national and regional scales of the ecological and recreational condition of lakes. This assessment was accomplished by collecting and analyzing data from across the conterminous United States.

The *National Lakes Assessment 2012: A Collaborative Survey of Lakes in the United States* (the Public Report) is not a technical document, but rather a report geared toward a broad, public audience. The NLA 2012 presents information from the second National Lakes Assessment. It provides national-scale assessments and also compares the condition of lakes to those from the earlier NLA 2007 conducted by EPA and its partners. You can find results for regional scales and comparisons between natural lakes and reservoirs using our interactive dashboard at https://nationallakesassessment.epa.gov/. The technical report is a supplemental document that serves as a technical reference to support findings presented in the public report and on-line.

1.2 Objectives of the National Lakes Assessment

The objective of the NLA is to characterize aspects of the biological, chemical, physical, and recreational condition of the nation's lakes throughout the conterminous United States. It employs a statistically-valid probability design stratified to allow estimates of the condition of lakes on a national and regional scale.

The NLA is designed to answer the following questions about lakes across the United States.

1. What is the current biological, chemical, physical, and recreational condition of lakes?
    a. What is the extent of degradation among lakes?
    b. Is degradation widespread (e.g., national) or localized (e.g., regional)?
2. Is the proportion of lakes in the most disturbed condition getting better, worse, or staying the same over time?
3. Which environmental stressors are most strongly associated with degraded biological condition in lakes?

A variety of chemical, physical, and biological data were collected and developed into indicators to address the NLA questions. For each of these indicators, this Technical Report focuses on the conceptual basis, methods, and procedures used for the NLA. The information described in this

Technical Report was developed through the efforts and cooperation of NLA scientists from EPA, technical experts, and participating cooperators from states, tribes, and academia. While this Technical Report serves as a comprehensive summary of the NLA procedures, it is not intended to present an in-depth report of the design, site evaluation process, field sampling, NLA results, or additional data analysis results. Please see the following documents for additional details on these aspects of the project.

2012 National Lakes Assessment: Quality Assurance Project Plan (EPA 841-B-11-006)

2012 National Lakes Assessment: Site Evaluation Guidelines (EPA 841-B-11-005)

2012 National Lakes Assessment: Field Operations Manual (EPA 841-B-11-003)

2012 National Lakes Assessment: Laboratory Operations Manual (EPA 841-B-11-004)

Chapter 2: Survey Design and Population Estimates

The NLA was designed to assess the condition of the population of lakes, reservoirs, and ponds in the conterminous United States. The NLA design allows characterization of lakes at national and regional scales using chemical, physical and biological indicators. It is not intended to represent the condition of individual lakes. The statistical design also accounts for the distribution of lakes across the country – some areas have fewer lakes than others – so that even in areas of the country where there are few sample sites regional and national results still apply to the broader target population.

2.1 Description of sample design

The target population for the NLA includes all lakes, reservoirs, and ponds within the 48 contiguous United States greater than 1 hectare (ha) in surface area that are permanent waterbodies. The word "lake" in the remainder of this document includes lakes, reservoirs and ponds. Lakes that are saline are excluded as are those used for aquaculture, disposal-tailings, sewage treatment, evaporation, or other unspecified disposal use.

To select sites for the NLA, EPA statisticians used a Generalized Random Tessellation Stratified (GRTS) (Stevens and Olsen, 1999; Stevens and Olsen 2004) survey design for a finite resource with stratification and unequal probability of selection. The design includes reverse hierarchical ordering of the selected lakes.

2.1.1 *Stratification*

The overall NLA survey design was stratified by state and by class (NLA12_CLS). NLA12_CLS has three classes:

- NLA07RVT – defined as all NLA 2007 lakes that were target and sampled,
- NLA12NEW – remaining lakes in NHD-Plus that are included in the sample frame, and
- Exclude – lakes in NHD-Plus that are excluded from the sample frame (see Sample Frame section below).

The design also included additional sites that states could use to conduct state-scale surveys. This was accomplished by adding additional sites to the primary draw such that each state had 50 sites. Each state design has two strata, ST_ NLA07RVT and ST_ NLA12NEW (where ST is replaced by two letter state abbreviation. The total number of strata is 96 (two for each state).

2.1.2 *Unequal Probability Categories*

The 48 state strata for lakes from the NLA 2007 visited again in 2012 was an equal probability design within each stratum. The 48 state strata NLA12NEW was an unequal probability design within each state stratum. The unequal probability categories were defined based on lake area: 1 to 4 ha, 4 to 10 ha, 10 to 20 ha, 20 to 50 ha and greater than 50 ha.

2.1.3 *Panels*

The survey design has four panels: NLA07RVT – identifies lakes from NLA 2007 that will be visited in 2012, NLA12NAT – identifies new lakes that will be sampled along the lakes in panel NLA07RVT as part of the NLA2012 national survey design, NLA12ST – identifies additional lakes that a state may sample to achieve a total sample size of 50 lakes for the state, and OverSamp – identifies lakes to be used to replace lakes that cannot be sampled for some reason (not a lake, denied access, physically inaccessible, etc).

The national survey design includes all lakes within a state that are in either panels NLA07RVT or NLA12NEW.

A state survey design includes all lakes within a state that are either in panels NLA07RVT, NLA12NEW or NLA12ST.

2.1.4 *Expected Sample Size*

The expected sample size depends on the strata, panels and lake area category. For the NLA07RVT strata, the objective was to resample 400 of the NLA 2007 lakes out of the 1028 lakes that were sampled in 2007, i.e., approximately 38% of the lakes. The sample size for each state in the strata was proportional to the number of lakes sampled in the state in 2007. Exceptions were made when a state implemented a state-level design in 2007. A total sample size of 1000 lakes (including revisit sites) was desired for the national design. The sample size for each state was proportional (approximately 60%) to the state's sample size in NLA 2007. The minimum number of lakes for a state was set at 8 and the maximum at 43. Although aggregated ecoregions were not explicitly used in the survey design or setting sample sizes, they are implicitly used since the NLA 2007 allocated sample sizes using aggregated ecoregions. Once these two sample sizes were set for a state, an additional sample size was allocated to a state so that the total number of sites in a state would be 50 lakes. See Table 2-1 for the expected sample size by state.

Lakes in the NLA 2007 Revisit stratum were selected with equal probability and did not depend on lake area (NLA 2007 did depend on lake area). New lakes in the design were selected with unequal probability based on five lake area categories. The total number of lakes for a state in this strata was divided by five and that sample size (approximately) was assigned to the "(10,20]" lake area category. Sample sizes for lake area categories "(20,50]" and ">50" were decreased successively by one and for lake area categories "(4,10]" and "(1,4]" were increased successively by one. This process was adjusted to meet the total sample size requirement for the stratum. The rationale for this assignment of sample sizes is based on experience that smaller lakes are more likely not to be lakes or be inaccessible than larger lakes. When lakes are replaced, the process is expected to more likely result in an equal number of lakes sampled by lake area category.

Table 2-1. National Lakes 2012 Initial Design.

State	National Lakes 2012 Design: Number of NLA2007 Lakes Revisited in NLA2012: Sampled Once	Number of NLA2007 Lakes Revisited in NLA2012: Sampled Twice	Number of New Lakes for NLA2012: Sampled Once	Number of New Lakes for NLA2012: Sampled Twice	Total Number of lakes to be Sampled	Lakes sampled twice in 2012	Total Number of Lake Visits 2012	Additional Lakes State Design	Number of lakes State Design	Over Sample Lakes	Total Lakes Selected
AL	3	1	3	1	8	2	10	42	50	92	142
AR	3	1	3	1	8	2	10	42	50	92	142
AZ	6	1	5	1	13	2	15	37	50	86	136
CA	7	1	15	1	24	2	26	26	50	84	134
CO	10	1	11	1	23	2	25	27	50	78	128
CT	4	1	4	1	10	2	12	40	50	90	140
DE	3	1	2	1	7	2	9	43	50	46	96
FL	8	1	6	1	16	2	18	34	50	82	132
GA	4	1	5	1	11	2	13	39	50	90	140
IA	6	1	7	1	15	2	17	35	50	86	136
ID	10	1	12	1	24	2	26	26	50	78	128
IL	5	1	6	1	13	2	15	37	50	88	138
IN	16	1	9	1	27	2	29	23	50	66	116
KS	7	1	6	1	15	2	17	35	50	84	134
KY	2	1	5	1	9	2	11	41	50	94	144
LA	5	1	7	1	14	2	16	36	50	88	138
MA	3	1	5	1	10	2	12	40	50	92	142
MD	3	1	3	1	8	2	10	42	50	46	96
ME	9	1	13	1	24	2	26	26	50	80	130
MI	17	1	19	1	38	2	40	12	50	64	114
MN	21	1	19	1	42	2	44	108	150	256	406
MO	6	1	9	1	17	2	19	33	50	86	136
MS	6	1	6	1	14	2	16	36	50	86	136

MT	13	1	16	1	31	2	33	19	50	72	122
NC	4	1	7	1	13	2	15	37	50	90	140
ND	13	1	27	1	42	2	44	8	50	72	122
NE	13	1	13	1	28	2	30	22	50	72	122
NH	4	1	5	1	11	2	13	39	50	90	140
NJ	3	1	6	1	11	2	13	39	50	92	142
NM	4	1	7	1	13	2	15	37	50	90	140
NV	5	1	8	1	15	2	17	35	50	88	138
NY	3	1	5	1	10	2	12	40	50	92	142
OH	6	1	8	1	16	2	18	34	50	86	136
OK	17	1	11	1	30	2	32	20	50	64	114
OR	12	1	15	1	29	2	31	21	50	74	124
PA	6	1	8	1	16	2	18	34	50	86	136
RI	3	1	3	1	8	2	10	42	50	92	142
SC	2	1	5	1	9	2	11	41	50	94	144
SD	13	1	28	1	43	2	45	7	50	72	122
TN	3	1	4	1	9	2	11	41	50	92	142
TX	15	1	24	1	41	2	43	9	50	68	118
UT	8	1	12	1	22	2	24	28	50	82	132
VA	7	1	12	1	21	2	23	29	50	84	134
VT	3	1	5	1	10	2	12	40	50	92	142
WA	11	1	18	1	31	2	33	19	50	76	126
WI	10	1	16	1	28	2	30	22	50	78	128
WV	2	1	4	1	8	2	10	42	50	93	143
WY	6	1	11	1	19	2	21	31	50	86	136
Sum	350	48	458	48	904	96	1000	1596	2500	4111	6611

Table 2-2. Number of Sites Sampled for NLA 2012 by Design Categories.

Number of Sites Sampled for NLA 2012

State	NLA07RVT		NLA12NEW		NLA12NEW_07RVT			
	Sampled Once	Sampled Twice	Sampled Once	Sampled Twice	Sampled Once	Sampled Twice	Total Sites	Total Site Visits
AL	3	1	3	1			8	10
AR	3	1	3	1			8	10
AZ	4	1	7	1			13	15
CA	7	1	28	1	1		38	40
CO	10	1	11	1			23	25
CT	5	1	4	1			11	13
DE	3	1	2	1			7	9
FL	7	2	5	2			16	20
GA	4	1	5	1			11	13
IA	6	1	7	1			15	17
ID	9	1	29	1			40	42
IL	3	1	8	1			13	15
IN	13	1	35	1			50	52
KS	6	1	8	1			16	18
KY	2	1	6			1	10	12
LA	5	1	7	1			14	16
MA	3	1	5	1			10	12
MD	3	1	3	1			8	10
ME	9	1	13	1			24	26
MI	17	1	34	1			53	55
MN	20	1	28	1			50	52
MO	6	1	9	1			17	19
MS	6	1	6	1			14	16
MT	11	1	19	1	1		33	35

NC	3	1	8	1			13	15
ND	12	1	30	1			44	46
NE	13	1	13	1			28	30
NH	4	1	5	1			11	13
NJ	3	1	6	1			11	13
NM	1	1	10	1			13	15
NV	5	1	7	1	1		15	17
NY	1	1	6	1			9	11
OH	6		8	1		1	16	18
OK	16	1	12	1			30	32
OR	11	1	15	1	1		29	31
PA	5	1	9	1			16	18
RI	2	1	3	1	1		8	10
SC	1	1	5	2			9	12
SD	11	1	31	1			44	46
TN	2	1	4	2			9	12
TX	11	1	34	1			47	49
UT	6	1	38	1			46	48
VA	6	1	12	1	1		21	23
VT	3	1	5	1			10	12
WA	10	1	19	1			31	33
WI	9	1	39	1			50	52
WV	1	1	5	1			8	10
WY	4	1	12	1			18	20
Total	**311**	**48**	**621**	**50**	**6**	**2**	**1038**	**1138**

2.2 Sample frame summary

The sample frame was derived from the National Hydrography Dataset (NHD). Once the initial shapefile that included all lake objects in NHD was prepared additional attributes were created to identify lakes included in the sample frame and other properties used to construct the survey design.

Lakes included in the sample frame were those lakes with DES_FYTPE values equal to:

Lake/Pond
Lake/Pond: Hydrographic Category = Perennial
Lake/Pond: Hydrographic Category = Perennial; Stage = Average WaterElevation
Lake/Pond: Hydrographic Category = Perennial; Stage = Normal Pool
Reservoir
Reservoir: Reservoir Type = Water Storage
Reservoir: Reservoir Type = Water Storage; Hydrographic Category = Perennial

Lakes excluded in the sample frame were those lakes with DES_FYTPE values equal to:

Lake/Pond: Hydrographic Category = Intermittent
Lake/Pond: Hydrographic Category = Intermittent; Stage = Date of Photography
Lake/Pond: Hydrographic Category = Intermittent; Stage = High Water Elevation
Playa
Reservoir: Reservoir Type = Aquaculture
Reservoir: Reservoir Type = Cooling Pond
Reservoir: Reservoir Type = Disposal
Reservoir: Reservoir Type = Evaporator
Reservoir: Reservoir Type = Tailings Pond
Reservoir; Reservoir Type = Treatment
Swamp/Marsh

Next, lakes were excluded that were evaluated during the NLA 2007 and were identified as lakes that did not meet definition of a lake for NLA 2012. These were lakes with evaluation codes of Lake_Saline, Lake_Shallow, Lake_Special_Purpose, Lake_Vegetated, Non_Target, or Not_Lake".

Finally, lakes that were less than or equal to 1 hectare were excluded.

2.3 Survey analysis

Any statistical analysis of data must incorporate information about the monitoring survey design. In particular, when estimates of characteristics from a statistical survey such as the NLA are made for the entire target population are computed, called ***population estimates,*** the statistical analysis must account for any stratification or unequal probability selection in the design. The statistical estimates for the NLA population estimates were completed using site weights (see the NLA 2012 Site Information - Data file at https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic-resource-surveys,) and the R package 'spsurvey' (Kincaid and Olsen 2013) which implements the methods described by Diaz-Ramos et al. (1996).

2.4 Estimated extent of the NLA lake population and implications for reporting

Crews evaluated sites from the NLA survey design using a variety of techniques including aerial photo interpretations, GIS analyses, local knowledge, etc. to identify locations that did not meet the definition of a lake for NLA. Crews also dropped sites from sampling during field reconnaissance if they were a non-target type or could not be assessed due to accessibility issues (land owner denial, too dangerous to access, etc.). Dropped sites were systematically replaced from a pool of replacement sites from the random design. This process is implemented to maintain the integrity of the random design and to sample sites consistent with the original number planned in different categories.

The treatment of sites eliminated from sampling affects how the final population results are estimated and reported including the total proportion of the target population that we can assess. Taking into account the sites identified as not being part of the target population (e.g., saline lakes, lakes less than 1 hectare in size, etc.), the NLA analysis estimated there were 159,652 lakes in the NLA target population across the conterminous U.S. The area represented by sites that were part of the target population, but not sampled because of accessibility issues, is excluded from the assessments because sites which had access issues cannot be assumed to be randomly distributed. For example, there may be a bias in land-ownership for sites where access was denied, or sites which were inaccessible may often occur in areas with limited disturbance. As a result, the final number of lakes represented by the probability sites sampled and reported by the NLA, i.e., the inference (or sampled) population, was 111,818 lakes or approximately 70% of the target population. Throughout this report, lake estimates as percentages are relative to the 111,818 lakes. Figure 2-1 shows the percent of the target population of lakes that was sampled and the proportions that fell into non-sampleable categories. The inference population is represented by 1038 probability sites. The not assessed component of the population is represented by sites 1) where access was denied, 2) that were inaccessible due to safety considerations or remote location and 3) with other reasons for dropping.

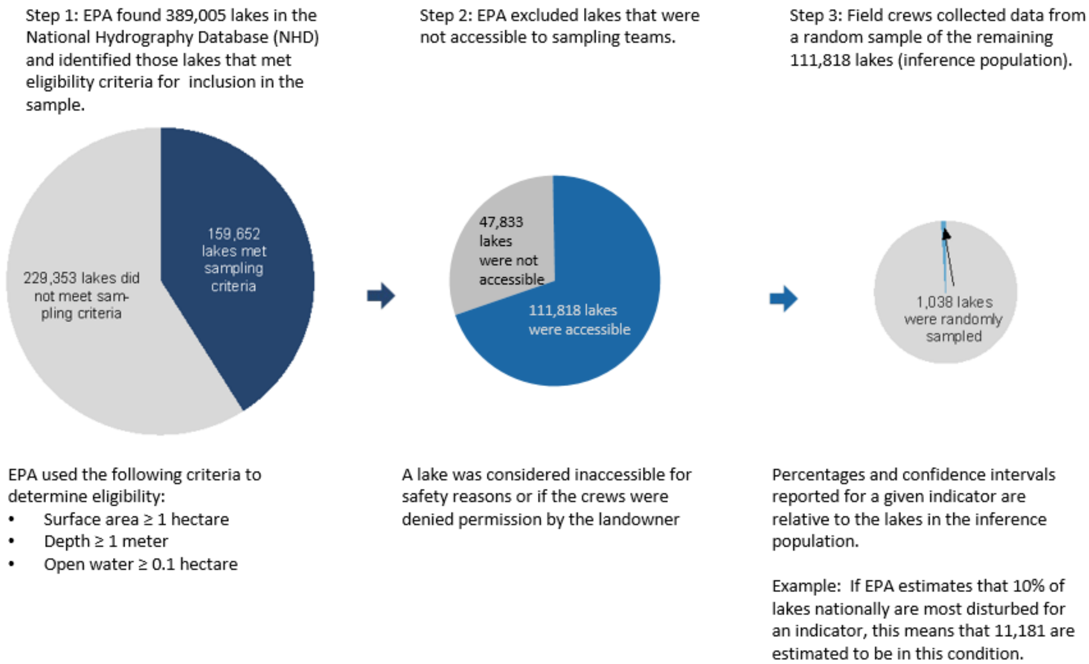

Figure 2-1. Proportion of Target Population Assessed Versus Not Assessed.

2.5 Literature cited

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Chapter 3: Reference Condition and Condition Benchmarks

3.1 Background information

NLA analysts used two processes for establishing the least disturbed, moderately disturbed, and most disturbed findings in the NLA report. For trophic status and recreational indicators, analysts used fixed, nationally consistent benchmarks. This approach is not covered in detail in this Technical Addendum although the specific benchmarks are identified in the appropriate sections. The second approach was to establish regionally consistent reference-based benchmarks. Detailed information on the regionally consistent approach is presented below. In refining benchmarks for the NLA 2012, some 2007 benchmark values were revised; therefore, direct comparisons should not be made between 2012 results and those reported in 2007. For purposes of identifying change in this report, 2007 results were recalculated based on new 2012 benchmarks.

To assess current ecological condition, it is necessary to compare measurements today to an estimate of “good” quality. Because of the difficulty of finding minimally disturbed sites in many parts of the country, NLA 2012 used “least disturbed condition” as the definition of reference condition. The use of least disturbed condition in the context of defining reference condition is different than the assessment category of least disturbed used in the NLA report. Least disturbed condition can be defined as the best available chemical, physical, and biological habitat conditions given the current state of the landscape – or “the best of what’s left” (Stoddard et al. 2006). Data from reference sites were used to develop ecoregion specific reference conditions against which test results could be compared. A total of four sets of reference sites were developed for use in establishing reference condition for the NLA report: one for the benthic macroinvertebrates indicator, one for the zooplankton indicator, one for the nutrient indicators, and one for the physical habitat indicators. This section describes the selection of the biological reference sites which also form the basis for all the nutrient and habitat reference sites.

3.2 Pre-sampling screening (hand-picked sites only)

In addition to the probability set of lakes, a smaller set of sites were hand selected *a priori* for sampling. We were trying to ensure that we captured samples from additional least disturbed lakes. Potential hand-picked sites were identified as high quality sites by EPA, states, tribes, and federal partners. When data were available, these potential sites were compared to water quality screens. When data were not available, sites underwent a high-level visual screen. The screen was used to minimize human disturbance around potential lakes (Herlihy et al., 2013). We identified 91 hand-picked lakes for sampling following this coarse screening process. The hand-picked sites were sampled during the 2012 index period using NLA sampling protocols, samples were processed and analyzed with the same analytical methods as the probability site samples, and then both the hand-picked sites and the probability sites were subjected to the post-sample screening process (Section 3.3). Regardless of whether sites were probability-based or hand-selected, only those that met the final screening criteria for the appropriate indicator (i.e. benthic macroinvertebrates, zooplankton, nutrients, and physical habitat) were used in developing reference conditions. In an update to 2007, ecoregion designations for each site were assigned based on the revised ecoregion GIS layer (2015) that accounted for updated Omernik ecoregion boundaries (Figure 3-1).

Figure 3-1. Nine aggregate ecoregions used for reference site classification.

3.3 Post-sampling screening for biological reference condition

To maximize the number of reference sites available for data analysis, hand-selected and probability-based sampled in either NLA 2007 or NLA 2012 were considered potential reference lakes. For benthic macroinvertebrates, only sites with at least 250 individuals in the sample were used to establish reference; this criterion did not apply to other sets of reference sites. Analysts used the chemical and physical data collected at each site to determine whether any given site was in least-disturbed condition for its aggregate ecoregion following the approach described by Herlihy et al. (2008). The nine aggregate ecoregions defined in NLA 2007 were used for the ecoregion classification although in some cases these ecoregions were further combined or lake types (natural vs. manmade) within an ecoregion treated differently (Figure

3-1). In the NLA, screening values were established for twelve chemical and physical parameters to screen for biological reference sites (Table 3-1). If measurements at a site exceeded the screening value for any one stressor, it was dropped from reference consideration. Given that expectations of least disturbed condition vary across regions, the criteria values for exclusion varied by ecoregion as well. Additional screening for physical habitat reference are described in Chapter 5.

Details on the calculation and naming of the shoreline habitat disturbance metrics is given in the physical habitat chapter (Section 5.3). Scoring of the disturbances on the visual assessment form for agricultural, residential, and industrial disturbance were simply done by summing the number of checked off disturbances on the form weighting for the noted level of disturbance. Low disturbance was weighted as 1 point, medium disturbances were weighted as 3 points, and high disturbances were weighted as 5 points. Fire was not summed in with the industrial disturbances as it could be an entirely natural disturbance.

All selected lake reference sites were also screened for excessive lake drawdown that was likely anthropogenic. Evidence of both horizontal and vertical lake level fluctuations were recorded by field crews. The square root of lake surface area was used as a surrogate for lake diameter and was used to scale horizontal exposure of littoral lake bottom. Similarly, lake maximum depth was used to scale vertical lake fluctuations. In addition, the drawdown criteria was relaxed for lakes with elevated levels of lakeshore disturbance, as indexed by HiiALL_syn > 0.75. A step by step key to defining NLA lakes impacted by drawdown is provided in Table 3-1. In NLA 2012, 13 otherwise reference lakes were removed due to excessive drawdown of likely anthropogenic origin.

Table 3-1. Least-disturbed reference screening filter thresholds for NLA2012.
If a lake exceeded any one of the thresholds it was not considered as a least-disturbed reference site for that ecoregion. Three filters were applied universally across all ecoregions, 1) ANC ≤ 25 ueq/L and DOC < 5 mg/L, 2) HifPany_Circa_syn& ≥ 0.9, and 3) no excessive lake drawdown (see Table 3-3).

Aggregate Ecoregion	TP (ug/L)	TN (ug/L)	Cl (ueq/L)	SO4 (ueq/L)	Turbidity (NTU)	Hii-NonAg&	Hii-Ag&	Assessment$ (Ag/Res/Ind)
WMT	>30@	>400	>100#	>200	>3	>0.6	>0	> 5/5/5
XER	>100	>1000	>500	>1000	>5	>1.5	>0.2	> 5/5/5
NPL	>150	>2000	>1000	---	>5	>1.5	>0.5	> 10/6/6
SPL	>150*	>2000*	>1000	---	>5	>1.5	>0.5	> 10/6/6
TPL	>120	>2000	>1000	>5000	>5.5	>1.7	>0.15	> 9/9/9
UMW	>40	>1200	>200	>200	>5	>0.6	>0	> 5/5/5
CPL	>50	>1200	>1000	>400	>5	>1.0	>0	> 6/10/6
SAP	>35	>800	>125	>300	>5	>0.9	>0	> 6/6/6
NAP	>30	>600	>100#	>300	>5	>0.6	>0	> 6/6/6

--- metric not used for screening

& HiiNonAg_syn, HiiAg_syn, and HifPany_Circa_syn are lakeshore physical habitat disturbance indices (see Section 5.3.4.6).
$ Assessment filters are based on indices of agricultural, residential, and industrial disturbance calculated from observations on the visual assessment form.
* No nutrient (TP, TN) or Turbidity filters applied in Sand Hills in SPL (Omernik Level III Ecoregion 44)
# No Chloride filter applied in Coastal Ecoregions in NAP (ecoregions 59,82), XER (ecoregion 6), and WMT (ecoregions 1,2,8)
@ No TP filter used in volcanic ecoregions in WMT (ecoregions 4,5,9,77)

In addition to selecting least disturbed reference sites, analysts also determined most disturbed sites for each ecoregion. These sites were used primarily in developing biotic MMIs that would be used in the biological assessment of the nation's lakes and in testing the strength of association of other indicators to anthropogenic stress. Similar to the reference lake selection process, thresholds were used to determine which lakes were to be considered most disturbed in each ecoregion (Table 3-2). If any site exceeded the most-disturbed threshold for any one of these screening criteria, then the site was classified as most-disturbed.

Note that the NLA did not use data on land-use in the watersheds for the final reference site screening—sites in agricultural areas (for example) may well be considered least disturbed, provided that their chemical and physical conditions are among the least-disturbed for the region. Additionally, the NLA did not use data from the biological assemblages themselves to define biological reference sites because the reference sites are being used to assess biological condition and to use biological data to then define reference would constitute circular reasoning.

Table 3-2. Most disturbed site screening thresholds for NLA2012.
If a lake exceeded any one of the thresholds it was considered a most-disturbed site for that ecoregion. One screen was applied universally across all ecoregions, ANC ≤ 0 ueq/L and DOC < 5 mg/L.

Aggregate Ecoregion	TP (ug/L)	TN (ug/L)	Cl (ueq/L)	SO4 (ueq/L)	Turbidity (NTU)	Hii-NonAg[&]	Hii-Ag[&]	Assessment[$] (Ag/Res/Ind)
WMT	>150[@]	>1500	>1500[#]	>1500	>10	>2.5	>0.9	> 15/15/15
XER	>400	>4000	---	---	>25	>3.5	>1.0	> 15/15/15
NPL	>400	>4000	---	---	>50	>3.5	>1.2	> 15/15/15
SPL	>400*	>4000*	---	---	>50	>3.5	>1.2	> 15/15/15
TPL	>500	>5000	>5000	>20,000	>50	>4.0	>1.2	> 15/18/15
UMW	>200	>2500	>2500	>2500	>20	>3.5	>0.9	> 15/15/15
CPL	>200	>3000	>5000	>2500	>30	>3.5	>1.0	> 15/15/15
SAP	>150	>2500	>1500	>1500	>20	>3.5	>0.9	> 15/15/15
NAP	>150	>2500	>1500[#]	>1500	>20	>3.5	>0.9	> 15/15/15

--- metric not used for screening
& HiiNonAg_syn and HiiAg_syn are lakeshore physical habitat disturbance indices (see Section 5.3.4.6)
$ Assessment filters are based on indices of agricultural, residential, and industrial disturbance calculated from observations on the visual assessment form.
* No nutrient (TP, TN) or Turbidity filters applied in Sand Hills in SPL (Omernik Level III Ecoregion 44)

No Chloride filter applied in Coastal Ecoregions in NAP (ecoregions 59,82), XER (ecoregion 6), and WMT (ecoregions 1,2,8)
@ No TP filter used in volcanic ecoregions in WMT (ecoregions 4,5,9,77)

Table 3-3. Dichotomous key for defining NLA lakes likely impacted by anthropogenic drawdown.

Based on field observations of horizontal lake level fluctuations (ΔH), vertical lake level fluctuations (ΔV), and human lakeshore disturbance (physical habitat summary metric HiiAll_syn).

1. ΔH < 10 m AND ΔV < 2 m
    Yes **- LAKE OK**
    No - go to 2
2. ΔH ≥ 10 m and ΔV ≥ 2 m
    Yes – **Lake Drawdown, Not Reference**
    No – go to 3
3. ΔV ≥ 2 m and ΔV/Maximum Lake Depth ≥ 10%
    Yes – **Lake Drawdown, Not Reference**
    No – go to 4
4. ΔH < 10 m
    Yes – **LAKE OK**
    No – go to 5
5. ΔH/sqrt(Lakearea) ≥ $5m^2$
    Yes – **Lake Drawdown, Not Reference**
    No – go to 6
6. Lake Disturbed, HiiAll_syn > 0.75
    Yes – **Lake Drawdown, Not Reference**
    No - **LAKE OK**

3.4 Post-sample screening for nutrient reference condition

Setting reference condition for nutrients requires a different process then the one used for biological reference condition evaluation. Because nutrients (TN, TP) were used to select biological reference sites, the biological reference sites could not be used as nutrient reference lakes due to circularity. During the development of nutrient reference sites, we compiled all sampled sites in NLA 2007 and 2012 as was done for the biological reference condition process described above. As was the case above, ecoregion designations for each site were assigned based on the 2015 revised ecoregion GIS layer that accounted for updated Omernik ecoregion boundaries. All sites were then passed through the NLA 2012 biological reference screening process for their ecoregion as described with one exception. To avoid complete circularity, TP and TN thresholds were removed as screening variables in the reference screening process. All told there were 418 initial reference sites in the combined data, 149 sampled in 2007 and 269 sampled in 2012. For cross-year repeat sites sampled in both years, only the 2012 data was used. Another modification was made for lakes in the Southern Plains. The nutrient conditions in the natural SPL lakes are so different than the man-made SPL lakes that they need to have different thresholds. We created SPLman and SPLnat surrogate ecoregions for this analysis.

Screening Reference Sites for Nutrient Thresholds

GIS Screening: There was a fairly strong disturbance signal in the reference sites as evidenced by looking at relationships with four GIS stressor variables (% Agriculture, %Urban, Road and Population density). Unfortunately, there was no road and population density available for the NLA 2007 data so GIS screening was only done using the %Ag and %Urban metrics. In order to remove this disturbance signal, a GIS stressor filtering approach was used to remove from the reference site pool those sites that failed the filtering. For %Ag, ecoregional criteria were used: NAP, WMT, XER (>10%); NPL, SAP, SPL, UMW (>25%); CPL (>40%); TPL (>50%). For %Urban, a >10% criteria was used for all ecoregions but the CPL where a >15% filtering criteria was used.

Out of the 418 initial nutrient reference sites, 375 passed the GIS stressor screening filter (Table 3-4). Dropped sites due to the GIS screen were most prevalent in the Plains. The TPL lost 11 of its 26 sites even with a 50% Ag screen. The man-made SPL lost 6 of 22 lakes.

Outlier Screening: As in the original Wadeable Streams Assessment and NLA 2007 threshold setting, we used a 1.5*IQR outlier screening test to drop outliers from the analysis (sites with values outside the range of Q1-1.5*IQR or Q3+1.5*IQR were dropped). Outlier screening removed 18 of the 375 GIS screened reference lakes for TP analysis and 13 of 375 lakes for TN analysis. For the GIS screened, outlier removed dataset, all ecoregions but the TPL had >10 sites, but only the CPL, NAP, SAP, UMW, and WMT had > 25 sites.

Table 3-4. Number of unique reference sites used in analysis – revised ecoregion data.

Eco	All Nutrient Ref (Initial screen)	GIS Screened Reference Sites	**GIS Screen with outliers removed (TP/TN)**
CPL	39	28	**27/26**
NAP	75	71	**68/69**
NPL	14	12	**12/12**
SAP	33	31	**30/30**
SPL-man	22	16	**15/16**
SPL-nat	19	19	**17/19**
TPL	26	15	**14/15**
UMW	59	56	**55/54**
WMT	103	103	**95/98**
XER	28	24	**24/23**
TOTAL	418	375	**357/362**

3.5 Literature cited

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Chapter 4: Benthic Invertebrates

4.1 Background information

The taxonomic composition and relative abundance of different taxa that make up the littoral macroinvertebrate assemblage present in a lake can be used to assess how human activities affect ecological condition. Two principal types of ecological assessment tools to assess condition based on macroinvertebrate assemblages are currently prevalent: multimetric indices and predictive models of taxa richness. The purpose of these indicators is to present the complex community taxonomic data represented within an assemblage in a way that is understandable and informative to resource managers and the public. For NLA 2012, we developed a multimetric index of macroinvertebrate condition.

Multimetric indicators have been used in the U.S. to assess condition based on fish and macroinvertebrate assemblage data (e.g., Karr and Chu, 2000; Barbour et al., 1999; Barbour et al., 1995). The multimetric approach involves summarizing various assemblage attributes (e.g., composition, tolerance to disturbance, trophic and habitat preferences) as individual "metrics" or measures of the biological community. Candidate metrics are then evaluated for various aspects of performance and a subset of the best performing metrics are then combined into an index, referred to as a multimetric index or MMI. In order to amass the largest dataset possible, macroinvertebrate data from both the NLA 2007 and NLA 2012 were combined and analyzed together to develop the MMI and calculate condition class thresholds. Thus, metrics and subsequent MMI scores were calculated in an identical manner for both NLA datasets.

4.2 Data preparation

4.2.1 *Standardizing counts*

The number of individuals counted in a sample was standardized to a constant number to provide an adequate number of individuals that was the same for the most samples and that could be used for multimetric index development. A subsampling technique involving random sampling without replacement was used to extract a true "fixed count" of 300 individuals from the total number of individuals enumerated for a sample (target lab count was 500 individuals). Samples that did not contain at least 300 individuals were used in the assessment because low counts can indicate a response to one or more stressors. Only those sites with at least 250 individuals, however, were used as reference sites.

4.2.2 *Autecological characteristics*

Autecological characteristics refer to specific ecological requirements or preferences of a taxon for habitat preference, feeding behavior, and tolerance to human disturbance. These characteristics are prerequisites for identifying and calculating many metrics. A number of state/regional organizations and research centers have developed autecological characteristics for benthic macroinvertebrates in their region. For the NLA 2012, a consistent “national” list of characteristics that consolidated and reconciled any discrepancies among the regional lists was needed before certain biological metrics could be developed and calibrated and an MMI could be constructed. The same autecological information used in WSA and NRSA was used in NLA. Members of the data analysis group pulled together autecological information from five existing sources: the EPA Rapid Bioassessment Protocols document, the National Ambient Water Quality Assessment (NAWQA) national and northwest lists, the Utah State University list, and the EMAP Mid-Atlantic Highlands (MAHA) and Mid-Atlantic Integrated Assessment (MAIA) list. These five were chosen because they were thought to be the most independent of each other and the most inclusive. A single national-level list was developed based on the following decision rules:

4.2.3 *Tolerance values*

Tolerance value assignments followed the convention for macroinvertebrates, ranging between 0 (least tolerant or most sensitive) and 10 (most tolerant). For each taxon, tolerance values from all five sources were reviewed and a final assignment made according to the following rules:

1. If values from different lists were all <3 (sensitive), final value = mean.
2. If values from different lists were all >3 and <7 (facultative), final value = mean.
3. If values from different lists were all >7 (tolerant), final value = mean.
4. If values from different lists spanned sensitive, facultative, and tolerant categories, best professional judgment was used, along with alternative sources of information (if available) to assign a final tolerance value.
5. Tolerance values of 0 to ≤3 were considered “sensitive” or “intolerant.” Tolerance values ≥7 to 10 were considered “tolerant,” and values in between were considered “facultative.”

4.2.4 *Functional feeding group and habitat preferences*

In many cases, there was agreement among the five data sources. When discrepancies in functional feeding group (FFG) or habitat preference (“habit”) assignments among the five primary data sources were identified, a final assignment was made based on the most prevalent assignment. In cases where there was no prevalent assignment, the workgroup examined why disagreements existed, flagged the taxon, and used best professional judgment to make the final assignment.

4.2.5 *Taxonomic resolution*

Taxonomic resolution is an import factor in the development of multimetric indices. Maintaining consistent taxonomic resolution for specific taxa across sites helps ensure that differences between sites are due to environmental factors and not an artifact of taxa identifications. For most taxa identified the taxonomic resolution was to the generic level, however the following groups had higher hierarchical taxonomic resolution: oligochaetes, mites, polychaetes were rolled up to family, ceratopogonids were rolled up to subfamily.

4.3 Multimetric index development

4.3.1 *Data Set*

The NLA macroinvertebrate 300 fixed count data was used to calculate the community metrics used in the MMI. A best ecoregional MMI was developed by scoring and summing the six metrics that performed best in each ecoregion. We combined the NLA 2007 and 2012 benthic metric files which were both calculated with common autecology and taxonomic resolution. All reference sites from both 2007 and 2012 data were defined using the NLA 2012 definitions described in Section 3 based on nine aggregate ecoregion criteria. The goal was to make the 2007 and 2012 data as comparable as possible so they could be combined for analysis. Reference sites that had less than 250 individuals were not used as reference for MMI development. All told, there were 2330 site visits (samples) in the data; 1132 from 2007 and 1198 from 2012. There were 1789 unique sites. Some sites were sampled twice in their respective years and some sites were sampled in both 2007 and 2012.

4.3.2 *Low Macroinvertebrate Numbers*

A large number of samples had a very low number of individuals. Examination of these low number sites did not suggest that this was primarily due to impairment. We think that it is related to field collection and lake bottom substrate composition. Samples with low bug numbers will have poor MMI scores because of the strong relationship between sample count and taxa richness. We decided that samples with less than 100 individuals were not sufficiently sampled and we would not assess them. They were removed from the process of MMI development and MMI scores will be set to missing values. These are identified as "not assessed" in the NLA. In the combined NLA 2007 and 2012 data, 182 samples had < 100 individuals. In the 2012 population, these represent 11,862 lakes (11% of the population).

4.3.3 *Ecoregion Classification*

For the NLA 2012 assessment, the nine national aggregate ecoregions (Figure 3-1) were aggregated into five aggregate biological ecoregions by combining some ecoregions together. Specifically, that consisted of making an Eastern Highlands (EHIGH) region by combining the SAP and NAP, a PLAINS ecoregion by combining the TPL, SPL, and NPL, and a Western ecoregion (WMTNS) by combing the WMT and XER regions. The CPL and UMW remain their own ecoregions. MMIs were developed independently for each of these 5 biological ecoregions. Ecoregion boundaries were defined by most current (2015) Omernik Ecoregion GIS layers.

4.3.4 *Metric Screening*

All 126 calculated benthic metrics were screened for both signal:noise (S:N) and discrimination of least-disturbed reference sites from most-disturbed sites (F-test). S:N ratios were calculated for each metric nationally and within each biological ecoregion using the visit 1 versus visit 2 variance within year as the noise and among site variance as the signal. For calculating F-tests, and all subsequent MMI development, we only used one visit per site (index visit). The first sample visit of the year with valid data was used. For sites with valid samples in both years, the 2012 first visit data were used (samples with less than 100 bugs were not considered valid data). F-tests were run on just the least disturbed reference (R) versus the most disturbed (T) sites.

Metrics had to pass both F and S:N screens in order to remain in consideration for inclusion in the final MMI. Metrics had to have S:N ≥ 1.5 either nationally or within their ecoregion in order to pass. For the F-test, only metrics that had F-values ≥ 4.0 passed. From this screening, 35 metrics from CPL, 42 from EHIGH, 44 from UMW, 29 from PLAINS, and 50 from WMTNS passed and were considered for the all subsets MMI selection.

4.3.5 *All Subsets MMI selection*

Passing metrics were assigned to one of the six basic metric classes used to assemble the MMI as done in the NARS stream MMI (Stoddard et al., 2008). An all subsets procedure was used to assemble all possible combinations of MMIs using the six metric class framework. There were 8,960 combinations of metrics in the CPL, 12,096 in the EHIGH, 36,855 in the UMW, 3360 in the PLAINS, and 65,280 in the WMTNs. For each possible MMI combination, the MMI S:N, F-test, metric correlations, and IQR box delta (separation between least and most disturbed) were calculated. For correlations, both the mean and maximum correlation among the six metrics were calculated. IQR box delta or separation is the difference between the 25th percentile of reference sites and the 75th percentile of most disturbed sites. Thus positive box deltas indicate separation between the least and most disturbed boxes, negative values indicate overlap in the IQRs (boxes of box and whisker plot) of the least and most disturbed sites.

To pick the best MMI from the all subsets results, all MMI candidates were first screened for S:N and maximum metric correlation. Only MMIs that had max correlation ≤ 0.7 and S:N ≥ 3 were considered. MMIs that passed this screen were evaluated for both box delta and F-value with the goal of picking the MMI that had the best combination of those two values. These two measures are highly correlated. To do this objectively, we ran a PCA on box delta and F-value and selected the MMI that had the highest PCA factor 1 score. The intent was to optimize and pick the model with the best combination of F-value and separation. The six metrics that make up the final (best) MMI are shown in Table 4-1.

Each of the six selected metrics were scored on a 0–10 scale by interpolating metrics between a floor and ceiling value. The six metric 0-10 point scaled scores were then summed and normalized to a 0–100 scale by multiplying by 100/60 to calculate the final MMI. Details of this process are described in Stoddard et al. (2008) for the NARS stream MMI but the NLA process is the same. The final metrics used in each ecoregion, metric direction, and floor and ceiling values are summarized in Table 4-1. Scoring equations are different depending on if the metric responds positively (high values good) or negatively (high values bad) with disturbance. For positive metrics, values above the ceiling get 10 points, and values below the floor get 0 points. For negative metrics, values above the ceiling get 0 points, and values below the floor get 10 points. The interpolation equations for scoring the 0-10 points for metrics between the floor and ceiling values are,

Positive Metrics: Metric Points = 10*((metric value-floor)/(ceiling-floor))
Negative Metrics: Metric Points = 10 * (1 - ((metric value-floor)/(ceiling-floor))).

For positive metrics, floor values are set at the 5th percentile of all samples in the ecoregion, ceiling values are the 95th percentile of reference sites in the ecoregion. Negative metric floor/ceilings are calculated the opposite way. Statistics for the final MMI in each ecoregion are shown in Table 4-2. The overall S:N of the MMI based on visit 1 vs. 2 revisits nationally across both years was 3.56. Box plots showing the R versus T discrimination of the final MMIs are shown in Figure 4-1.

Table 4-1. Final NLA 2007-2012 biological ecoregion benthic MMI metrics and their floor/ceiling values for MMI scoring.

Ecoregion	Metric Class	Metric name*	Direction	Floor Value	Ceiling Value
Coastal Plains	Composition	NOINPTAX	Negative	21.88	55.17
Coastal Plains	Diversity	CHIRDOM3PIND	Negative	38.57	96.08
Coastal Plains	Feeding Group	PREDRICH	Positive	6.00	23.0
Coastal Plains	Habit	SPWLRICH	Positive	5.00	15.0
Coastal Plains	Richness	EPT_RICH	Positive	1.00	8.00
Coastal Plains	Tolerance	NTOLPIND	Positive	6.33	64.33
E. Highlands	Composition	NOINPTAX	Negative	13.79	48.72
E. Highlands	Diversity	CHIRDOM3PIND	Negative	39.87	85.94
E. Highlands	Feeding Group	COGARICH	Positive	8.00	27.0
E. Highlands	Habit	CLNGRICH	Positive	3.00	12.0
E. Highlands	Richness	EPOTRICH	Positive	2.00	14.0
E. Highlands	Tolerance	TL23RICH	Positive	1.00	9.00
Plains	Composition	DIPTPTAX	Negative	16.67	60.00
Plains	Diversity	HPRIME	Positive	0.65	3.17
Plains	Feeding Group	PREDRICH	Positive	2.00	19.0
Plains	Habit	CLMBPTAX	Positive	10.0	33.33
Plains	Richness	EPOTRICH	Positive	0	10.0
Plains	Tolerance	TL23PIND	Positive	0	19.67
Upper Midwest	Composition	NOINPIND	Negative	5.33	89.0
Upper Midwest	Diversity	CHIRDOM3PIND	Negative	36.51	87.91
Upper Midwest	Feeding Group	SHRDPIND	Negative	2.67	50.67
Upper Midwest	Habit	CLNGRICH	Positive	3.00	14.0
Upper Midwest	Richness	CRUSRICH	Negative	0	3.00
Upper Midwest	Tolerance	TL23PTAX	Positive	2.17	23.81
Western Mts.	Composition	ODONPIND	Negative	0	17.33
Western Mts.	Diversity	CHIRDOM5PIND	Positive	7.33	98.25
Western Mts.	Feeding Group	SCRPRICH	Negative	0	5.00
Western Mts.	Habit	CLNGRICH	Positive	1.00	8.00
Western Mts.	Richness	TRICRICH	Positive	0	4.00
Western Mts.	Tolerance	TL23PTAX	Positive	0	21.43

*Metric Names
NOINPTAX= % Non-Insect Taxa (Non-Insect Taxa Richness / Total Taxa Richness*100)

DIPTPTAX = % Diptera Taxa (Diptera Taxa Richness / Total Taxa Richness*100)

NOINPIND = % Non-Insect Individuals

ODONPIND = % Odonata Individuals

CHIRDOM3PIND = % Chironomid Individuals in Top 3 most abundant Chironomid Taxa

CHIRDOM5PIND = % Chironomid Individuals in Top 5 most abundant Chironomid Taxa

HPRIME = Shannon Diversity Index

PREDRICH = Predator Taxa Richness

COGARICH = Collector-Gatherer Taxa Richness

SHRDPIND = % Shredder Individuals

SCRPRICH = Scraper Taxa Richness

SPWLRICH = Sprawler Taxa Richness

CLNGRICH = Clinger Taxa Richness

CLMBPTAX = % Climber Taxa (Climber Taxa Richness / Total Taxa Richness *100)

EPT_RICH = Ephemeroptera + Plecoptera + Trichoptera Taxa Richness

EPOTRICH = Ephemeroptera + Plecoptera + Trichoptera + Odonata Taxa Richness

CRUSRICH = Crustacean Taxa Richness

TRICRICH = Trichoptera Taxa Richness

NTOLPIND = % Individuals with pollutant tolerance values < 6

TL23RICH = Taxa Richness of taxa with pollutant tolerance values ≥ 2.0 and < 4.0

TL23PIND = % Individuals with pollutant tolerance values ≥ 2.0 and < 4.0

TL23PTAX = % Taxa with pollutant tolerance values ≥ 2.0 and < 4.0

Table 4-2. Final NLA 2007-2012 biological ecoregion benthic MMI statistics.

Ecoregion	F-test	Box Delta	Max Corr.	Mean Corr.	S:N
Coastal Plain	54.7	12.7	0.45	0.17	3.45
E. Highlands	69.0	1.85	0.50	0.26	3.12
Plains	36.2	-2.26	0.68	0.41	3.35
Upper Midwest	64.5	10.4	0.57	0.24	3.00
Western Mts.	88.9	4.46	0.48	0.16	3.66

F-test=F-score for difference between reference and trash site means; Box Delta=Separation difference between Reference Q1 and most-disturbed Q3 in MMI units; Corr=Pearson correlation among six MMI metrics; S:N = Ecoregional within year S:N ratio.

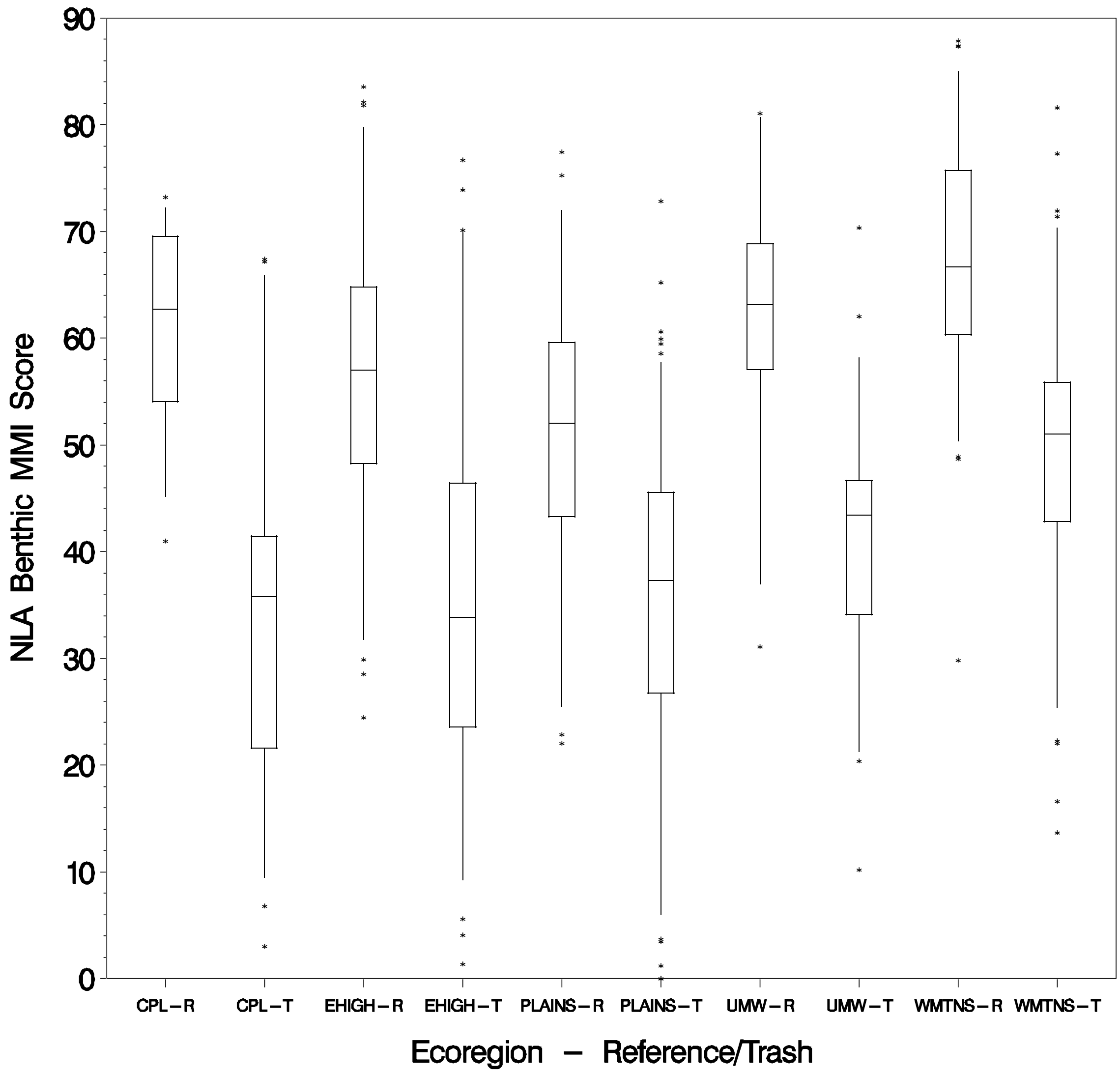

Figure 4-1. Box and whisker plots showing discrimination between reference (R) and trash (T) sites by biological ecoregion. Whiskers show the 5th and 95th percentiles

4.3.6 *Setting MMI Thresholds*

Previous large-scale assessments have converted MMI scores into classes of assemblage condition by comparing those scores to the distribution of scores observed at least-disturbed reference sites. See Section 3.3 for information on selecting reference sites. If a site's MMI score was less than the 5th percentile of the reference distribution, it was classified as in most disturbed condition; scores between the 5th and 25th percentile were classified as moderately disturbed and scores in the 25th percentile or higher were classified as least disturbed. This approach assumes that the distribution of MMI scores at reference sites reflects an approximately equal, minimum level of human disturbance across those sites. But this assumption did not appear to be valid for some of the ecoregions.

Percentile-based thresholds were adjusted for reference site quality by regressing MMI versus a PCA Factor 1 disturbance score. For the PCA disturbance factor, all variables used in the NLA reference site screening (TP, TN, Cl, SO4, Turbidity, physical habitat disturbance indices, and assessment indices – Table 3-1) were put into the PCA. Values were log transformed before analysis. The first principal component (Factor 1) of this PCA well represented a generalized gradient of human disturbance. There were 247 NLA reference sites with full disturbance data that was required to calculate the PCA disturbance factor score. Before threshold calculation, a 1.5*IQR outlier analysis was done on the reference site MMIs to remove outliers. Three sites were dropped as outliers (2 in the UMW and 1 in the WMTNS) leaving 244 reference sites for analysis.

MMI scores at the reference sites were weakly, but significantly, related to this disturbance gradient (Figure 4-2). Thus, MMI reference distributions from these regions may be biased downward, because they include somewhat disturbed sites which may have lower MMI scores. Herlihy et al. (2008) developed a process that used this PCA disturbance gradient to reduce the effects of disturbance on threshold values within the reference site population. The process uses multiple regression modeling to develop adjusted thresholds analogous to the 5^{th} and 25^{th} percentiles of reference sites in each ecoregion based on the slope of the MMI-disturbance relationship in each ecoregion. Briefly, the process involves setting the goal for disturbance to the 25^{th} percentile of the Factor 1 disturbance score for reference sites in each ecoregion. The ecoregion MMI value at that goal is predicted from the MMI-disturbance regression as,

MMIpred = (GOAL * SLOPE) + INTERCEPT.

Then the percentiles to be used as the adjusted thresholds are calculated assuming there is a normal distribution around this predicted mean using the RMSE of the regression model as the standard error,

Least-Moderately Disturbed 25^{th} threshold = MMIpred - 0.675 * RMSE
Moderately-Most Disturbed 5^{th} threshold = MMIpred - 1.650 * RMSE.

The best regression model from the NLA reference site data had a common slope and separate intercepts by ecoregion. The pooled model RMSE was 11.01, the common slope was –7.953 and the intercepts were 65.45 in the CPL, 54.30 in the EHIGH, 60.14 in the UMW, 61.47 in the Plains, and 61.73 in the WMTNS. The resulting adjusted MMI threshold values for the condition classes in each ecoregion used in the NLA 2012 report are given in Table 4-3.

Table 4-3. NLA2012 macroinvertebrate MMI thresholds.

Ecoregion	# of Ref Sites	Adjusted 25th Least-Disturbed Threshold	Adjusted 5th Most Disturbed Threshold
Coastal Plains	23	≥ 54.8	< 44.1
East. Highlands	70	≥ 51.5	< 40.8
Plains	48	≥ 46.8	< 36.1
Upper Midwest	35	≥ 58.1	< 47.3
Western Mountains	68	≥ 64.8	< 54.1

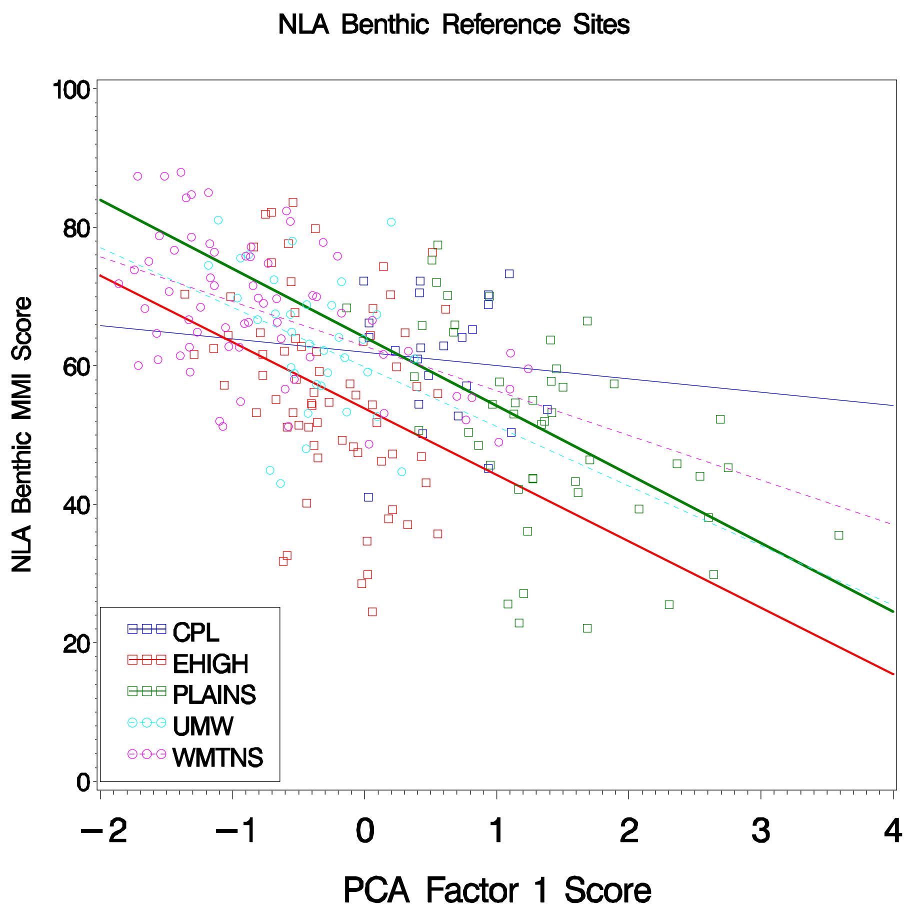

Figure 4-2. MMI score versus PCA factor 1 disturbance score for NLA macroinvertebrate reference sites. Higher PCA factor 1 scores indicate more disturbance.

4.4 Literature cited

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Chapter 5: Physical Habitat

5.1 Background information

Near-shore physical habitat structure in lakes has only recently been addressed by the U.S. Environmental Protection Agency (EPA) in its National Aquatic Resource Surveys (NARS) monitoring efforts (e.g., USEPA 2009, Kaufmann et al. 2014a,b,c). Like human activities, aquatic and riparian biota are concentrated near lakeshores, making near-shore physical habitat ecologically important, but exposed and vulnerable to anthropogenic perturbation (Schindler and Scheuerell 2002, Strayer and Findlay 2010, Hampton et al. 2011). Littoral and riparian zones are positioned at the land-water interface, and tend to be more structurally complex and biologically diverse than either pelagic areas or upland terrestrial environments (Polis et al. 1997, Strayer and Findlay 2010). This complexity promotes interchange of water, nutrients, and biota between the aquatic and terrestrial compartments of lake ecosystems (Benson and Magnuson 1992, Polis et al. 1997, Palmer et al. 2000, Zohary and Ostrovsky 2011). Structural complexity and variety of cover elements in littoral areas provide diverse opportunities for supporting assemblages of aquatic organisms (Strayer and Finlay 2010; Kovalenko et al 2012), while intact riparian vegetation and wetlands surrounding lakes increase near-shore physical habitat complexity (e.g., Christensen et al. 1996, Francis and Schindler 2006) and buffer lakes from the influence of upland land use activities (Carpenter and Cottingham 1997, Strayer and Findlay 2010). Human activities on or near lakeshores can directly or indirectly degrade littoral and riparian habitat (Francis and Schindler 2006). Increased sedimentation, loss of native plant growth, alteration of native plant communities, loss of physical habitat structure, and changes in littoral cover and substrate are all commonly associated with lakeshore human activities (Christensen et al. 1996, Engel and Pederson 1998, Whittier et al. 2002, Francis and Schindler 2006, Merrell et al. 2009). Such reductions in physical habitat structural complexity can deleteriously affect fish (Wagner et al. 2006, Taillon and Fox 2004, Whittier et al. 1997, 2002, Halliwell 2007, Jennings et al. 1999, Wagner et al. 2006), aquatic macroinvertebrates (Brauns et al. 2007), and birds (Kaufmann et al. 2014b).

The EPA developed standardized, rapid field methods to quantify physical habitat structure and near-shore anthropogenic disturbances (Kaufmann and Whittier 1997), and piloted them in the Northeastern U.S. (Larsen and Christie 1993, Whittier et al. 2002b, Kaufmann et al. 2014b). These methods were modified (USEPA 2007a, Kaufmann et al. 2014a) and applied in 2007 for the first U.S. national survey of lake physical habitat condition (US EPA 2009, Kaufmann et al. 2014c). The EPA's lake physical habitat methods were once again modified to explicitly assess habitat structure in exposed drawdown zones (USEPA 2012), and applied in the NLA 2012 survey as part of the EPA's second national survey of the ecological condition of lakes in the United States (USEPA 2016). The NLA 2012 field method modifications were structured so that we were able to duplicate of all the lake habitat condition indices that were used in the previous (2007) national assessment. We calculated habitat metrics and indices described by Kaufmann et al. 2014a,c) to quantify the variety, structural complexity, and magnitude of areal cover from physical habitat elements within the near shore zones of lakes in the NLA 2012 survey.

Our objectives in this chapter are to describe how we calculated physical habitat indices based on near-shore physical habitat data collected in the NLA survey, and how we derived physical habitat condition thresholds relative to least-disturbed conditions. We only briefly describe the NLA field methods and data reduction procedures, which are published elsewhere (USEPA 2012; Kaufmann 2014a). Finally we evaluate the precision of NLA's key indices of physical habitat condition and examine their association with anthropogenic disturbances.

5.2 Data preparation

We took the following eight steps to assess physical habitat condition in U.S. lakes based on the NLA 2012 national probability sample of lakes and reservoirs.

1) Field crews made measurements and observations of near-shore physical habitat structure and human activities on a national probability sample of lakes and reservoirs (described by USEPA 2016, and Kaufmann et al. 2014a);
2) Classified survey lakes by aggregated ecoregion (ECOWSA9_2015), and by their relative levels of anthropogenic disturbance within those ecoregions (RT_NLA12_2015).
3) Calculated a set of physical habitat metrics as described by Kaufmann et al. (2014a) for NLA 2007, but adapted calculations to adjust for the NLA 2012's field method change that assessed riparian vegetation cover, littoral cover, and human disturbance in the drawdown zone separate from those above the typical high water mark or inundated by water in the littoral zone;
4) Calculated multimetric indices of lakeshore anthropogenic disturbance and nearshore physical habitat cover and structure as described by Kaufmann et al. (2014c) for NLA 2007, and assigned variants of these indices according to aggregated Ecoregions (ECOWSA9_2015); also defined a new indicator of lake drawdown;
5) Estimated lake-specific expected ("E") values for physical habitat indices from region-specific regression models of factors predicting physical habitat in the combined set of least-disturbed lakes from the NLA 2007 and 2012 surveys. Our modeling approach is very similar to that employed by Kaufmann et al. (2014c) in the Western Mountain and Xeric ecoregions for the NLA 2007 report;
6) Set criteria for low, medium and high lakeshore anthropogenic disturbance (good, fair, poor) based on professional judgement; good, fair, and poor littoral and riparian physical habitat condition based on deviation from the central tendency of observed/expected (O/E) values within the group of least-disturbed lakes; and small, medium, and large lake drawdown based on percentiles of the indicator values themselves in least-disturbed lakes.
7) Examined the precision of NLA 2012 key physical habitat indicators.
8) Examined the association between NLA 2012 physical habitat indicators and anthropogenic disturbances, comparing the regional distributions of habitat condition in least-disturbed reference lakes with those in highly disturbed lakes.

5.3 Methods

5.3.1 *Study area and site selection*

The NLA field sampling effort targeted all lakes and reservoirs in the 48 conterminous U.S. with surface areas >1 ha and depths greater than 1 m. Field crews visited 1131 lakes and reservoirs between May and October 2012. Of these, 1038 had been selected as a probability sample from the USGS/EPA National Hydrography Dataset (NHD) with a spatially-balanced, randomized systematic design that excluded the Great Lakes and Great Salt Lake (Peck et al. 2013). The remaining 91 lakes were hand-selected to increase the number of lakes in least-disturbed condition, which were used to estimate potential condition and evaluate response of the indices to disturbance (following Stoddard et al. 2006). For the NLA 2012 report, we used physical habitat data collected from 1109 of the 1131 survey lakes, which were those having surface areas <10,000 ha (1026 probability-selected and 83 hand-picked lakes). Probability and hand-selected lakes from both 2012 and 2007 were used to develop expected physical habitat condition models and distributions of O/E values in least-disturbed lakes. Random subsets of 90 probability lakes from NLA 2007 and 88 from NLA 2012 were visited twice during their respective summer sampling periods to estimate the precision of NLA indicators, including the habitat measurements and indices (Kaufmann et al. 2014a).

5.3.2 *Field sampling design and methods*

Our lake physical habitat field methods (USEPA 2007a, USEPA 2012, Kaufmann et al. 2014a) produced information concerning 7 dimensions of near-shore physical habitat: 1) water depth and surface characteristics, 2) substrate size and type, 3) aquatic macrophyte cover and structure, 4) littoral cover for biota, 5) riparian vegetation cover and structure, 6) near-shore anthropogenic disturbances, and 7) bank characteristics that indicate lake level fluctuations and terrestrial-aquatic interactions. At each lake, field crews characterized these 7 components of near-shore physical habitat at 10 equidistant stations along the shoreline. Each station included a littoral plot (10m × 15m) abutting the shoreline, a riparian plot (15m × 15m) extending landward from the typical high-water mark, and in a 15m wide drawdown zone plot that extended a variable distance landward, depending on the amount of lake level drop compared with typical high water levels (Figure 5-1). Littoral depth was measured 10 m off-shore at each station. Metrics and indices were calculated for the variable-width drawdown zone plots, the 15m x 15m riparian plots and the 10m x 15m littoral plots. To match the riparian and near-shore human disturbance indices to those used in the previous (NLA 2007) assessment, we used information from riparian and drawdown plots along with drawdown horizontal extent information. These index values are equivalent to the 2007 index values that were directly calculated from observation the near-shore zone extending from the lake water’s edge 15m outward. See Kaufmann et al. (2014a) for further description of field methods, our approach for calculating whole-lake physical habitat metrics, and a detailed assessment of habitat metric precision.

5.3.3 *Classifications*

5.3.3.1 *Ecoregions*

We report findings nationally, and by 9 aggregated Omernik (1987) level III ecoregions (Paulsen et al. 2008): the Northern Appalachians (NAP), Southern Appalachians (SAP), Coastal Plains (CPL), Upper Midwest (UMW), Temperate Plains (TPL), Northern Plains (NPL), Southern Plains (SPL), Western Mountains (WMT), and Xeric West (XER) (Figure 3-1). We used ecoregions as a first-level classification for defining and evaluating near-shore riparian and littoral condition indicators (*RVegQ, LitCvrQ, and LitRipCvrQ)* and their variants (e.g., RVegQ_2, LitCvrQ_b, LitRipQ_2d). Ecoregions are useful predictors of many characteristics of landform, geology, climate, hydrology, and potential natural vegetation (Omernik 1987, Paulsen et al. 2008) that influence physical habitat in lakes (Kaufmann et al. 2014c). Kaufmann et al. (2014c) used a multivariate classification of lake characteristics including lake chemistry and depth to assign variants of *LitCvrQ*, suggesting that such classifications would capture aspects of in-lake habitat cover complexity better than would ecoregions. We reexamined the 2007 data and found no substantial difference in assignment of LitCvrQ variants according to Ecoregion (WSAECO9) versus multivariate cluster analysis (CLUSB). For some aspects of habitat index development, we grouped ecoregions into broader ecoregions: the Eastern Highlands (EHIGH = NAP + SAP), the Plains and Lowlands (PLNLOW = CPL + UMW + TPL + NPL + SPL), Central Plains (CENPL = TPL+ NPL+SPL), and the West (WMT + XER).

5.3.3.2 *Anthropogenic disturbance and least-disturbed reference site screening*

We used region-specific screening based on water chemistry, near-shore human influences, and evidence of anthropogenic lake drawdown in NLA survey lakes, 1109 from NLA 2012 and 1101 from NLA 2007, to classify all NLA lakes according to their level of anthropogenic disturbance (low, medium, high), as described in Chapter 3. Lakes meeting low-disturbance screening criteria served as least-disturbed reference sites for best-available condition. Low-disturbance stress (least-disturbed) lakes within each Ecoregion were identified on the basis of chemical variables (total phosphorus, total nitrogen, chloride, sulfate, acid neutralizing capacity, dissolved organic carbon, and dissolved oxygen in the epilimnion) and direct observations of anthropogenic disturbances along the lake margin (proportion of lakeshore with non-agricultural influences, proportion of lakeshore with agricultural influences, and the relative extent and intensity of human influences of all types together). For each aggregated ecoregion, a threshold value representing least-disturbed conditions was established as a "pass/fail" criterion for each parameter (Table 3-1). Thresholds were values that would be very unlikely in least-disturbed lakes within each region, and varied by lake type to account for regional variations in water chemistry and littoral-riparian human activities (Herlihy et al. 2013). A lake was considered least-disturbed if it passed the screening test for all parameters, and we identified 214 least-disturbed lakes from NLA 2012 and 168 from NLA 2007. We used the 2012 survey data for the 44 lakes from NLA 2007 that were again sampled in NLA 2012, and still passed the reference screening, so 124 NLA 2007 lakes remained in the reference set (Table

5-1). Lakes that were not classified as least-disturbed were provisionally considered intermediate in disturbance. The intermediate disturbance lakes were then screened with a set of high-disturbance thresholds applied to the same variables (Table 3-2) Lakes that exceeded one or more of the high disturbance thresholds were considered highly disturbed. To avoid circularity in defining physical habitat alteration, we did not use any of the physical habitat cover complexity indices or their subcomponent metrics in defining lake disturbance classes.

Our screening process identified 382 least-disturbed, 1309 intermediate, and 519 highly disturbed lake visits. Of the 338 least-disturbed lakes that did not overlap survey years, 190 were in the WMT, NAP, and UMW aggregated ecoregions (Table 5-1). Even with relaxed disturbance screening criteria, it was more difficult to find least-disturbed lakes in some other ecoregions. Respectively, only 11, 20, and 23 least-disturbed lakes were identified in the NPL, XER, and TPL ecoregions. To increase the useable sample size for estimating expected lake condition, we grouped least-disturbed lakes from the NPL, SPL, TPL into the Central Plains (CENPL), and the WMT and XER into the West (for some models). Because of insufficient numbers of least-disturbed lakes relative to the large amount of lake variability within ecoregions, we needed all available reference lakes for modeling expected conditions, so were unable to use totally independent subsets of lakes for developing and validating those models.

5.3.4 *Calculation of lake physical habitat metrics*

5.3.4.1 *Names of habitat metrics*

Our variable names are those from the publicly-available NLA 2007 and NLA 2012 datasets released by the U.S. EPA (http://water.epa.gov/type/lakes/NLA_data.cfm). The first several letters in the NLA variable names denote the category and type of metric. The initial letters ***"hi..."*** identify human influence metrics. The initial letters ***"hifp..."*** specify human influence frequency of presence metrics and ***"hii..."*** specify indices of aggregated or summed human influences. Riparian vegetation mean presence metrics begin with ***"rvfp ..."*** and mean riparian vegetation cover metrics begin with ***"rvfc...",*** whereas ***"rvi..."*** denotes riparian vegetation cover sums (e.g., two types of woody cover). The initial letters ***"fc..."*** and ***"am..."*** indicate, respectively, fish cover and aquatic macrophyte metrics. These letters followed by ***"...fp...", "..fc...", or "..i..."*** indicate, respectively mean frequency of presence among stations, mean areal cover, and indices created by summing various metrics. Littoral bottom and exposed shoreline substrate metrics, respectively, are identified by ***"bs..."*** and ***"ss..."***. The summary habitat indices described by Kaufmann et al. (2014c), and used to define habitat condition in the NLA (*RVegQ, LitCvrQ,* and *LitRipCvQ*) all end in the upper case *Q*, and the NLA summary human disturbance index is *RDis_IX* (Riparian Disturbance Intensity and eXtent). Kaufmann et al. (2014a) describe in detail the definitions and calculation of NLA physical habitat metrics and quantify their precision.

Many of the physical habitat metrics for NLA 2012 are additionally identified by the suffixes ***_rip, _lit, and _DD*** (e.g., *rviWoody_rip, rviWoody_DD, fciNatural_lit, fciNatural_DD*), designating that the habitat observations or measurements were from, respectively, the set of riparian, littoral, or drawdown plots (Figure 5-1).

5.3.4.2 *Drawdown Zone Apportioning to match NLA 2007 Riparian and Human Disturbance metrics:*

NLA 2012 retained the measures of "bathtub ring" height and horizontal extent exactly as done in NLA 2007 to quantify lake drawdown and seasonal lake level fluctuations. However, the near-shore plot designs of the two surveys differ. In NLA 2007, the 15m x 15m riparian plots abutted the shoreline. Consequently, exposed littoral bottom may comprise 0 to 100% of NLA 2007 plots, depending upon the extent of drawdown. Near-shore habitat was accurately depicted in the NLA 2007 data, but because cover and disturbances were not separately assessed in the drawdown zone, there was no accurate way to separately assess changes in habitat condition attributable to drawdown (vs. riparian vegetation removal, for example). The NLA 2012 field methods have separate measures of vegetation and human disturbances for the riparian and drawdown zone plots, and separate fish cover estimates in littoral and drawdown zone plots. These field plot changes improve the separation of lake level changes and drawdown from other stressors in a diagnosis of likely causes of poor nearshore habitat condition in NLA 2012.

We used cover and human disturbance tally data from the riparian and drawdown plots to calculate cover estimates or disturbance tallies simulating the set of ten 15m x 15m near-shore plots abutting the shoreline, as had been used in the NLA 2007 field methods. We calculated Rc_{syn}, as a synthetic estimate of cover in the 15m band around the shoreline by summing the areal covers in the drawdown and riparian plots, after weighting each by the proportion of the 15m band that was, respectively, within the drawdown zone or not within the drawdown zone:

$$Rc_{syn} = (Rp_{draw} \times Rc_{draw}) + (Rp_{rip} \times Rc_{rip}) \qquad \text{(Eq 1)}$$

where:

Rc_{syn} = Calculated cover in 15 x 15 m shoreline PHab plot, synthesizing metric values equivalent to those used in NLA 2007, which represent the riparian condition in the 15m near-shore band adjacent to the wetted edge of the lake.

Rp_{draw} and Rp_{rip} are the proportions of the 15x15m shoreline PHab plot that are, respectively, occupied by the drawdown zone and the riparian zone above the high water mark.

Rp_{draw} = (Horizontal Distance to high water)/(15m) = (*bfxHorizDist/*15m), and Rp_{draw} =1.0 if ***bfxHorizDist***>15m.

$Rp_{rip} = (1 - Rp_{draw})$ ----- by definition because $Rp_{rip} + Rp_{draw} = 1.0$

Rc_{draw} and Rc_{rip} are, respectively, the areal cover of vegetation in the drawdown and riparian zones; Rc_{rip} could be single cover type (e.g., canopy layer, or barren ground), or could be a sum of cover types (e.g., sum of woody cover in 3 layers).

Calculated $\boldsymbol{Rc_{syn}}$ for a hypothetical lake with a mean horizontal drawdown of 10m (est. by *bfxHorizDist*), and 100% canopy cover above the high water mark, but 0% cover in the drawdown zone is as follows:

$\boldsymbol{Rp_{draw}}$ = 10/15 = 0.67

$\boldsymbol{Rp_{rip}}$ = (1.0 - 0.67) = 0.33

Drawdown Canopy cover: $\boldsymbol{Rc_{draw}}$ = 0%

Riparian Canopy cover: $\boldsymbol{Rc_{rip}}$ = 100%

$\boldsymbol{Rc_{syn}}$ = (0.67 x 0%) + (0.33 x 100%) = 33%

The loss or gain in near-shore riparian habitat cover resulting from lake drawdown or natural lake level declines can be estimated by the difference in cover between the riparian cover above the high water mark ($\boldsymbol{Rc_{rip}}$) and that within 15 m of the lakeshore ($\boldsymbol{Rc_{syn}}$).

We conducted a volunteer Drawdown Pilot Survey in 2011 to determine whether modification of the NLA 2007 field protocols could be made without jeopardizing our ability to track changes or trends in riparian habitat over time (Anne Rogers 2012 NALMS; Kaufmann et al. Jan 9, 2012 webinar presentation to NLA steering committee and states). NLA 2007 and NLA 2012 field protocols were applied simultaneously at 210 stations on 21 lakes spread over a range of drawdown conditions in the states of Texas, Wisconsin, Washington, Oregon, Wyoming, North Dakota, and Colorado. Kaufmann et al. (2012 webinar) demonstrated that 2007 metric values for lakeshore vegetation and human disturbances were calculated accurately from the new (2012) protocol, preserving ability to track changes/trends. The regressions predicting the measured values of key physical habitat metric values from the NLA 2007 protocol from values calculated by Eq 1 were virtually 1:1 lines with intercepts very close to 0.0, slopes very close to 1.0, and R^2 between 0.87 and 0.94. The drawdown pilot analysis also showed that there was virtually no difference in whole-lake metric values obtained by applying Eq 1 at each station, versus applying it once per lake based on values of drawdown extent and cover averaged over the 10 riparian and drawdown plots on each lake. The drawdown pilot results also demonstrated that adding separate determinations of habitat cover elements in the drawdown zone was logistically feasible and resulted in very minor increases in field time.

5.3.4.3 *Drawdown Zone Apportioning to Estimate littoral habitat changes due to drawdown:*

We used a calculation similar to Eq 1 to simulate the amount of littoral cover that would be present if, hypothetically, the amount of lake drawdown were zero:

$$\boldsymbol{Lc_{sim} = (Lp_{draw} \times Lc_{draw}) + (Lp_{lit} \times Lc_{lit})} \qquad \text{(Eq 2)}$$

where:

$\boldsymbol{Lc_{sim}}$ = Calculated littoral cover simulating the amount of real or potential cover in a 10 x 15 m littoral plot abutting the high-water mark, ie., simulating littoral cover that might be present if there were no drawdown.

$\boldsymbol{Lp_{draw}}$ **and** $\boldsymbol{Lp_{lit}}$ are the estimated proportions of a hypothetical 10m x 15m littoral PHab plot abutting the highwater mark that are, respectively, occupied by the drawdown zone (dry) and the littoral zone (wet).

$\boldsymbol{Lp_{draw}}$ = (Horizontal Distance to high water)/(10m) = (*bfxHorizDist/*10m), and $\boldsymbol{LP_{draw}}$ =1.0 if ***bfxHorizDist***>10m.

$\boldsymbol{Lp_{lit} = (1 - Lp_{draw})}$ ----- by definition because $\boldsymbol{Lp_{rip} + Lp_{draw}}$ = 1.0

$\boldsymbol{Lc_{lit}}$ and $\boldsymbol{Lc_{draw}}$ are, respectively, the areal cover of fish habitat elements in the littoral plot, and exposed (dry) in the drawdown zone, *Lc* could be single cover type (e.g., *fcfcSnags*) or could be a sum of cover types (e.g., sum of non-anthropogenic cover types: *fcfcNatural*).

Calculated $\boldsymbol{Lc_{sim}}$ for a hypothetical lake with a mean horizontal drawdown of 10m and 100% Snag cover in the drawdown zone (dry and exposed), but 0% Snag cover in the littoral (wet) zone is as follows:

$\boldsymbol{Lp_{draw}}$ = 10/10 = 1.00

$\boldsymbol{Lp_{lit}}$ = (1.00 – 1.00) = 0

Drawdown Snag cover: $\boldsymbol{Lc_{draw}}$ = 100%

Littoral Snag cover: $\boldsymbol{Lc}_{lit}$ = 0%

$\boldsymbol{Lc_{sim}}$ = (1.00 x 100%) + (0 x 0%) = 100%

The loss or gain in littoral habitat cover resulting from lake drawdown or natural lake level declines can be estimated as the difference between the littoral cover simulated for zero drawdown conditions ($\boldsymbol{Lc_{sim}}$) the observed cover actually existing in the littoral at the time of sampling ($\boldsymbol{Lc_{lit}}$).

5.3.4.4 *Use of Variable suffixes in this report:*

Riparian cover or human disturbance metrics calculated by Eq 1 are synthetic values that match the 2007 metrics, and are designated by the suffixes ***_syn*** (e.g., *rviWoody_syn* and *hiiAll_syn*) in the EPA database. **For simplicity, we will drop the suffixes on riparian vegetation and human disturbance metrics in the remainder of this article, and it is understood that we are using the synthesized variables when no suffix is present (*_*syn*), and NOT the drawdown zone (*_*DD*), or riparian plot (*_*rip*) versions of those variables**.

Littoral cover metrics designated with the suffix ***_lit*** are based on field observations that are conceptually and procedurally identical to those used in NLA 2007. **For simplicity, we will drop the suffixes on littoral cover metrics in the remainder of this article, and it is understood that we are using the innudated littoral plot version of those variables when no suffix is present (*_*lit*), and NOT the drawdown zone (*_*DD*) or zero-drawdown simulated values (*_*sim*) versions of those variables**. Littoral cover metrics calculated using Eq 2 simulate littoral cover that would be present in the near-shore littoral area if the amount of drawdown were zero, and are designated by the suffix ***_sim*** (eg., *fciNatural_sim*).

5.3.4.5 *Near-shore disturbance metrics*

We calculated extent of shoreline disturbance around the lakeshore (*hifpAnyCirca*) as the proportion of stations at which crews recorded the presence of at least one of the 12 anthropogenic disturbance types as described by Kaufmann et al. (2014a). We calculated the disturbance intensity metric *hiiAll* as the sum of the 12 separate proximity-weighted means for all shoreline disturbance types observed at the 10 shoreline stations (Kaufmann et al. 2014a). We also calculated subsets of total disturbance intensity by summing metrics for defined groups of disturbance types. For example, *hiiAg* sums the proximity-weighted presence metrics for row crop, orchard, and pasture; *hiiNonAg* sums the proximity-weighted presence metrics for the remaining 9 non-agricultural disturbance metrics: 1) buildings, 2) commercial developments, 3) parks or man-made beaches, 4) docks or boats, 5) seawalls, dikes, or revetments, 6) trash or landfill, 7) roads or railroads, 8) power lines, and 9) lawns.

5.3.4.6 *Riparian vegetation metrics*

Field data consisted of visual areal cover % class assignments of the vegetation type and areal cover for each of 3 layers: canopy (>5 m high), mid-layer (0.5–5 m high), and ground cover (<0.5 m high). Crews estimated large (diameter at breast height [DBH] > 0.3 m) and small (DBH < 0.3 m) diameter tree cover separately in the canopy and mid-layer, distinguished woody from herbaceous vegetation in the mid-layer and ground cover, and distinguished barren ground from vegetation inundated by water in the ground layer. To characterize riparian vegetation in the near-shore zone of the lake, we converted field cover class observations to mean cover estimates for all the types and combinations of vegetation data (Kaufmann et al. 2014a). We assigned cover class arithmetic midpoint values to each plot's cover-class observations (i.e., absent = 0%, sparse (>0-10%) = 5%, moderate (>10-40%) = 25%, heavy (>40-75%) = 57.5%, and very heavy (>75-100%) = 87.5%), and then calculated lakeshore vegetation cover as the average of those cover values across all 10 plots. Metrics for combined cover types (e.g., sum of woody vegetation in 3 layers) were calculated by summing means for the single-types (see Kaufmann et al. 1999, 2014a). Metrics describing the proportion of each lakeshore with presence (rather than cover) of particular features were calculated as the mean of presence (0 or 1) over the 10 riparian plots.

5.3.4.7 *Littoral cover and aquatic macrophyte metrics*

The NLA survey crews made observations of the areal cover attributable to 8 littoral cover types within each of the 10 littoral plots: rock ledges, boulders, brush, inundated live trees, snags, overhanging vegetation, aquatic macrophytes, and human structures. Additionally field crews made separate visual estimates of areal cover for emergent, floating, and submerged aquatic macrophytes within each of the 10 littoral plots. They used the same % cover classes for these observations as used for riparian vegetation. Metrics describing the mean cover (and mean presence) of littoral physical habitat features and aquatic macrophytes were calculated from these cover class observations as described above for riparian vegetation. Metrics for combined cover types (e.g. sum of natural types fish cover, floating and emergent aquatic macrophyte cover) were calculated by summing means for single types.

5.3.4.8 *Littoral and shoreline substrate metrics*

NLA field crews visually estimated the percent areal cover of 8 substrate types (bedrock, boulder, cobble, gravel, sand, silt/clay/muck, woody debris, and organic detritus) at each of the 10 near-shore stations (Figure 5-1). These estimates were made separately for the 1 m shoreline band above the lake margin and for the lake bottom within the littoral plot. In cases where the bottom substrate could not be observed directly, crews viewed the bottom through a viewing tube, felt the substrate with a 3 m PVC sounding tube, or observed sediments adhering to the boat anchor as it was retrieved from the bottom. Cover classes were the same as for riparian vegetation. We calculated metrics describing the lake-wide mean cover of near-shore littoral and shoreline substrate in each size category by averaging the cover estimates at each station, based on the cover class midpoint approach described above.

We adapted the approach of Faustini and Kaufmann (2007) and Kaufmann et al. (2009) for estimating geometric mean and variance of substrate diameters from systematic pebble-counts. In this approach (Kaufmann et al. 2014a), we assigned the geometric mean between the upper and lower diameter bound of each size class for each cover observation before calculating the cover-weighted mean size index. We calculated the geometric mean diameters (D_{gm}) of littoral and shoreline substrate (*bsxLdia* and *ssxLdia*) as follows:

$$D_{gm}=\text{Antilog}\{\text{Sum}_i\{P_i\{[\log_{10}(D_{iu})+\log_{10}(D_{il})]/2\}\}, \quad \text{(Eq. 3)}$$

where:

P_i =areal cover proportion for diameter class i;

D_{iu} =diameter (mm) at upper limit of diameter class i;

D_{il} =diameter (mm) at lower limit of diameter class i;

Sum_i =summation across diameter classes; and

Nominal size class midpoint diameters of 5660 and 0.0077 mm were set, respectively, for the largest (bedrock and hardpan) and smallest (silt, clay, and muck) diameter classes.

Our calculations are identical to those of Faustini and Kaufmann (2007), except that here the percent cover estimates used to weight diameters were the mean values of 10 visual cover estimates rather than areal streambed cover determinations derived from the pebble-count percentages for individual particles in each diameter class.

5.3.4.9 *Littoral depth, Lake level fluctuations, bank and water surface characteristics*

Field crews measured littoral depth, estimated water level fluctuations and bank heights, and, and observed water surface and bottom sediment color and odor at each of the 10 nearshore stations (Figure 5-1). SONAR, sounding lines, or sounding tubes were used to measure lake depth 10 m offshore. NLA field crews used hand-held levels, survey rods, and laser rangefinders (rather than unaided visual estimates) to measure vertical and lateral (horizontal) lake level fluctuation. Field indications of short to medium term fluctuation, drawdown and/or declines in lake levels were based on measurement of the vertical height and horizontal extent of exposed lake bottom ("Bathtub Ring") field evidence.

Crews recorded the presence of surface films or scums, algal mats, oil slicks, and sediment color and odor. They visually estimated the bank angle in the 1 m-wide shoreline band and the vertical and lateral range in lake level fluctuations, based on high and low water marks. We calculated whole lake metrics for mean littoral depth and water level fluctuations as arithmetic averages (*sixDepth, bfxVertHeight* and *bfxHorizDist*) and standard deviations of the measured values at the 10 stations. For bank angle classes and qualitative observations of water surface condition and sediment color and odor, we calculated the proportion of stations having observations in each class.

5.3.5 *Calculation of summary physical habitat condition indices*

We calculated 4 multimetric indices of physical habitat condition and an index of lake drawdown:

RDis_IX: Lakeshore Anthropogenic Disturbance Index (Intensity and Extent),

RVegQ: Riparian Vegetation Cover Complexity Index,

LitCvrQ: Littoral Cover Complexity Index,

LitRipCvQ: Littoral-Riparian Habitat Complexity Index, and

Drawdown Index: based on *bfxVertHeight* and *bfxHorizDist*

5.3.5.1 *Lakeshore Anthropogenic Disturbance Index (RDis_IX)*

This index was calculated as:

$$RDis\_IX = (Disturbance\ Intensity + Disturbance\ Extent)/2; \qquad \text{(Eq 4)}$$

where :

disturbance intensity was represented by separate sums of the mean proximity-weighted tallies of near-shore agricultural and non agricultural disturbance types and extent was expressed as the proportion of the shore with presence of any type of disturbance.

$$RDis\_IX = \frac{\left\{1 - \left[\frac{1}{\left[1 + hiiNonAg + (5 \times hiiAg)\right]}\right] + hifpAnyCirca\right\}}{2}; \qquad \text{(Eq 5)}$$

where:

hiiNonAg = Proximity-weighted mean disturbance tally (mean among stations) of up to 9 types of non-agricultural activities.

hiiAg = Proximity-weighted mean tally of up to 3 types of agriculture-related activities (mean among stations).

hifpAnyCirca = Proportion of the 10 shoreline stations with at least 1 of the 12 types of human activities present *within* their 10 x 15 m littoral plots, drawdown plots, or within 15m of the lake shore in their 15 x 15 m riparian plots.

Field procedures classified only 3 types of agricultural disturbances, versus 9 types of non-agricultural disturbances, limiting the potential ranges to 0-3 for *hiiAg* and 0-9 for *hiiNonAg*. In the combined NLA 2007 and 2012 surveys, the observed ranges of these variables also differed: *hiiAg* ranged from 0 to 1.55, whereas *hiiNonAg* had an observed range almost 5 times as great (0 to 7.125). To avoid under-representing agricultural disturbances and over-representing non-agricultural disturbances in the index, we weighted the disturbance intensity tallies for agricultural land use by a factor of 5 in Equation 2. This weighting factor (ratio of observed ranges in non-agricultural to agricultural disturbance types) effectively scales agricultural land-uses equal in disturbance potential to those for non-agricultural land uses. We scaled the final index from 0 to 1, where 0 indicates absence of any anthropogenic disturbances and 1 is the theoretical maximum approached as a limit at extremely high disturbance. We applied a single formulation of the disturbance index *RDis_IX* throughout the NLA survey in the U.S.

5.3.5.2 *Riparian Vegetation Cover Complexity Index (RVegQ)*

This index is based on visual estimates of vegetation cover and structure in three vegetation layers at the 10 near-shore riparian plots along the lake shore. The cover metrics were calculated for the variable-width drawdown zone plots (metrics with suffix "*_DD"*) and the 15m x 15m riparian plots (with suffix "*_rip"*). For the NLA 2012 report, we used areal cover information from both types of plots along with drawdown horizontal extent information to calculate *RVegQ* estimates matching those for the previous report, which are for the near-shore zone extending from the lake water's edge 15m outward (see Eq. 1). Because the potential vegetation cover differs among regions, we calculated three variants of the Riparian Vegetation Cover-Complexity Index (*RVegQ_2*, *RVegQ_7*, or *RVegQ_8*) for application to different aggregated ecoregions (Table 5-2). The region-specific formulations reduce the among-region variation in index values in least-disturbed lakes and reduce ambiguity in their response to anthropogenic disturbances. If component metrics had potential maximum values >1, their ranges were scaled to range from 0 to 1 by dividing by their respective maximum values based on the NLA 2007 data (see Table 3 in Kaufmann et al. 2014a). Each variant of the final index was calculated as the mean of its component metric values. Index values range from 0 (indicating no vegetative cover at any station) to 1 (40 to 100 % cover in multiple layers at all stations).

$$RVegQ\_2 = \frac{\left[\left(\frac{rviWoody}{2.5}\right) + rvfcGndInundated\right]}{2}; \quad \text{(Eq 6)}$$

$$RVegQ\_7 = \frac{\left[\left(\frac{rviLowWood}{1.75}\right) + rvfcGndInundated\right]}{2}; \quad \text{(Eq 7)}$$

$$RVegQ\_8 = \frac{\left[\left(\frac{rviWoody}{2.5}\right) + rvfpCanBig + rvfcGndInundated + ssiNATBedBld\right]}{4}; \quad \text{(Eq 8)}$$

where:

rviWoody = Sum of the mean areal cover of woody vegetation in 3 layers: canopy (large and small diameter trees), understory, and ground layers *(rvfcCanBig + rvfcCanSmall + rvfcUndWoody + rvfcGndWoody)*.

rviLowWood = Sum of mean areal cover of woody vegetation in the understory and ground cover layers *(rvfcUndWoody + rvfcGndWoody)*.

rvfcGndInundated = Mean areal cover of inundated terrestrial or wetland vegetation in the ground cover layer.

rvfpCanBig = Proportion of stations with large diameter (>0.3 m dbh) trees present.

ssiNATBedBld = Sum of mean areal cover of naturally-occurring bedrock and boulders *(ssfcBedrock + sfcBoulders)*, and where the value of *ssiNATBedBld* was set to 0 in lakes that have a substantial amount of human-built seawalls and revetments (i.e., *hipwWalls* $\geq$0.10).

We used *RVegQ_2* for mesic ecoregions with maximum elevations <2,000 m (NAP, SAP, UMW, CPL) where tree vegetation can be expected in relatively undisturbed locations (Table 5-2). *RVegQ_2* sums the woody cover in three lakeside vegetation layers (*rviWoody*) and includes inundated groundcover vegetation (*rvfcGndInundated)* as a positive characteristic.

We used *RVegQ_7* for Central Plains ecoregions (NPL, SPL and TPL). Whereas perennial woody groundcover and shrubs can be expected on undisturbed lake shorelines throughout the Central Plains (West and Ruark 2004), the presence or absence of large trees (>5m high) along lake margins in this region has ambiguous meaning without floristic information (Johnson 2002, Barker and Whitman 1988, Huddle et al. 2011). *RVegQ_7* accommodates lack of tree canopy in least-disturbed lakes by summing only the lower 2 layers of woody vegetation (*rviLowWood)* and includes inundated ground cover vegetation as a positive characteristic.

We used *RVegQ_8* for the West (WMT, XER), where climate ranges from wet to arid, and where lakeshores may have the potential to grow large diameter riparian trees but may lack vegetated lake shorelines at high elevations, or where rock precludes vegetation (Table 5-2). *RVegQ_8* sums the woody cover in 3 lakeside vegetation layers and includes inundated groundcover vegetation as a positive characteristic; it also includes the proportional presence of large diameter trees around the lakeshore as a positive characteristic. *RVegQ_8* includes natural rock as an undisturbed riparian cover type to avoid penalizing relatively undisturbed lakes in arid areas or at high elevations above timberline. For lakes where there is a substantial extent or abundance of constructed seawalls, dikes, or revetments along the shoreline, the substrate metric was set at 0.

5.3.5.3 *Littoral Cover Complexity Index (LitCvrQ)*

This index was based on the station-averages for visual estimates of the areal cover of 10 types of littoral features, including aquatic macrophytes but excluding human structures, within each of the 10 littoral plots (see Kaufmann et al. 2014a). Note that littoral metrics used to calculate *LitCvrQ* are those with the suffix "*_lit*", which match exactly the NLA 2007 littoral cover metrics having no suffix. We calculated 3 variants, for application in different ecoregions (Table 5-2). Each variant of the index was calculated as the mean of its component metric scores, so index values range from 0 (no cover present at any station) to 1 (very heavy cover at all 10 stations). Component metrics with potential maximum values >1 were scaled from 0-1 by dividing by their respective maximum values in the NLA 2007 dataset.

$$LitCvrQ\_b = \frac{\left[fciNatural + \left(\frac{fcfcSnag}{0.2875} \right) \right]}{2}; \quad \text{(Eq 9)}$$

$$LitCvrQ\_c = \frac{\left[fciNatural + \left(\frac{fcfcSnag}{0.2875} \right) + \left(\frac{amfcFltEmg}{1.515} \right) \right]}{3}; \quad \text{(Eq 10)}$$

$$LitCvrQ\_d = \frac{\left[\left(\frac{SomeNatCvr}{1.5} \right) + \left(\frac{fcfcSnag}{0.2875} \right) + \left(\frac{amfcFltEmg}{1.515} \right) \right]}{3}; \quad \text{(Eq 11)}$$

where:

fciNatural = summed areal cover of non-anthropogenic fish cover elements (*fcfcBoulders + fcfcBrush + fcfcLedges + fcfcLivetrees + fcfcOverhang + fcfcSnag + fcfcAquatic*).

SomeNatCvr = summed cover of natural fish cover elements excluding snags and aquatic macrophytes (*fcfcBoulders + fcfcBrush + fcfcLedges +fcfcLivetrees + fcfcOverhang*).

amfcFltEmg = summed cover of emergent plus floating aquatic macrophytes (*amfcEmergent + amfcFloating*).

fcfcAquatic = total cover of aquatic macrophytes of any type.

All three variants of *LitCvrQ* include an expression of the summed cover of naturally occurring fish or macroinvertebrate cover elements. Snag cover is recognized as a particularly important element of littoral habitat complexity (Francis and Schindler 2006, Christensen et al. 1996, Miranda et al. 2010). Therefore, we included snags as a separate contributing cover component in all three variants of the index, and divided cover metrics by their maximum values in the NLA 2007 data to make the weightings of snag cover equal to those of the other two littoral cover sums. For *LitCvrQ_c* and *LitCvrQ_d,* we increased the emphasis on emergent and floating-leaf aquatic macrophytes relative to other littoral components in response to their reported importance as cover and their sensitivity to human disturbances in many lake types and regions (Radomski and Geoman 2001, Jennings et al. 2003, Merrell et al. 2009, Beck et al. 2013).

We used *LitCvrQ_b* for lakes in the CPL, which includes many generally shallow, warm, low conductivity lakes. We used *LitCvrQ_c* for lakes in the SAP, which are all reservoirs, where disturbed sites commonly have substantial erosion of clay-rich upland soils, large water level fluctuations, and bare-soil shorelines. These conditions generate abiotic turbidity that suppresses submerged macrophytes, thereby diminishing the association of abundant submerged aquatic macrophytes with anthropogenic nutrient inputs that is typically seen in other regions. *LitCvrQ_c* emphasizes floating and emergent aquatic macrophytes in addition to snags, but still includes submerged aquatic macrophytes along with other aquatic macrophytes and cover types in *fciNatural*. *LitCvrQ_d* excludes submerged aquatic macrophytes, and we used it in the remaining ecoregions (NAP, TPL, NPL, SPL, WMT, and XER), where submerged aquatic macrophytes provide valuable cover, but high submerged cover is frequently associated with anthropogenic eutrophication (Hatzenbeler et al. 2004, Merrell et al. 2009).

5.3.5.4 *Littoral-Riparian Habitat Complexity Index (LitRipCvrQ)*

We averaged the lake values of the littoral cover complexity and riparian vegetation cover complexity indices to calculate the littoral-riparian habitat compl**exity index *LitRipCvrQ*:**

$$\boldsymbol{LitRipCvrQ} = \frac{(\boldsymbol{RVegQ\_n} + \boldsymbol{LitCvrQ\_x})}{2}; \qquad \text{(Eq 12)}$$

where:

RVegQ_n = variant of the riparian vegetation cover complexity index (n=2, 7 or 8, depending on ecoregion, Table 5-2.

LitCvrQ_x = variant of littoral cover-complexity index (x = b, c, or d, depending on ecoregion, Table 5-2.

5.3.5.5 *Lake Level Drawdown Index (combined use of bfxVertHeight and bfxHorizDist)*

We used the mean lake values estimating Lake Level Vertical Fluctuation (*bfxVertHeight*) in combination with Lake Level Horizontal Fluctuation (*bfxHorizDist*) to characterize lake drawdown and natural lake level declines. These metrics are, respectively, the height (meters) measured from the present lake level to high water, and the horizontal (lateral) distance in meters from the lake shore to the high water mark in meters. NLA field crews made these determinations based on the extent and location of vegetation intolerant to frequent or prolonged inundation, location of flotsom deposits ("trash racks"), evidence of wave action, and exposed lake bottom. The lake bottom exposure measured by these methods characterizes seasonal lake level declines and fluctuations on timescales shorter than that required for disintegration of flotsom at the high water mark, or encroachment of perennial terrestrial vegetation onto the exposed lake bottom area. In most regions, these measurements should be adequate to document trends in lake level declines attributable to climate change, water withdrawals, and reservoir management over a decadal timescale. However, more rigorous tracking of such trends over longer timescales would require that field crews measure lake levels in relation to established permanent (monumented) reference elevations and/or staff gauges at sample lakes.

5.3.6 *Deriving expected index values under least-disturbed conditions*

We based expectations for *bfxVertHeight* and *bfxHorizDist* on “Null Models”: the expected value and its dispersion are represented by the central tendency and distribution of these variables in regional sets of least-disturbed reference sites. In the CENPL and WEST, expectations were set separately for natural lakes versus man-made reservoirs.

We used lake-specific predictive regression models to estimate physical habitat expectations for *RVegQ, LitCvrQ*, and *LitRipCvrQ* under least-disturbed condition (Table 5-3). We compared the performance of these regression models with null models (Table 5-4), for which expectations were simply the mean of $\log_{10}$-transformed physical habitat index scores among least-disturbed lakes from each ecoregion. Our motivation for using lake-specific models of expected (“E”) condition was to reduce the variance in physical habitat condition indices (in this case O/E values of *RVegQ, LitCvrQ*, and *LitRipCvrQ*) among least-disturbed reference lakes. Air temperature, precipitation, soils and lithology can vary greatly across ecoregions, resulting in corresponding variations in potential natural vegetation among least-disturbed lakes. In turn, that variation results in differences in the amount and complexity of littoral cover, especially for those elements derived from riparian vegetation. We derived lake-specific expected values by modeling the influence of important non-anthropogenic environmental factors in relatively undisturbed lakes, an approach analogous to that used to predict least-disturbed conditions for multimetric fish assemblage indices (Esselman et al. 2013, Pont et al. 2006, 2009).

For calculating lake-specific expected (E) values of *RVegQ, LitCvrQ*, and *LitRipCvrQ* under least-disturbed condition, we conducted the multiple linear regression (MLR) modeling in 7 aggregated ecoregions (Table 5-3 and Appendix A). These models were based on least-disturbed lakes from the combined 2007 and 2012 NLA surveys within each region (Table 5-1). The lake habitat index MLRs employed one to four predictors from among the following: Latitude, Longitude, Elevation, ElevXLatitude, ElevXLongitude, Lake surface area, Lake origin (man-made reservoir or natural lake), near-shore anthropogenic disturbance of all types (*RDis_IX*), and near-shore anthropogenic agricultural disturbance (*hiiAg*). Latitude, longitude, elevation, and ecoregion are surrogates for temperature, precipitation, soil, and other characteristics that influence potential natural vegetation and littoral cover. Field measurements of *bfxVertHeight* and *bfxHorizDist* were good predictors of riparian and littoral cover in most of the regions. However, we chose not to use these indicators of level fluctuation and drawdown to predict expected condition because their use would confound interpretations and obscure the effects of drawdown on habitat condition. We also did not use lake depth measurements (like maximum depth or littoral mean depth, because of their association with lake level change. Similarly, survey year was a good predictor of lake physical habitat metrics in regions where there were marked differences in the amount of lake drawdown between surveys. We chose not to use survey year as a predictor of expected condition because it would confound analysis of temporal trends and change between surveys.

Ideally, calculations of expected cover and complexity would be based only on minimally-disturbed lakes. However, the least-disturbed lakes in most regions include sometimes substantial disturbances, necessitating inclusion of near-shore disturbance predictors in our models if they were associated with variance in the habitat indices. The use of *RDis_IX* or *hiiAg* as predictors was supported by the data for all three habitat indicators in the NPL, CPL and CENPL, and the littoral cover indicator in the SAP (Table 5-3). For predicting expected *LitCvrQ* and *LitRipCvrQ* in the NAP, we had to combine least-disturbed with moderately disturbed lakes and reservoirs (RT_NLA12_2015 = R or S) to span lake size and elevation gradients affecting riparian vegetation and littoral cover in that region. The weak association of human disturbance with habitat indices would not have warranted including *RDis_IX* as a predictor within NAP least disturbed sites alone (RT_NLA12_2015=R). However, the human disturbance gradient introduced by including moderately disturbed NAP lakes (RT_NLA12_2015=S), and the effect of that disturbance on littoral habitat in the NAP made it necessary to include *RDis_IX* as a predictor. Inclusion of *RDis_IX* or *hiiAg* as predictors of expected lake habitat index values was not supported by the data for lakes and reservoirs in the UMW, WMT, and XER. As in most of the other regions, lake level fluctuation indicators were good predictors of riparian and littoral cover in the UMW and WEST, but were not used as predictors for reasons we stated in the previous paragraph.

For regions where *RDis_IX* or *hiiAg* were used in modeling expected habitat condition, we set the value of these variables in the predictive MLR equation to the minimum value observed in the region before calculating expected values of *RVegQ, LitCvrQ*, and *LitRipCvrQ*. In all regions and subregions there were sites with RDis_IX and hiiAg values of 0 (See Appendix A). Setting the reference expected lake habitat index values slightly higher in this way results in the central tendency for reference site O/E to be less than 1.0.

5.3.7 *Condition Criteria for Nearshore Lake Physical habitat*

For the lakeshore anthropogenic disturbance index *RDis_IX*, we used uniform criteria for all lakes. For *RVegQ*, *LitCvrQ*, and *LitRipCvQ* we set condition criteria based on the distribution of O/E values of these indices observed in least-disturbed lakes. For *bfxVertHeight* and *bfxHorizDist*, we set condition criteria based on the distribution of the metric values themselves in least-disturbed lakes (Null model).

5.3.7.1 *Condition Criteria for Lakeshore Anthropogenic Disturbance Intensity and Extent*

Because *RDis_IX* is a direct measure of human activities, we based criteria for high, medium, and low levels of disturbance on judgment:

Good (Low Disturbance): *RDis_IX* ≤0.20
Fair (Medium Disturbance): *RDis_IX* >0.20 but ≤ 0.75
Poor (High Disturbance): *RDis_IX* >0.75

Lakes with *RDis_IX* ≤0.20 have very low levels of lake and near-lake disturbance, typically having anthropogenic disturbance on <8% of their shorelines. Those with *RDis_IX* >0.75 have very high levels of disturbance, typically having human activities evident on 100% of their shorelines. For perspective, <21% of the 2364 sample site visits in the combined 2007 and 2012 NLA surveys had *RDis_IX* <0.20, and <21% had *RDis_IX* >0.75. Most of the reference sites in the WMT, UMW, and NAP regions have *RDis_IX* <0.20, most of those in SAP, SAP, XER, TPL, and CPL have *RDis_IX* <0.40, most NAP reference sites have *RDis_IX* between 0.40 and 0.6, and no reference sites have *RDis_IX* >0.70 (Figure 5-3).

5.3.7.2 *Condition Criteria for RVegQ, LitCvrQ, and LitRipCvQ*

We calculated physical habitat index observed/expected (O/E) values of *RVegQ_OE*, *LitCvrQ_OE*, and *LitRipCvQ_OE* for each sample lake by dividing the observed index value at each lake by the lake-specific expected value derived from regressions in Table 5-3 and Appendix A. The calculated O/E values of the habitat metrics for each lake express the degree of deviation of that lake from an estimate of its expected value under least-disturbed conditions. No model perfectly predicts expected indicator values (E-values) in lakes under least-disturbed conditions, and field measurements of indicator values ("O" values) include error and temporal variation. Consequently, O/E values of these indices among reference lakes have a dispersion (variance) that decreases with the performance of predictive models (i.e., how precisely does the model predict reference condition?), and with the precision of the habitat indicator measurements (i.e., how well do the field methods measure observed condition?). We set condition criteria for *RVegQ*, *LitCvrQ*, and *LitRipCvQ* with reference to the distributions of these indices among least-disturbed lakes within each of the 7 merged ecoregions Table 5-5.

The small number of lakes meeting our low-disturbance criteria in most regions precluded obtaining reliable percentiles of *RVegQ*, *LitCvrQ*, and *LitRipCvQ* directly from the least-disturbed lake distributions. Consequently, for all regions, we used the central tendency and variance of index O/E values in least-disturbed lakes values to model their distributions and to estimate percentiles (Snedecor and Cochran 1980). The $\log_{10}$-transformed O/E values in the least-disturbed lakes had symmetrical, approximately normal distributions. We calculated means and standard deviations of $\log_{10}$-transformed O/E values (Table 5-5, columns 3 and 4), and estimated the 5^{th} and 25^{th} percentiles (Table 5-5, columns 7 and 8) based on the log-normal approximation of the index distributions in least-disturbed lakes within each ecoregion. Because the means and SD's are all log values, a range of ± 1SD would be calculated, for example, by multiplying and dividing the geometric mean by the geometric SD (see Table 5-5 legend for details, including handling of the log-transformation constant).

Lakes with O/E values (MLR model) that are ≥25th percentile for least-disturbed lakes within their regions were considered to have habitat in good condition (i.e., similar to that in the population of least-disturbed lakes of the region). Similarly, lakes with index or O/E values <5th percentile of least-disturbed lakes were considered to have poor habitat quality (i.e., they have significantly lower cover and complexity than observed within the sub-population of least-disturbed lakes of the region). Those with index or O/E values between the 5th and 25th percentiles of least-disturbed lakes were scored as fair condition.

We emphasize that our designations of good, fair and poor are relative to the least disturbed sites available in each ecoregion. We define good condition as habitat quality not distinguishable from the distribution of habitat in least-disturbed sites; and poor condition as habitat quality that is not likely to be found within the distribution of least-disturbed sites of the ecoregion. Our designations of poor condition do not indicate impaired water body status. Conversely, our designations of good condition mean that habitat is similar to the least-disturbed sites available in a region, which does not mean pristine, only the best available, which can be relatively disturbed in extensively and highly disturbed regions.

5.3.7.3 *Condition Criteria for Lake Drawdown*

We based our assessment of Lake Drawdown condition on null models of the expected amount of drawdown in least disturbed lakes. Specifically, we examined the empirical distributions of the metrics quantifying vertical and horizontal lake level fluctuations (*bfxVertHeight* and *bfxHorizDist*) in least disturbed lakes within aggregated ecoregions, sometimes stratified by lake origin (natural lakes versus man-made reservoirs). We used separate null models for the NAP, SAP, UMW, and CPL regions. For the CENPL (TPL+SPL+NPL) and the West (WMT+XER), we used separate null models for natural lakes versus man-made reservoirs. Vertical and horizontal drawdown were considered small if they were ≤75th percentile of their respective reference distributions; large if >95th percentile, and medium if in-between (Table 5-6). Overall lake drawdown condition was considered small if both vertical and horizontal drawdown were small; medium if one or both were medium (but not large); and large if vertical, horizontal or both were large.

5.4 Least-disturbed reference distributions and regressions (from sections 5.3.6 and 5.3.7)

5.4.1 *Disturbance within least-disturbed reference sites*

Near shore human disturbance indexed by *RDis_IX* varied considerably among least-disturbed reference sites, and among regions. Reference site *RDis_IX* was lowest in the WMT and UMW, intermediate in the NAP, then steadily increasing through SAP, SPL, XER, TPL and CPL to their highest values in the NPL (Figure 5-2). The level of *RDis_IX* among all sites within regions did not cleanly follow their ordering by increasing reference site *RDis_IX.* For example, the UMW reference sites had very low *RDis_IX* in relation to the general level of *RDis_IX* in that region (Figure 5-2). Conversely, *RDis_IX* in reference sites of the NPL did not greatly differ from the distribution of rather high *RDis_IX* for sites in general within that region.

5.4.2 *Null Model Results for RVegQ, LitCvrQ, and LitRipCvQ:*

Geometric means for *RVegQ*, *LitCvrQ*, and *LitRipCvQ* in least-disturbed lakes differed among regions (Table 5-4), but these unscaled null model values are not directly comparable because the habitat index formulations differed among regions. The *RVegQ*, *LitCvrQ*, and *LitRipCvQ* null-model logSD's and geometric SD's (Columns 4 and 6 of Table 5-4) were calculated from log-transformed variables, and therefore are expressions of the proportional variance among least-disturbed lakes of each region. Whether scaled (divided by the mean) or not, they are directly comparable as measures of model precision among regions with different geometric means, or between null and MLR modeling approaches.

Comparing indicators, the precision in modeling least-disturbed condition using null models was generally better (smaller SDs) for *LitRipCvQ* than for *RVegQ or LitCvrQ*, and null models for *RVegQ* were generally more precise than for *LitCvrQ* (Table 5-4, columns 4 and 6). The most obvious differences, however, were among regions, and the differences were associated with the level of disturbance in the reference sites. We ordered the seven NLA lake habitat modeling ecoregions according to increasing reference site median RDis_IX for examining variance in the other lake habitat indicators (Figure 5-3). The regions with the greatest amount of disturbance in their reference sites (the CENPL, including NPL, SPL, TPL, the CPL, and the XER) generally had higher within-reference site variance all three lake habitat indices, with the exception of low variance in all three indicators within reference sites of the relatively high-disturbance CPL reference sites (Figure 5-4). The precision in modeling least-disturbed condition using null models was generally best in the UMW and NAP (i.e., lowest gSDs). The smaller the SD of index values (or O/E values) among least-disturbed lakes, the easier it is to confidently distinguish disturbed lakes from least-disturbed lakes. The null model SD's serve as an upper bound for the variance of the indicators among regional reference sites, and are analogous to the RMSE's of the regressions in Table 5-3. Removing the variance attributed to the predictors reduces the unexplained variance among reference sites.

5.4.3 *O/E Model Results for RVegQ, LitCvrQ, and LitRipCvQ:*

The LogSD's of *RVegQ_OE*, *LitCvrQ_OE*, and *LitRipCvQ_OE* among reference sites (Table 5-5, column 4) were consistently, and in some cases substantially, lower than those for null models in their respective regions, as evidenced by comparing open circles and black dots plotted in Figure 5-4. The CPL, CENPL, XER and WMT showed the largest reduction of reference site variance compared with corresponding null models, denoting improvement in O/E model performance over null models. As for the null models, however, O/E models in regions with relatively disturbed reference sites had higher reference site variance (the expected condition models were less precise). Again, with the exception of the CPL, regions with more disturbance in their reference sites still had higher SD's than those in regions with less disturbance.

Conversely, the four regions with the lowest level of human disturbance in their reference sites (WMT, UMW, NAP, and SAP) also had the lowest O/E model variance among their reference sites. These results reinforce the idea that human disturbances are likely responsible for a large amount of the variance in lake physical habitat structure in reference sites within the disturbed regions. Therefore, further effort to capture this variance by modeling only non-anthropogenic ("natural") controls would not likely be successful in reducing the variance in O/E values among reference sites.

Except for regions where O/E models incorporated human disturbance variables (NAP, CPL, CENPL and *LitCvr_OE* in SAP), the central tendency of reference site O/E values (Table 5-5, column 6) was very close to 1 (0.98 to 1.01). This is to be expected. Where E-Models contained human disturbance predictors, reference O/E values regained the variance modeled out when observed values were divided by expected values determined with human disturbance predictors (*RDis_IX* or *hiiAg*) set to regional minimum values. If human disturbances decrease the observed value, the mean O/E will be <1. Accordingly, reference site mean O/E values for MLR Models in the NAP, CPL, and CPL (and *LitCvr_OE* in SAP) ranged from 0.79 to 0.91. We regressed the reference O/E values against the *RDis_IX* or *hiiAg* values to obtain y-intercepts for expected O/E for the minimum disturbance observed in these regions. These are shown in the Table 5-5 rows with "$_{\text{OE Yint}}$" subscripted after their Ecoregion designation. For example the $\text{NAP}_{\text{OEYint}}$ row is the result of this final adjustment on reference O/E results from the $\text{NAP}_{\text{MLRModel}}$ row.

Anthropogenic disturbance among reference sites tends to increase the variance in O/E values within regions, even after the minimum disturbance adjustment. There is a strong relationship between the LogSDs of null and adjusted O/E models for lake habitat among reference lakes and the regional level of near-shore anthropogenic disturbance in reference sites (Figure 5-4). Our modeling improves these models, but it is likely that disturbances other than those captured by *RDis_IX* contribute to the uncertainty in predicting habitat characteristics in minimally-disturbed lakes. These results reinforce the idea that human disturbances are likely responsible for a large amount of the variance in lake physical habitat structure among least-disturbed reference sites in the disturbed regions. Therefore, further effort to capture this variance by modeling only non-anthropogenic ("natural") controls would not likely be successful in reducing the variance in O/E values among reference sites.

5.4.4 *Null Model Results for Lake Drawdown and Level Fluctuations:*

Least-disturbed reference lakes and reservoirs in the NAP, SAP and UMW experienced less drawdown and level fluctuation than those in the CPL, CENPL, and WEST; particularly in comparison with marked drawdown observed in man-made reservoirs of the CENPL and WEST (Table 5-6). Not surprisingly, least-disturbed natural lakes in the CENPL and WEST also experienced less drawdown and level fluctuation than their human-constructed counterparts. As a result, the criteria for assessing substantial drawdown in lakes of the Appalachians and UMW were much smaller than those for lakes (and particularly reservoirs) in the CENPL and WEST.

5.5 Precision of physical habitat indicators

In our synoptic survey context, σ^2_{lake} is the signal of interest, and σ^2_{rep} is noise variance; we define their ratio as *S/N*. The methods we used to quantify precision, the precision of NLA lake physical habitat metrics and key habitat condition indices, and the implications of varying precision levels for monitoring and assessment, are comprehensively evaluated by Kaufmann et al. (1999, 2014a). Here we summarize findings for key physical habitat indicators based on the NLA 2012 survey data.

The key NLA physical habitat indices had moderate to high *S/N* (2.2 – 11.0) over the entire NLA 2012 survey (Table 5-7). Compared with the other composite indices, the human disturbance index *RDis_IX* and horizontal drawdown index had the highest *S/N* (9.1-11), whereas the littoral cover O/E index had the lowest S/N (2.2). The advantage of *S/N* as a precision measure is its relevance to many types of statistical analysis and detecting differences in subpopulation means (Zar 1999). High noise in habitat descriptions relative to the signal (i.e., low signal: noise ratio, *S/N*) diminishes statistical power to detect differences among lakes or groups of lakes. Imprecise data limit the ability to detect temporal trends (Larsen et al. 2001, 2004). Noise variance also limits the maximum amount of variance that can be explained by models such as multiple linear regression (Van Sickle et al. 2005, Kaufmann and Hughes 2006). By reducing the ability to quantify associations between variables (Allen et al. 1999, Kaufmann et al. 1999), imprecision compromises the usefulness of habitat data for discerning likely controls on biota and diagnosing probable causes of impairment. The adverse effects of noise variance on these types of analysis are negligible when *S/N* >10; becoming minor as *S/N* decreases to 6, increasing to moderate as *S/N* decreases to 2, and finally becoming severely limiting as *S/N* approaches 0 (Paulsen et al. 1991, Kaufmann et al. 1999). At *S/N*=0, all the metric variance observed among lakes in the survey can be attributed to measurement "noise". Based on these guidelines, the effects of imprecision are minor for all the indicators except for the Littoral Cover index, for which the effects are minor-to-moderate.

Kaufmann et al. (2014a) explain that the *S/N* ratio may not always be a good measure of the potential of a given metric to discern ecologically important differences among sites. For example, a metric may easily discriminate between sparse and abundant littoral cover for fish, but S/N for the metric would be low in a region where littoral cover does not vary greatly among lakes. In cases where the signal variance (σ^2_{lake}) observed in a regional survey reflects a large range of habitat alteration or a large range in natural habitat conditions, *S/N* would be a good measure of the precision of a metric relative to what we want it to measure. However, in random surveys or in relatively homogeneous regions, σ^2_{lake} and consequently *S/N*, may be less than would be calculated for a set of sites specifically chosen to span the full range of habitat conditions occurring in a region. To evaluate the potential usefulness of metrics, Kaufmann et al. (2014a) suggested that an alternate measure of relative precision, σ_{rep} divided by its potential or observed range (Rg_{pot} or Rg_{obs}) offers additional insight. The minimum detectable difference in means between 2 lakes (or between two times in one lake) is given by D_{min} =

$1.96\sigma_{rep}(2n)^{1/2} = 2.77\sigma_{rep}$, using a 2-sided Z-test with $\alpha = 0.05$ (Zar 1999). Thus, to detect any specified difference between 2 lakes in a metric relative to its potential or observed range (Rg_{pot} or Rg_{obs}, the standardized within-lake standard deviation, σ_{rep}/Rg, cannot exceed (D_{min}/Rg)/2.77. By the criteria in Kaufmann et al. (2014a - Table 2), the key NLA physical habitat indices were precise or moderately precise, with σ_{rep}/Rg_{obs} between 0.052 – 0.107 (Table 5-7). Depending on the index, they have the potential to discern differences between single lakes (or one lake at two different times) that are between 1/3rd and 1/8th the magnitude of the observed ranges of these indices.

5.6 Physical habitat index responses to anthropogenic disturbance

In the U.S. as a whole, *RVegQ_OE, LitCvrQ_OE,* and *LitRipCvQ_OE* were significantly higher ($p<0.0001$) in least-disturbed lakes (RT_NLA12_2015=R) than in highly-disturbed lakes (RT_NLA12_2015=T) (Table 5-8, Figure 5-5). The differences were substantial for *RVegQ_OE,* and *LitRipCvQ_OE ,* and discrimination was good (no or nearly no overlap in interquartile ranges). For *LitCvrQ_OE***,** there was an overlap of approximately one-third of the interquartile range. RDis_IX was a major screening variable used to disqualify potential reference sites, so it is not surprising that the entire range of *RDis_IX* among reference sites had very little overlap with that for highly disturbed sites. Note that a site with very low *RDis_IX* could be classified as highly-disturbed on the basis of many other variables, but the converse is not true because reference sites must all have low *RDis_IX.* Like *RDis_IX ,* both vertical and horizontal drawdown were significantly lower ($p<0.0001$) in least-disturbed lakes than in highly-disturbed lakes (Table 5-8, Figure 5-5). Except for lake drawdown, contrasts were very similar for the 2007 and 2012 NLA surveys (Figure 5-6). Although the t test between reference and highly disturbed lakes was similar in both years, the positive relationship between disturbance and in lake level drawdown was much less evident in the drier year (2007) than in 2012. In 2012 fewer than 5% of reference lakes showed any drawdown at all, whereas 75 to 95 % of reference lakes showed drawdown in 2007 – with a lot of overlap in the inter-quartile ranges of reference and highly disturbed sites.

RVegQ_OE, LitCvrQ_OE, and *LitRipCvQ_OE* in sub-sets and sub-regions of the U.S. universally showed the same pattern of response as the nation, with the mean of reference sites significantly greater than those for highly-disturbed sites (Table 5-9). Discrimination was generally greater for *RVegQ_OE* and *LitRipCvQ_OE* than for *LitCvrQ_OE* or the drawdown indices. Discrimination of these 3 indices was somewhat greater for natural lakes than for reservoirs, but good in both. *RVegQ_OE* was strongly and clearly associated with disturbance (RT_NLA12) in all regions and years except for NPL, and SPL in the NLA 2007 survey year. *LitCvrQ_OE* was strongly related to disturbance class in the CPL and NPL, moderately related to disturbance in the NAP, TPL (2012), SPL, and XER; and associations were with disturbance were weakest in the SAP, WMT, and TPL (2007). *LitRipCvQ_OE* was strongly and clearly associated with disturbance (RT_NLA12) in all regions and both years.

5.7 Discussion

The NLA and other lake survey and monitoring efforts increasingly rely upon biological assemblage data to define lake condition. Information concerning the multiple dimensions of physical and chemical habitat is necessary to interpret this biological information and meaningfully assess ecological condition. The controlling influence of littoral structure and complexity on lake biota has been long recognized, and recent research highlights the roles of habitat structural components like littoral woody debris in providing refuges from predation and affecting nutrient cycling and littoral production. NLA field crews characterized lake depth, water surface characteristics, bank morphology and evidence of lake level fluctuations, littoral and shoreline substrate, fish concealment features, aquatic macrophytes, riparian vegetation cover and structure, and human land use activities. These littoral and riparian physical habitat measurements and visual observations were made in a randomized array of 10 near-shore littoral-riparian plots systematically spaced along the shoreline of each sample lake. Metrics describing a rich variety of lake characteristics were calculated from this raw data, and many of these were determined with moderate precision in the national dataset. For the NLA, we summarize this information with four integrative measures of lake condition, and one measure of lake drawdown and lake level fluctuation: *RDis_IX*, incorporating measures of the extent and intensity of near-shore human land and water use activities; *RVegQ*, incorporating the structure and cover in three layers of riparian vegetation, including inundated vegetation; *LitCvrQ*, a combined biotic cover complexity measure including large woody snags, brush, overhanging vegetation, aquatic macrophytes, boulders, and rock ledges; and *LitRipCvrQ*, which combines *RipVegQ* and *LitCvrQ*. The measure of lake level drawdown incorporates both horizontal and vertical fluctuation, comparing them to the regional mean values observed in least-disturbed lakes and reservoirs.

We modeled expected values of *RVegQ, LitCvrQ, and LitRipCvrQ* and their divergence from reference conditions in least-disturbed lakes using regression-based O/E models. The precision of these O/E indices was moderate to high, and showed good discrimination between least-disturbed and highly-disturbed lakes nationally, and within ecoregions. These results show that, compared with least-disturbed reference lakes, those with moderate or high human disturbances in the same region have reduced cover and extent of multi-layered riparian vegetation or natural wetlands. In addition, those with moderate or high disturbance generally also have reduced snag, brush and emergent aquatic macrophyte cover. These results complement the results of the NLA 2012 Assessment report and those of Kaufmann et al. 2014b, 2014c), confirming our general expectation that near-shore wetland and multi-layered riparian vegetation and abundant, complex fish concealment features foster native fish, macroinvertebrate, zooplankton, and avian assemblage integrity, whereas extensive and intensive shoreline human activities that reduce natural riparian vegetation and reduce littoral cover complexity are detrimental to these biotic assemblages.

We believe that the metrics and indices derived from the NLA physical habitat field approach and the O/E indices expressing their divergence from least-disturbed reference conditions describe ecologically-relevant characteristics of lake habitat with sufficient precision to evaluate near-shore lake habitat structure in national, state, and ecoregional assessments. Their association with gradients of human disturbance demonstrates that they also describe lake attributes that are vulnerable to anthropogenic degradation and potential for productive restoration through lake and land management.

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Table 5-1. NLA reference sites from combined 2007 & 2012 surveys.
Selected using consistent criteria (Alan Herlihy's RT_NLA12_2015, choosing 2012 visit for sites sampled in both years). Bold font indicates grouping of reference sites used for modeling expected values for ***RVegQ***, ***LitCvrQ***, and ***LitRipCvrQ***.

ECO9	ECOp5	Total	2007	2012
NAP	APPAL	**67**	23	44
SAP	APPAL	**31**	14	17
	APPAL	(98)	(37)	(61)
CPL	CPL	**28**	5	23
UMW	UMW	**49**	18	31
TPL	CENPL	23	7	16
NPL	CENPL	11	3	8
SPL	CENPL	35	21	14
	CENPL	**(69)**	(31)	(38)
WMT	WEST	**74**	29	45
XER	WEST	**20**	4	16
	WEST	(94)	(33)	(61)
Totals for lower 48 states		**338**	**124**	**214**

Table 5-2. Assignment of riparian vegetation cover complexity, littoral cover complexity, and littoral-riparian habitat complexity index variants by aggregated ecoregion.

Aggregated Omernik Ecoregion	***Riparian Vegetation Cover Complexity Index (RVegQ)***	***Littoral Cover Complexity Index (LitCvrQ)***	***Littoral-Riparian Habitat Complexity Index (LitRipCvrQ)***
CPL	*RVegQ_2*	*LitCvrQ_b*	*LitRipCvrQ_2b*
SAP	*RVegQ_2*	*LitCvrQ_c*	*LitRipCvrQ_2c*
NAP, UMW	*RVegQ_2*	*LitCvrQ_d*	*LitRipCvrQ_2d*
TPL, NPL, SPL	*RVegQ_7*	*LitCvrQ_d*	*LitRipCvrQ_7d*
WMT, XER	*RVegQ_8*	*LitCvrQ_d*	*LitRipCvrQ_8d*

Table 5-3. Summary of regression models used in estimating lake-specific expected values of Lake Physical Habitat variables *RVegQx*, *LitCvrQx* and *LitRipCvrQx* under least-disturbed conditions.
See Appendix A for model details.

REGION	*y = RVegQ*	*y = LitCvrQ*	*y = LitRipCvrQ*
NAP	*Ly* = f(Lat, Lon, LkOrig, **RDisIX**,)* (R^2=23%, RMSE=0.162L**)	*Ly = f(L_LkArea, **RDisIX**)* (R^2= 12%, RMSE=0.281L)	*Ly = f(Lat, Lon, LkOrig, **RDisIX**)* (R^2=24%, RMSE=0.168L)
SAP	*Ly = f(Lon)* (R^2=16%, RMSE=0.119L)	*Ly = f(ElevXLon, **RDisIX**)* (R^2=19%, RMSE=0.267L)	*Ly = f(Lon, ElevXLon, Elev)* (R^2=31%, RMSE= 0.148L)
CPL	*y = f(ElevXLat, **RDisIX**)* (R^2=39%, RMSE=0 .0896)	*y = f(L_Elev, **RDisIX**)* (R^2=25%, RMSE= 0.174)	*y = f(L_Elev, **RDisIX**)* (R^2=44%, RMSE=0.093)
UMW	*Ly = (mean LRVegQ)* (R^2=0%, RMSE=0.153L)	*Ly = (mean LitCvrQ)* (R^2=0%, RMSE=0.199L)	*Ly = (mean LitRipCvrQ)* (R^2=0%, RMSE=0 .115L)
CENPL	*Ly = f(**hiiAg**)* (R^2=15%, RMSE=0.318L)	*Ly = f(LkOrig, **hiiAg**)* (R^2=9%, RMSE=0.276L)	*Ly = f(**hiiAg**)* (R^2=15%, RMSE=0.233L)
WMT	*Ly = f(Lat, Elev, L_LkArea, LkOrigin)* (R^2=28%, RMSE=0.167L)	*Ly = f(Lat, Elev, L_LkArea, LkOrigin)* (R^2=16%, RMSE=0.244L)	*Ly = f(Lat, Elev, L_LkArea, LkOrigin)* (R^2=29%, RMSE=0.145L)
XER	*Ly = f(Lat, Elev)* (R^2=24%, RMSE=0.284L)	*Ly = f (Lat, Elev)* (R^2=16%, RMSE=0.290L)	*Ly = f(Lat, Elev)* (R^2=21%, RMSE=0.265L)

**Ly* refers to Log_{10}-transformed lake habitat metric values.
**L refers to RMSE's that are in Log_{10} units (e.g., 0.162L

Table 5-4. Null Model Geometric Means (gMean), geometric Standard Deviations (gSD), 5th percentiles, and 25th percentiles of habitat index values in least-disturbed reference lakes in the aggregated ecoregions of the NLA. The gMeans and gSDs are antilogs of mean and SD of log_{10}-transformed index values (LogMean and LogSD). ***Bold, italicized*** text identifies minimum LogSD and gSD values, i.e., the most precise models for each index. **Bold, underlined text** marks the least precise models. gSDs calculated from log-transformed variables are expressions of the proportional variance of these distributions, so are directly comparable among regions with different gMeans. A range of ±1LogSD is equivalent to *multiplying and dividing* the gMean by the gSD. For example, the gMean ±1 gSD for the riparian vegetation cover complexity index in least-disturbed NAP lakes translates to a range of *RVegQ* from 0.182 to 0.338: the geometric mean habitat index value of 0.2482 multiplied and divided by 1.363. The 5th and 25th percentiles were estimated, respectively, as the mean of log-transformed index values minus 1.65 and 0.67 times the SD of log-transformed habitat index values (see Table 5-2 for the variant of each index used). All percentiles are expressed in the units of the habitat indices, i.e., as antilogs of log-transformed values. (Note that the constant 0.01 is subtracted from all antilogs because it was added when O/E values were log-transformed).

Aggregated ecoregion	Index	Ref$_{0712}$ LogMean	Ref$_{0712}$ LogSD	Ref$_{0712}$ gMean	Ref$_{0712}$ gSD	Ref$_{0712}$ est 5th%	Ref$_{0712}$ est 25th %
Riparian Vegetation Cover Complexity:							
NAP$_{NULL}$	*RVegQ*	-0.5881	0.1345	0.2482	1.363	0.1449	0.1998
SAP$_{NULL}$	*RVegQ*	-0.6111	***0.1277***	0.2348	***1.342***	0.1407	0.1911
UMW$_{NULL}$	*RVegQ*	-0.6130	0.1533	0.2338	1.423	0.1262	0.1824
CPL$_{NULL}$	*RVegQ*	-0.6645	0.2810	0.2065	1.910	0.0644	0.1304
CENPL$_{NULL}$	*RVegQ*	-0.8346	**0.3427**	0.1364	**2.201**	0.0298	0.0760
TPL$_{NULL}$	*RVegQ*	-0.7295	0.3129	0.1764	2.055	0.0468	0.1050
NPL$_{NULL}$	*RVegQ*	-1.1352	0.2500	0.0632	1.778	0.0183	0.0398
SPL$_{NULL}$	*RVegQ*	-0.8093	0.3402	0.1451	2.189	0.0326	0.0817
WMT$_{NULL}$	*RVegQ*	-0.5900	0.1922	0.2470	1.557	0.1138	0.1811
XER$_{NULL}$	*RVegQ*	-0.8301	0.3070	0.1379	2.028	0.0360	0.0821
Littoral Cover Complexity:							
NAP$_{NULL}$	*LitCvrQ*	-0.8174	0.2418	0.1423	1.745	0.0508	0.9049
SAP$_{NULL}$	*LitCvrQ*	-0.6469	0.2873	0.2155	1.938	0.0657	0.1347
UMW$_{NULL}$	*LitCvrQ*	-0.8756	***0.1994***	0.1232	***1.583***	0.0524	0.0879
CPL$_{NULL}$	*LitCvrQ*	-0.4883	0.2331	0.3049	1.710	0.1240	0.2167
CENPL$_{NULL}$	*LitCvrQ*	-1.0164	0.2880	0.0863	1.941	0.0222	0.0518
TPL$_{NULL}$	*LitCvrQ*	-0.9927	**0.3190**	0.0917	**2.084**	0.0203	0.0522
NPL$_{NULL}$	*LitCvrQ*	-0.9974	0.2116	0.0906	1.628	0.0350	0.0626
SPL$_{NULL}$	*LitCvrQ*	-1.0389	0.2929	0.0814	1.963	0.0200	0.0482
WMT$_{NULL}$	*LitCvrQ*	-1.0162	0.2578	0.0863	1.811	0.0262	0.0547
XER$_{NULL}$	*LitCvrQ*	-1.1457	0.2990	0.0615	1.991	0.0130	0.0351
Littoral-Riparian Habitat Complexity:							
NAP$_{NULL}$	*LitRipCvrQ*	-0.6740	0.1404	0.2018	1.382	0.1143	0.1606
SAP$_{NULL}$	*LitRipCvrQ*	-0.6069	0.1690	0.2372	1.476	0.1201	0.1805
UMW$_{NULL}$	*LitRipCvrQ*	-0.7083	***0.1149***	0.1857	***1.303***	0.1165	0.1541
CPL$_{NULL}$	*LitRipCvrQ*	-0.5391	0.1687	0.2796	1.475	0.1422	0.2128
CENPL$_{NULL}$	*LitRipCvrQ*	-0.8820	0.2508	0.1212	1.782	0.0406	0.0791
TPL$_{NULL}$	*LitRipCvrQ*	-0.8230	0.2813	0.1403	1.911	0.0416	0.0874
NPL$_{NULL}$	*LitRipCvrQ*	-1.0442	0.1887	0.0803	1.544	0.0341	0.0575

Aggregated ecoregion	Index	Ref_{0712} LogMean	Ref_{0712} LogSD	Ref_{0712} gMean	Ref_{0712} gSD	Ref_{0712} est 5^{th}%	Ref_{0712} est 25^{th} %
SPL_{NULL}	*LitRipCvrQ*	-0.8698	0.2305	0.1902	1.700	0.0462	0.0846
WMT_{NULL}	*LitRipCvrQ*	-0.7369	0.1677	0.1733	1.471	0.0869	0.1315
XER_{NULL}	*LitRipCvrQ*	-0.9455	**0.2818**	0.1034	**1.913**	0.0289	0.0634

Table 5-5. O/E Physical Habitat Model means (LogMean, gMean), standard deviations (LogSD, gSD), and percentiles of the distribution of habitat index O/E values for least-disturbed reference lakes in the aggregated ecoregions of the NLA.

See Table 5-3 for the variant of each index used. The gMean and gSD are antilogs of mean and SD of $\log_{10}$-transformed index values (LogMean and LogSD). Percentiles were estimated, respectively, as the log-transformed index O/E value of 0.0 (see text) minus 1.65 and 0.67 times the SD of log-transformed habitat index values. ***Bold, italicized*** *text identifies* minimum SD values, i.e., the most precise models for each index. **Bold, underlined text** marks the least precise models. gSDs calculated from log-transformed variables are expressions of the proportional variance of these distributions, so are directly comparable among regions with different geometric means. A range of ±1SD is calculated by *multiplying and dividing* the gMean by the gSD. For example, the LogMean ± 1LogSD for the riparian vegetation cover complexity O/E index in least-disturbed lakes of the NAP (0.04276 ± 0.1255) translates to a range of O/E values from 0.78 to 1.31: the geometric mean habitat index O/E value of 1.00 (antilog of +0.04276 = 1.10 minus log-transform constant 0.10) multiplied and divided by 1.34, the antilog of 0.1255. All percentiles expressed as antilogs of log-transformed values minus constant 0.10. We based physical habitat condition criteria based on the distribution of O/E index values in least-disturbed lakes within each region. The 5th and 25th percentiles, respectively, were set as the upper bounds for poor and fair condition.

Aggregated ecoregion	Index	Ref 0/E LogMean	Ref 0/E LogSD	Ref O/E gMean	Ref O/E gSD	Ref O/E 5th %tile	Ref O/E 25th %tile
NAP MLR Model	*RVegQ_OE*	(-0.00811)	(0.1255)	(0.88)	(1.34)	-------	-------
NAP OE Yint	" "	+0.04276	0.1255	1.00	1.34	0.5850	0.8092
SAP MLR Model	*RVegQ_OE*	+0.04226	***0.1105***	1.00	***1.29***	0.6244	0.8295
UMW MLR Model	*RVegQ_OE*	+0.0428	0.1442	1.00	1.39	0.5381	0.7835
CPL MLR Model	*RVegQ_OE*	(-0.0617)	(0.2113)	(0.87)	(1.63)	-------	-------
CPL OE Yint	" "	-0.00067	0.2129	0.90	1.63	0.3449	0.6191
CENPL MLR Mode	*RVegQ_OE*	(-0.02799)	**<u>(0.3165)</u>**	(0.84)	**<u>(2.07)</u>**	-------	-------
l CENPL OE Yint	" "	+0.04688	0.2928	1.01	1.96	0.2663	0.6091
WMT MLR Model	*RVegQ_OE*	+0.04290	0.1535	1.00	1.42	0.5162	0.7711
XER MLR Model	*RVegQ_OE*	+0.04199	0.2656	1.00	1.84	0.3016	0.6312
NAP MLR Model	*LitCvrQ_OE*	(+0.04502)	(0.2330)	(1.01)	(1.71)	-------	-------
NAP OE Yint	" "	+0.04665	0.2330	1.01	1.71	0.3594	0.6772
SAP MLR Model	*LitCvrQ_OE*	(-0.05093)	(0.2500)	(0.79)	(1.78)	-------	-------
SAP OE Yint	" "	+0.04287	0.2440	1.00	1.75	0.3368	0.6575
UMW MLR Model	*LitCvrQ_OE*	+0.04422	0.1954	1.00	1.57	0.4245	0.7152
CPL MLR Model	*LitCvrQ_OE*	(-0.03310)	***(0.1909)***	(0.83)	***(1.55)***	-------	-------
CPL OE Yint	" "	-0.00743	0.1940	0.88	1.56	0.3704	0.6288
CENPL MLR Model	*LitCvrQ_OE*	(+0.00495)	(0.2870)	(0.91)	(1.94)	-------	-------
CENPL OE Yint	" "	+0.02752	0.2839	0.97	1.92	0.2624	0.5876
WMT MLR Model	*LitCvrQ_OE*	+0.03770	0.2528	0.99	1.79	0.3174	0.6385
XER MLR Model	*LitCvrQ_OE*	+0.03451	**<u>0.2983</u>**	0.98	**<u>1.99</u>**	0.2486	0.5834
NAP MLR Model	*LitRipCvrQ_OE*	(+0.00344)	(0.1321)	(0.91)	(1.36)	-------	-------
NAP OE Yint	" "	+0.04230	0.1321	1.00	1.36	0.5672	0.7990
SAP MLR Model	*LitRipCvrQ_OE*	+0.04326	0.1329	1.00	1.36	0.5667	0.7999
UMW MLR Model	*LitRipCvrQ_OE*	+0.04199	***0.1110***	1.00	***1.29***	0.6252	0.8296
CPL MLR Model	*LitRipCvrQ_OE*	(-0.0248)	(0.1230)	(0.84)	(1.33)	-------	-------
CPL OE Yint	" "	+0.01615	0.1234	0.94	1.33	0.5494	0.7580
CENPL MLR Model	*LitRipCvrQ_OE*	(-0.0121)	(0.2413)	(0.87)	(1.74)	-------	-------
l CENPL OE Yint	" "	+0.04303	0.2246	1.00	1.68	0.3703	0.6808

Aggregated ecoregion	Index	Ref 0/E LogMean	Ref 0/E LogSD	Ref O/E gMean	Ref O/E gSD	Ref O/E 5th %tile	Ref O/E 25th %tile
$WMT_{MLR\ Model}$	*LitRipCvrQ_OE*	+0.04200	0.1366	1.00	1.37	0.5556	0.7922
$XER_{MLR\ Model}$	*LitRipCvrQ_OE*	+0.04012	**0.2552**	1.00	**1.80**	0.3159	0.6398

Table 5-6. Empirical 75th and 95th percentiles of the distribution of vertical and horizontal drawdown. As interpreted from indicators of lake level fluctuation (*bfxVertHeight* and *bfxHorizDist*) at least-disturbed reference lakes sampled by NLA in 2007 and 2012. We used the 75th and 95th percentiles to define the boundaries between small, medium and large magnitude of drawdown.

		Number of Reference Lakes (2007+2008)			Vertical Drawdown (m) (*bfxVertHeight*)			Horizontal Drawdown (m) (*bfxHorizDist*)		
Ecogion	**Lake Origin**	**Total**	**Natural**	**Man-Made**	**median**	**75th%**	**95th%**	**median**	**75th%**	**95th%**
NAP	All	**67**	54	13	0.000	**0.12**	**0.470**	0.00	**0.25**	**1.65**
SAP	All	**31**	0	31	0.000	**0.20**	**0.760**	0.00	**0.20**	**2.15**
UMW	All	**49**	49	0	0.000	**0.11**	**0.50**	0.00	**0.51**	**2.65**
CPL	All	**28**	5	23	0.000	**0.03**	**1.00**	0.00	**0.10**	**4.00**
CENPL	Natural	**29**	29	0	0.000	**0.06**	**0.28**	0.00	**0.10**	**2.85**
" "	Man-Made	**39/40**	0	39/40	0.010	**0.36**	**1.20**	0.21	**1.55**	**14.63**
WEST	Natural	**69**	69	0	0.021	**0.33**	**1.00**	0.00	**0.64**	**9.43**
" "	Man-Made	**25**	0	25	0.232	**1.05**	**2.00**	0.27	**4.39**	**11.37**

Table 5-7. Precision of the key NLA Physical Habitat indices used as the primary physical habitat condition measures in the NLA.

Precision expressed as: 1) the pooled standard deviation of repeat visits (σ_{rep}), 2) precision relative to potential or observed range (σ_{rep}/Rg_{pot} and σ_{rep}/Rg_{pot}), and 3) the signal: noise ratio, where signal is among-lakes variance and noise is within-lake variance during the same year and season (S/N = $\sigma^2_{lake}/\sigma^2_{rep}$). Analysis was based on NLA field measurements on a summer probability sample of 1203 lakes in the 48 conterminous U.S. states, with repeat sampling on a random subset of 88 of those lakes during the summer of 2012. Six of the sample lakes showed very large changes in water level, which affected the littoral and riparian indicator values. We excluded these 6 lakes in this analysis, except for values within perentheses. *RDis_IX* is the Near-shore human disturbance index, *RVegQ$_c$* is the Riparian vegetation cover & structure index, *Log(RVegQ$_{c3}$OE)* is the log-transformed O/E index for Riparian vegetation cover & structure, *LitCvrQ$_c$* is the Littoral cover complexity index, *Log(LitCvrQ$_{c3}$OE* is the log-transformed O/E index for Littoral cover complexity *, LitRipCvrQ$_c$* is the Littoral-riparian habitat complexity index, *Log(LitRipCvrQ$_{c3}$OE)* is the log-transformed O/E index for Littoral-riparian habitat complexity, *L_VertDD* = Log_{10}(Vertical drawdown +0.1m), and *L_HorizDD* = Log_{10}(Horizontal drawdown + 1m).

NLA PHab Indices	σ_{rep}	Rg_{obs}	σ_{rep}/Rg_{obs}	S/N
RDis_IX	0.098	0.0 - +0.950	0.103	9.1
L_RVegQ$_c$	0.144	-2.0 - -0.266	0.083	6.6
L_RVegQ$_{c3}$OE	0.130	-1.0 - +0.666	0.078	5.0
L_LitCvrQ$_c$	0.190	-2.0 - +0.0266	0.094	3.4
L_LitCvrQ$_{c3}$OE	0.188	-1.0 - +0.759	0.107	2.2
L_LitRipCvrQ$_c$	0.134	-2.0 - -0.135	0.072	5.6
L_LitRipCvrQ$_{c3}$OE	0.122	-1.0 - +0.681	0.073	4.1
L_VertDD	0.193 (0.266)	-1.0 - +1.654	0.073 (0.100)	5.9 (2.7)
L_HorizDD	0.148 (0.283)	0.0 - +2.873	0.052 (0.099)	11.0 (3.8)

Table 5-8. Association of NLA-2012 Physical Habitat Indices with high and low anthropogenic disturbance stress classes (RT_NLA12 = R and T), defined as least-disturbed and most disturbed within NLA regions. The t-values test the null hypothesis that the mean value of the habitat index in Reference sites minus the mean in most-disturbed sites was zero in the NLA 2012 survey. Positive t_{RT} values indicate that habitat index values are greater in least-disturbed sites; negative values indicate higher index values in disturbed sites. See Figure 5-6 for box and whisker plots by NLA regions, presented separately for the NLA 2012 and 2007 surveys.

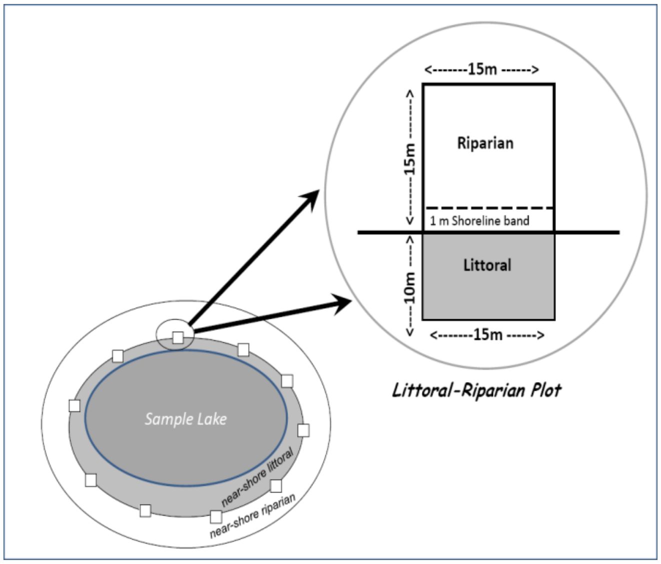
NLA Physical Habitat Indices	t_{RT}	$p_{RT} > \| t_{RT} \|$
RDis_IX – Near-shore human disturbance index	-25*	<0.0001*
L_RVegQ$_c$ – Riparian vegetation cover & structure index	13	<0.0001
L_RVegQ$_{c3}$OE - O/E index for Riparian vegetation cover & structure	14	<0.0001
L_LitCvrQ$_c$ – Littoral cover complexity index	8.3	<0.0001
L_LitCvrQ$_{c3}$OE-- O/E index for Littoral cover complexity	9.3	<0.0001
L_LitRipCvrQ$_c$ –Littoral-riparian habitat complexity index	13	<0.0001
L_LitRipCvrQ$_{c3}$OE -- O/E index for Littoral-riparian habitat complexity	14	<0.0001
L_VertDD – Log_{10}(Vertical drawdown +0.1m)	-4.3*	<0.0001*
L_HorizDD– Log_{10}(Horizontal drawdown +1.0m)	-4.7*	<0.0001*

* Note that *RDis_IX* was one of the screening variables used to define least-disturbed reference sites (RT_NLA12=R) and highly-disturbed sites (RT_NLA12=T), and was a very influential. The drawdown variables *bfxVertHeight* and *bfxHorizDist* were also used in the screening process, but had only a minor influence on the definition of sites.

Table 5-9. Association of NLA 2007 and 2012 Physical Habitat Indices with high and low anthropogenic disturbance stress classes (RT_NLA12 = R and T), defined as least-disturbed and most disturbed within NLA regions. The t-values test the null hypothesis that the mean value of the habitat index in Reference sites minus the mean in most-disturbed sites was zero in the Domain specified in column 1. Positive t_{RT} values indicate that habitat index values are greater in least-disturbed sites; negative values indicate higher index values in disturbed sites. See Figure 5-6 for box and whisker plots by NLA regions, presented separately for the NLA 2012 and 2007 surveys.

DOMAIN	*L_RVegOE*	*L_LitCvrOE*	*L_LitRipCvrOE*	*L_HorizDD*
National 07&12	19****	12****	19****	-7.7****
National 07&12				
Natural	14****	9.6****	14****	-3.5***
Man-Made	13****	6.6****	12****	-6.0****
National 2007	13****	7.3****	13****	-6.3****
2012	14****	9.3****	14****	-4.7****
APPAL 2007	6.4****	3.0***	4.4****	+1.9
2012	6.4****	5.1****	4.1****	-3.2***
NAP 2007	4.0***	2.4**	4.1***	+1.1
2012	3.8***	3.8***	4.3****	-2.4*
SAP 2007	4.8****	1.1	2.9**	-0.2
2012	6.3****	1.4	3.3**	-2.4*
CENPL 2007	4.4****	2.5**	5.0****	-4.0****
2012	6.2****	5.5****	6.4****	-0.6
TPL 2007	4.0***	0.3	2.9**	-1.2
2012	3.6***	3.3**	3.7***	0.6
NPL 2007	1.3	4.6***	4.8***	-5.1****
2012	2.4*	2.4*	2.2*	+1.6*
SPL 2007	1.4	2.1*	2.2**	-1.2
2012	6.0****	4.4****	6.1****	-2.2*
CPL 2007	4.5***	1.4	4.6****	-1.3
2012	3.6***	4.2****	5.4****	-0.5
UMW 2007	6.5****	6.2****	7.2****	+4.4****
2012	6.1****	3.3***	6.5****	-0.5
WEST 2007	8.7****	3.4***	7.7****	-8.1****
2012	8.3****	3.2***	7.2****	-5.3****
WMT 2007	6.3****	1.6*	5.4****	-5.7****
2012	6.7****	2.3*	6.0****	-5.6****
XER 2007	6.2****	3.5***	5.8****	-4.6****
2012	4.5****	2.0*	3.6**	-1.4

Near-Shore Station NLA-2007:



Near-Shore Station NLA-2012:

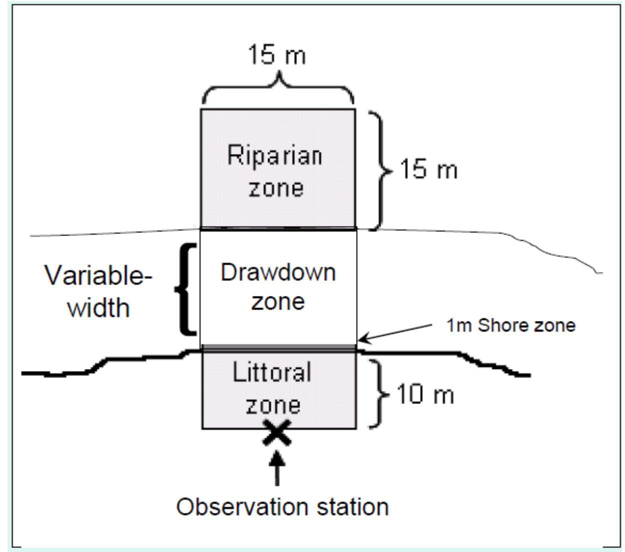


Figure 5-1. Field sampling design with 10 near-shore stations at which data were collected to characterize near shore lake riparian and littoral physical habitat in the 2007 and 2012 National Lakes Assessment (NLA) surveys.
The 10 stations were systematically spaced around the shore of the lake from random starting point. Insert shows riparian plot, shoreline band, littoral plot, and (for NLA-1012 only) drawdown zone plot located at each station.

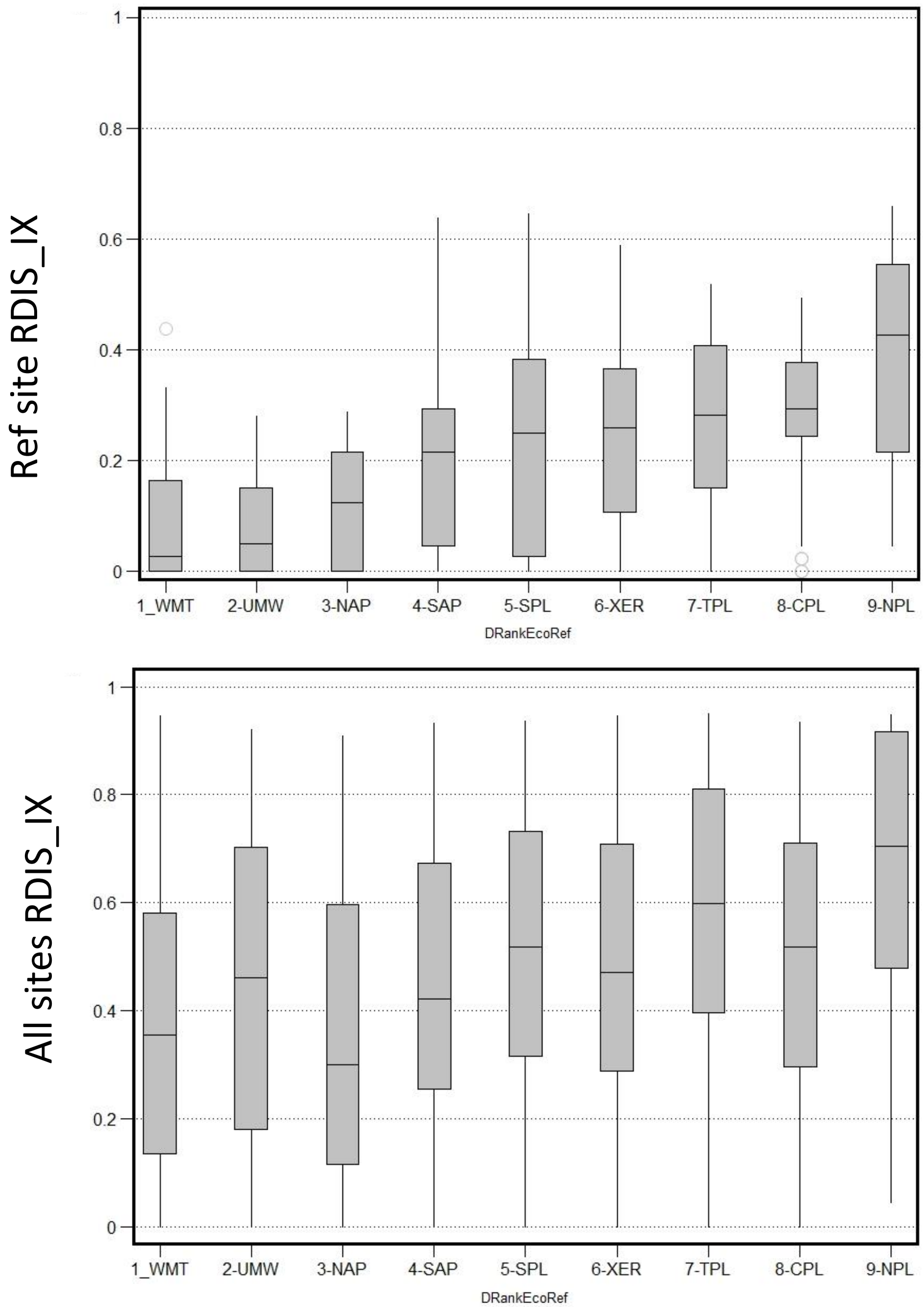


Figure 5-2. Near-shore anthropogenic disturbance (*RDis_IX)* in NLA0712 regions, ordered by their median Reference site RDis.
Upper plot: Least-disturbed reference sites. Lower plot: all sites. Unweighted sample statistics are shown; box midline and lower and upper ends show median and 25th and 75th percentile values, respectively; whiskers show maximum and minimum observations within 1.5 times the interquartile range above / below box ends; circles show outliers.

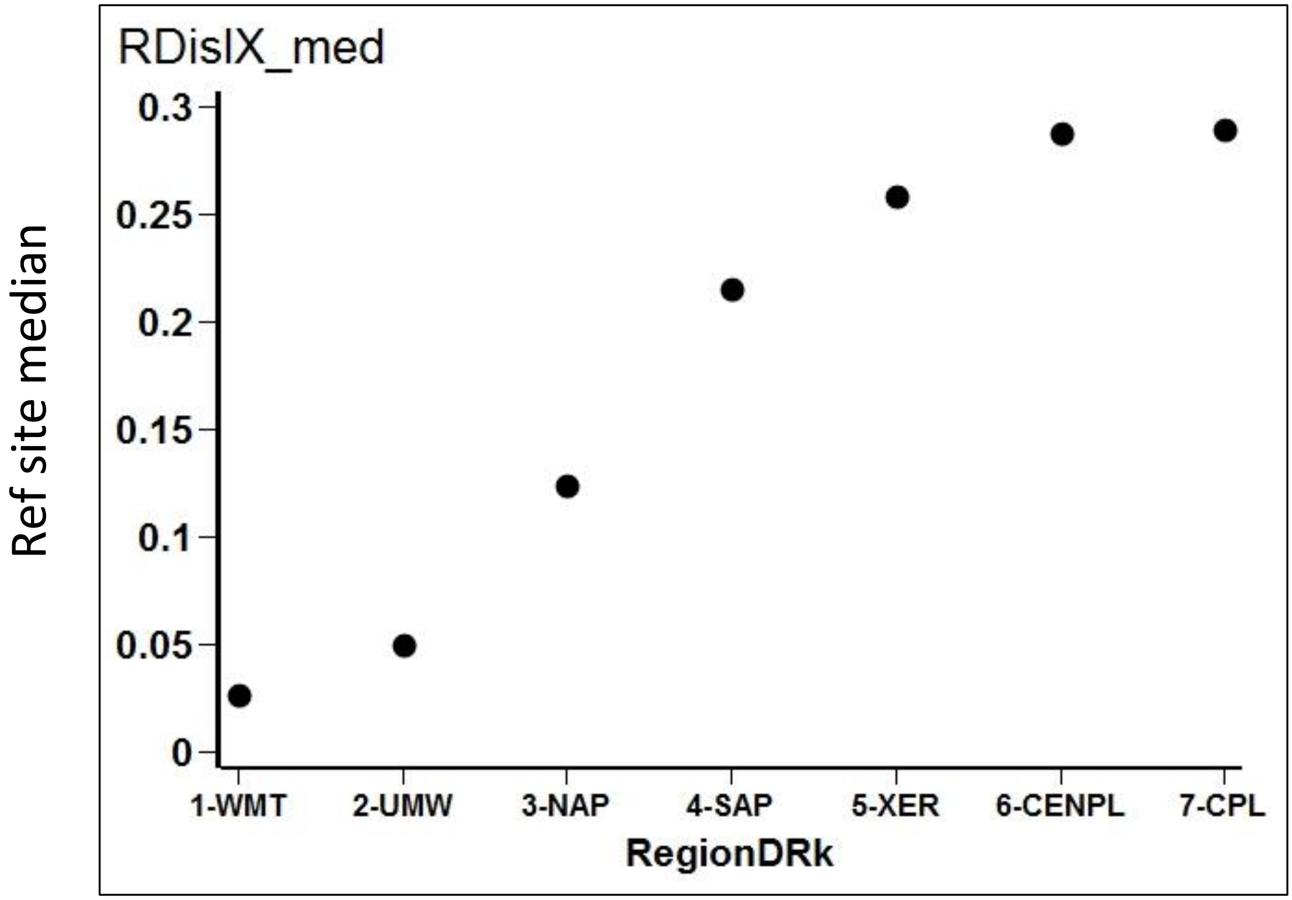
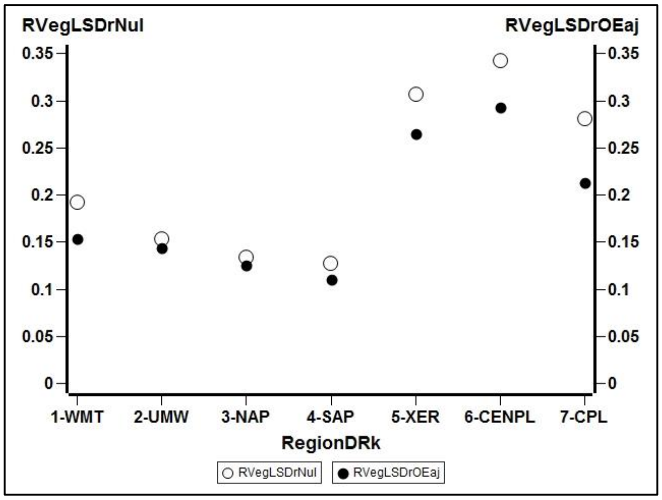
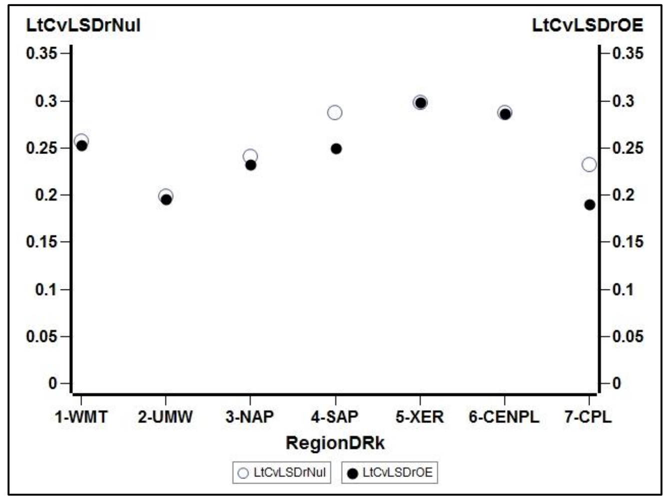


Figure 5-3. Near-shore anthropogenic disturbance in NLA0712 least-disturbed reference sites (median RDis_IX), ordered by aggregated region according to the same median level of near-shore disturbance.
The NLA ECO9 regions NPL, SPL, and TPL are combed into the Central Plains (CENPL) region.

Log(RVegQ):



Log(LitCvrQ):



Log(LitRipCvrQ):

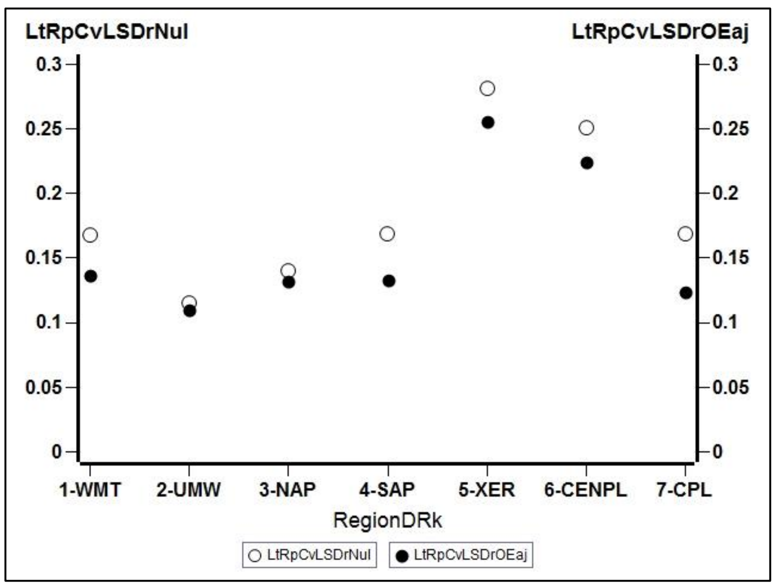


Figure 5-4. LogSD's for Null-Model and regression-based O/E model for Near-shore *RVegQ*, *LitCvrQ*, and *LitRipCvrQ* in the set of least-disturbed lakes and reservoirs (Table 5-1) sampled in the combined 2007 and 2012 NLA surveys.

X-axis shows the 7 modeling regions ordered by increasing median RDis_IX in the reference sites. The NLA ECO9 regions NPL, SPL, and TPL are combed into the Central Plains (CENPL) region. Low variance among reference sites denotes greater precision in estimating expected reference condition. The smaller variance in regression-based O/E models (black dots) illustrate their greater precision compared with null models (open circles) for a given indicator and region.

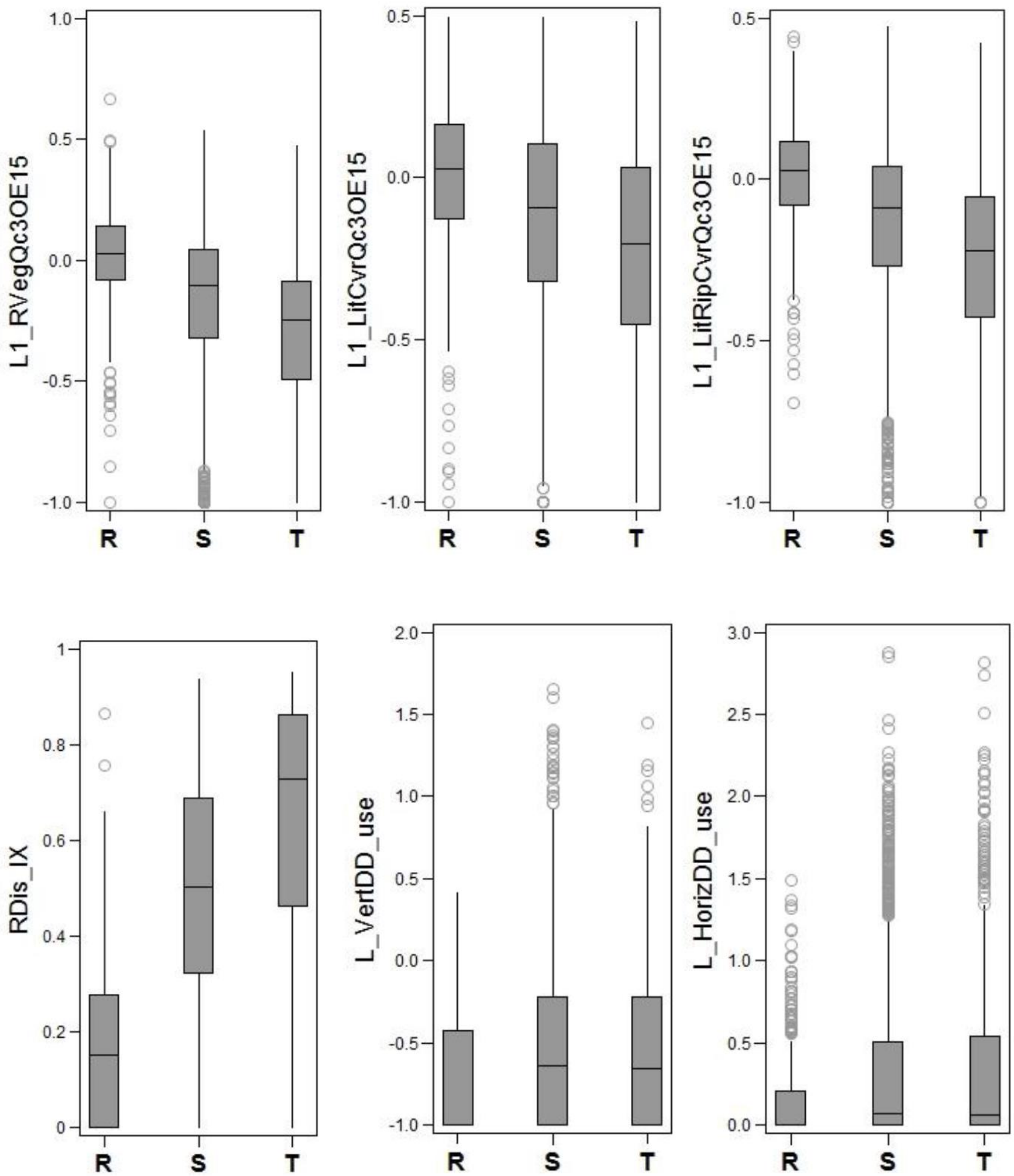


Figure 5-5. Contrasts in key NLA physical habitat index values among least-disturbed reference (R), intermediate (S), and highly disturbed (T) lakes in the contiguous 48 states of the U.S. based on combined NLA 2007 and 2012 data.
Unweighted sample statistics are shown; box midline and lower and upper ends show median and 25th and 75th percentile values, respectively; whiskers show maximum and minimum observations within 1.5 times the interquartile range above ⁄ below box ends; circles show outliers. See Table 5-9 for *t* and p values for the differences between means for reference (R) and disturbed (T) sites.

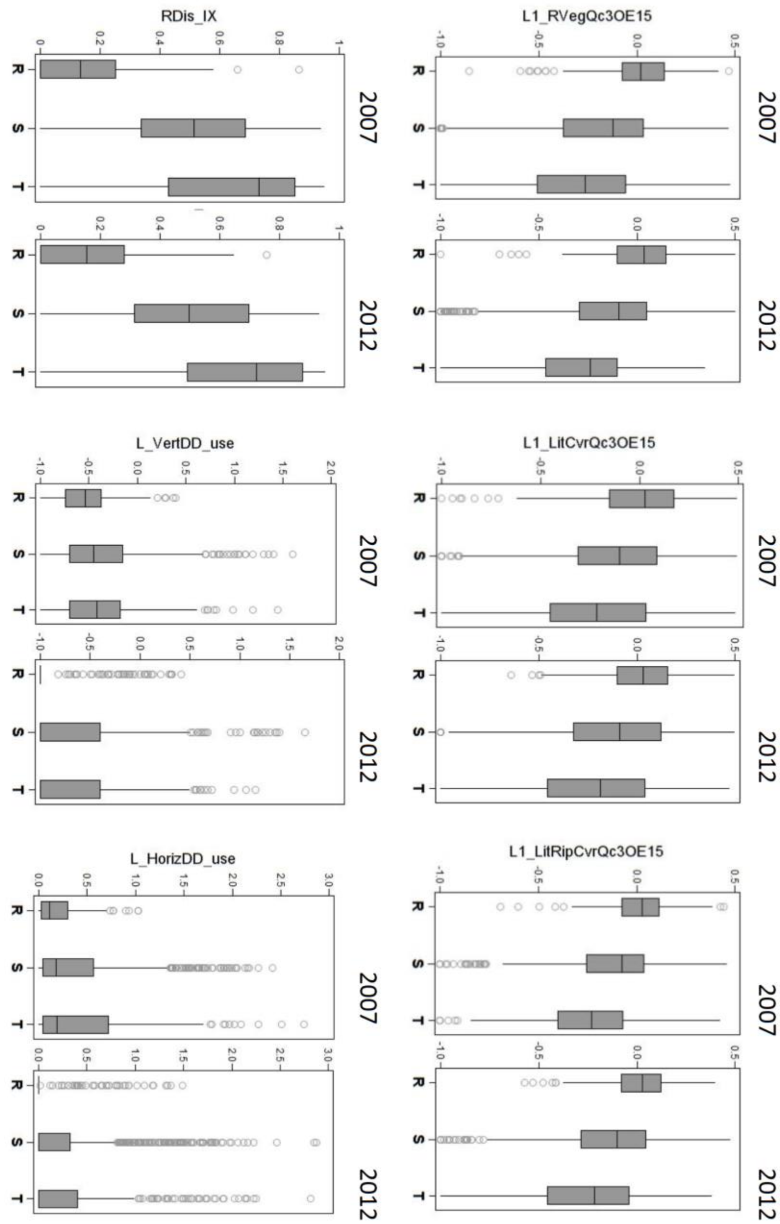


Figure 5-6. Contrasts in key NLA physical habitat index values among least-disturbed reference (R), intermediat (S), and highly disturbed (T) lakes in the contiguous 48 states of the U.S. shown separately for the NLA 2007 and 2012 surveys.
Unweighted sample statistics are shown; box midline and lower and upper ends show median and 25th and 75th percentile values, respectively; whiskers show maximum and minimum observations within 1.5 times the interquartile range above ⁄ below box ends; circles show outliers. See Table 5-9 for *t* and p values for the differences between means for reference (R) and disturbed (T) sites.

Lake Physical Habitat Expected Condition Models Appendix A

Table 3 from TSD Chapter. Summary of regression models used in estimating lake-specific expected values of Lake Physical Habitat variables *RVegQx*, *LitCvrQx* and *LitRipCvrQx* under least-disturbed conditions. Variable definitions and model details on following pages.

REGION	*y = RVegQ*	*y = LitCvrQ*	*y = LitRipCvrQ*
NAP	*Ly* = f(Lat, Lon, LkOrig,* ***RDisIX****,)* (R^2=23%, RMSE=0.162L**)	*Ly = f(L_LkArea,* ***RDisIX****)* (R^2= 12%, RMSE=0.281L)	*Ly = f(Lat, Lon, LkOrig,* ***RDisIX****)* (R^2=24%, RMSE=0.168L)
SAP	*Ly = f(Lon)* (R^2=16%, RMSE=0.119L)	*Ly = f(ElevXLon,* ***RDisIX****)* (R^2=19%, RMSE=0.267L)	*Ly = f(Lon, ElevXLon, Elev)* (R^2=31%, RMSE= 0.148L)
CPL	*y = f(ElevXLat,* ***RDisIX****)* (R^2=39%, RMSE=0 .0896)	*y = f(L_Elev,* ***RDisIX****)* (R^2=25%, RMSE= 0.174)	*y = f(L_Elev,* ***RDisIX****)* (R^2=44%, RMSE=0.093)
UMW	*Ly = (mean LRVegQ)* (R^2=0%, RMSE=0.153L)	*Ly = (mean LitCvrQ)* (R^2=0%, RMSE=0.199L)	*Ly = (mean LitRipCvrQ)* (R^2=0%, RMSE=0 .115L)
CENPL	*Ly = f(****hiiAg****)* (R^2=15%, RMSE=0.318L)	*Ly = f(LkOrig,* ***hiiAg****)* (R^2=9%, RMSE=0.276L)	*Ly = f(****hiiAg****)* (R^2=15%, RMSE=0.233L)
WMT	*Ly = f(Lat, Elev, L_LkArea, LkOrigin)* (R^2=28%, RMSE=0.167L)	*Ly = f(Lat, Elev, L_LkArea, LkOrigin)* (R^2=16%, RMSE=0.244L)	*Ly = f(Lat, Elev, L_LkArea, LkOrigin)* (R^2=29%, RMSE=0.145L)
XER	*Ly = f(Lat, Elev)* (R^2=24%, RMSE=0.284L)	*Ly = f (Lat, Elev)* (R^2=16%, RMSE=0.290L)	*Ly = f(Lat, Elev)* (R^2=21%, RMSE=0.265L)

**Ly* refers to Log_{10}-transformed lake habitat metric values.
**L refers to RMSE's that are in Log_{10} units (e.g., 0.162L)

VARIABLE DEFINITIONS

On following pages variables are defined as follows:

Observed Habitat Indicator values are: (in the TSD text, these are abbreviated as *RVegQ, LitCvrQ,* and *LitRipCvrQ*)
RVegQc15, LitCvrQc15, LitRipCvrQc15
L_RVegQc15 = Log_{10}***(RVegQc15*** +0.01)
L_LitCvrQc15 = Log_{10}***(LitCvrQc15*** +0.01)
L_LitRipCvrQc15 = Log_{10}***(LitRipCvrQc15*** +0.01)

Expected Condition Regression Models have the form (in the TSD text, Expected condition variables are abbreviated as *RVegQX, LitCvrQX,* and *LitRipCvrQX*):
L_RVegQc3x15 = f(predictors) or ***RVegQc3x15*** = f(predictors)
L_LitCvrQc3x15 = f(predictors) or ***LitCvrQc3x15*** = f(predictors)
L_LitRipCvrQc3x15 = f(predictors) or ***LitRipCvrQc3x15*** = f(predictors)

Observed/Expected Condition Variables are defined as follows (in the TSD text, O/E variables are abbreviated as *RVegQ_OE, LitCvrQ_OE,* and *LitRipCvrQ_OE*):
RVegQc3OE15= (RVegQc15/RVegQc3x15) and ***L1_RVegQc3OE15 =*** Log_{10}***(RVegQc3OE15*** +0.1)
LitCvrQc3OE15= (LitCvrQc15/LitCvrQc3x15) and ***L1_LitCvrQc3OE15 =*** Log_{10}***(LitCvrQc3OE15*** +0.1)
LitRipCvrQc3OE15= (LitRipCvrQc15/LitRipCvrQc3x15) and ***L1_LitRipCvrQc3OE15 =*** Log_{10}***(LitRipCvrQc3OE15*** +0.1)

Predictors defined from variables in prk datafile NLA12_pc.nla_lakeinfo_all_20150415 are as follows:
LATdd_use = ***LAT_DD_N83*** = latitude in decimal degrees
LONdd_use = ***LON_DD_N83*** = longitude in decimal degrees
ELEV_use = ***ELEVATION*** = lake surface elevation (meters above mean sea level)
L_ELEV_use = Log_{10}(***ELEV_use***)
LkArea_km2 = LAKEAREA = lake surface area (km^2)
L_LkAreakm2 = Log_{10}(***LkArea_km2***)
Lake_Origin_use = LAKE_ORIGIN (with values: 'NATURAL' or 'MAN-MADE')
Reservoir = an indicator variable of Lake Origin, where
If ***Lake_Origin_use*** = 'MAN-MADE' then ***Reservoir***=1;
If ***Lake_Origin_use*** = 'NATURAL' then ***Reservoir***=0;

Field human disturbance variables:
RDis_IX ---- index of near-shore human disturbance intensity and extent (see TSD text equation 5)
hiiAg ------- proximity-weighted mean tally of up to 3 near-shore agricultural disturbances (mean among stations).

NAP Expected PHab Reference Condition Models:

L_RVegQc3x15 = 2.34593-(0.03705****LATdd_use***)+(0.01723****LONdd_use***)-(0.07954****Reservoir***)
-(0.31865****RDis_IX***);
Note: *Reservoir* = 0 for natural lakes, 1 for man-made reservoirs.
Rsq=0.2331 RMSE=0.16177 p<.0001 n=166/170;
Sites: All non-overlapping 2007-2012 NAP RT_NLA12=R or S;
Set *RDis_IX* to zero (14% of 2007-&12 NAP sample sites have RDis_IX=0);
RVegQc3x15=10**(***L_RVegQc3x15***)-0.01;
Applied simple dirty models for LitCvr and LitRipCvr (see powerpoint file of regressions 6/13/14) that better define the influence of lake area --- but then MUST include RDis_IX, because it is the strongest predictor of any of the 3 PHab indices if RT_NLA12_2015 S or T sites are included with reference (R) sites;

Adjustment for reference distribution of O/E values:
L_RVegQc3OE15= +0.04276 - (0.29150 ***RDis_IX***);
Rsq= 0.2026 RMSE=0.14469 p<0.0001 n=166/170;
Sites: All non-overlapping 2007-2012 NAP RT_NLA12=R or S;

Ref O/E distribution based on Y-intercept of adjustment regression, but SD of ref sites only (not S sites)

L_LitCvrQc3x15= -0.8598 -(0.08109****L_LkAreakm2***) - (0.28562****RDis_IX***);

Rsq=0.1228 RMSE=0.2808 p<0.0001 n=166/170;
Set *RDis_IX* to zero (14% of 2007-2012 NAP sample sites have RDis_IX=0);
Sites: All non-overlapping 2007-2012 NAP RT_NLA12_2015=R or S;
LitCvrQc3x15=10**(***L_LitCvrQc3x15***)-0.01;

Adjustment for reference distribution of O/E values:
L_LitCvrQc3OE15= +0.04665 - (0.28240 ***RDis_IX***);
Rsq= 0.0592 RMSE=0.26819 p=0.0009 n=166/170;
Sites: All non-overlapping 2007-2012 NAP RT_NLA12=R or S;

Ref O/E distribution based on Y-intercept of adjustment regression, but SD of ref sites only (not S sites)

L_LitRipCvrQc3x15= 2.41606-(0.03964****LATdd_use***)+(0.01798****LONdd_use***) -(0.08301* ***Reservoir***)
-(0.34039****RDis_IX***);
Note: *Reservoir* = 0 for natural lakes, 1 for man-made reservoirs.
Rsq=0.2407 RMSE=0.16783 p<0.0001 n=166/170;
Set *RDis_IX* to zero (14% of 2007-2012 NAP sample sites have RDis_IX=0);
Sites: All non-overlapping 2007-2012 NAP RT_NLA12_2015=R or S;
LitRipCvrQc3x15=10**(***L_LitRipCvrQc3x15***)-0.01;

Adjustment for reference distribution of O/E values:
L_LitRipCvrQc3OE15= +0.04230 - (0.31323 ***RDis_IX***);
Rsq= 0.2075 RMSE=0.15095 p<0.0001 n=166/170;
Sites: All non-overlapping 2007-2012 NAP RT_NLA12=R or S;
Ref O/E distribution based on Y-intercept of adjustment regression, but SD of ref sites only (not S sites).

SAP -- Expected PHab Condition Models:

L_RVegQc3x15= 0.24710 +(0.01012****LONdd_use***);

Rsq=0.1637 RMSE=0.11878 p=0.0240 n=31/31 ;
Sites: All non-ovelapping 2007-2012 SAP RT_NLA12_2015=R;
RVegQc3x15=10**(***L_RVegQc3x15***)-0.01;

Ref O/E distribution based on mean and SD of ref sites.

L_LitCvrQc3x15= -0.66613 -(0.00000410****ElevXLon_use***) -(0.51350****RDis_IX***);

Rsq=0.1942 RMSE=0.26697 p=0.0487 n=31/31;
Set *RDis_IX* to zero (2% of 2007-2012 SAP sample sites have RDis_IX=0);
Sites: All non-overlapping 2007-2012 SAP RT_NLA12_2015=R;
LitCvrQc3x15=10**(***L_LitCvrQc3x15***)-0.01;

Adjustment for reference distribution of O/E values:
L_LitCvrQc3OE15= +0.04287 - (0.46211 ***RDis_IX***);
Rsq= 0.0790 RMSE=0.24397 p=0.1255 n=31/31;
Sites: All non-overlapping 2007-2012 SAP RT_NLA12=R;

Ref O/E distribution based on Y-intercept and RMSE of adjustment regression.

L_LitRipCvrQc3x15=1.92708 -(0.000115130****ElevXLon_use***) + (0.03141****LONdd_use***) - (0.00923****ELEV_use***);

Rsq=0.3083 RMSE=0.14817 p=0.0175 n=31/31;
Sites: All non-overlapping 2007-2012 SAP RT_NLA12_2015=R;
LitRipCvrQc3x15=10**(***L_LitRipCvrQc3x15***)-0.01;

Ref O/E distribution based on mean and SD of ref sites.

CPL Expected PHab Condition Models:

RVegQc3x15=0.35438 -0.00003019(***ElevXLat_use***) - 0.15193(***RDis_IX***);

Rsq= 0.3868 RMSE=0.08963 p<0.0001 n=28/28;
Sites: All non-overlapping 2007-2012 CPL RT_NLA12_2015=R;
Set *RDis_IX* to lowest value in the region (4.4% have RDis_IX=0 in CPL);

Adjustment for reference distribution of O/E values:
L_RVegQc3OE15= -0.0006653 - (0.22746 ***RDis_IX***);
Rsq= 0.0235 RMSE=0.21279 p=0.4362 n=28/28;
Sites: All non-overlapping 2007-2012 CPL RT_NLA12=R;
Note: Regression keeping one low outlier with very little leverage;

Ref O/E distribution based on Y-intercept and RMSE of adjustment regression.

LitCvrQc3x15= 0.71804 - (0.19300****L_Elev_use***) - (0.12565****RDis_IX***);

Rsq= 0.2526 RMSE=0.17393 p<0.0001 n=28/28;
Sites: All non-overlapping 2007-2012 CPL RT_NLA12_2015=R;
Set *RDis_IX* to lowest value in the region (0 in CPL);

Adjustment for reference distribution of O/E values:
L_LitCvrQc3OE15= -0.00743 - (0.09579 ***RDis_IX***);
Rsq= 0.0051 RMSE=0.1940 p=0.7178 n=28/28;
Sites: All non-overlapping 2007-2012 CPL RT_NLA12=R;

Ref O/E distribution based on Y-intercept and RMSE of adjustment regression.

LitRipCvrQc3x15= 0.59561 - (0.15322****L_Elev_use***) – (0.14358* ***RDis_IX***);

Rsq= 0.4423 RMSE=0.09293 p<0.0001 n=28/28;
Sites: All norepeat 2007-2012 CPL RT_NLA12_2015=R;
Set *RDis_IX* to lowest value in the region (0 in CPL);

Adjustment for reference distribution of O/E values:
L_LitRipCvrQc3OE15= 0.01615 - (0.15265 ***RDis_IX***);
Rsq= 0.0312 RMSE=0.1234 p=0.3685 n=28/28;
Sites: All non-overlapping 2007-2012 CPL RT_NLA12=R;

Ref O/E distribution based on Y-intercept and RMSE of adjustment regression.

UMW Expected PHab Condition Models:

L_RVegQc3x15= -0.61298;

****Dropped LON and LkArea -- USED geometric (Log mean) NULL MODEL;
Rsq=0 RMSE=0.15333 n=49/50 ;
Sites: All non-overlapping 2007-2012 UMW RT_NLA12_2015=R;
RVegQc3x15=10**(***L_RVegQc3x15***)-0.01;

Ref O/E distribution based on mean and SD of ref sites.

L_LitCvrQc3x15= -0.87559;

****Dropped survey year -- USED geometric (Log mean) NULL MODEL;
Rsq=0 RMSE=0.19944 p=N/A n=49/50;
Sites: All non-overlapping 2007-2012 UMW RT_NLA12_2015=R;
LitCvrQc3x15=10**(***L_LitCvrQc3x15***)-0.01;

Ref O/E distribution based on mean and SD of ref sites.

L_LitRipCvrQc3x15=-0.70830;

***** Dropped Lake Area -- USED geometric (Log mean) NULL MODEL;
Rsq=0 RMSE=0.11487 p=N/A n=49/50;
Sites: All non-overlapping 2007-2012 UMW RT_NLA12_2015=R;
LitRipCvrQc3x15=10**(***L_LitRipCvrQc3x15***)-0.01;
LitCvrQc3x15=10**(***L_LitCvrQc3x15***)-0.01;

Ref O/E distribution based on mean and SD of ref sites.

<u>CENPL (NPL + SPL + TPL) Expected PHab Condition Models</u>:

L_RVegQc3x15=-0.75460- (0.0.86385****hiiAg***);
Rsq=0.1532 RMSE=0.3178 p<0.0009 n=69/71;
Sites: All non-overlapping 2007-2012 CENPL_2015 RT_NLA12_2015=R, Excluding KS-R02 SD-101 (Oahi Res) which has inadequate no of transects, but Includes Mound City res KS-R02 with corrected Elevation;
Set *hiiAg* to lowest value in the region (0)

Note: 2007-2012 NLA sites in CENPL with hiiAg=0 in NPL(>25%) SPL(>50%) TPL(75%)

RVegQc3x15=10**(***L_RVegQc3x15***)-0.01;

<u>Adjustment for reference distribution of O/E values</u>:
L_RVegQc3OE15= 0.04688 - (0.80799 ***hiiAg***);
Rsq= 0.1571 RMSE=0.29278 p=0.0007 n=69/71;

Ref O/E distribution based on Y-intercept and RMSE of adjustment regression.

L_LitCvrQc3x15= -1.03378 + 0.10822****Reservoir*** -(0.38197****hiiAg***);
Note: *Reservoir* = 0 for natural lakes, 1 for man-made reservoirs.
Rsq=0.0855 RMSE= 0.27579 p<0.0572 n=69/71;
Sites: All non-overlapping 2007-2012 CENPL_2015 RT_NLA12_2015=R
Set *hiiAg* to lowest value in the region (0)

Note: 2007-2012 NLA sites in CENPL with hiiAg=0 in NPL(>25%) SPL(>50%) TPL(75%)

LitCvrQc3x15=10**(***L_LitCvrQc3x15***)-0.01;

<u>Adjustment for reference distribution of O/E values</u>:
L_LitCvrQc3OE15= 0.02752 - (0.35038 ***hiiAg***);
Rsq= 0.0359 RMSE=0.28386 p=0.1255 n=69/71;

Ref O/E distribution based on Y-intercept and RMSE of adjustment regression.

L_LitRipCvrQc3x15=-0.82455-(0.61960****hiiAg***);
Rsq=0.1471 RMSE=0.23336 p=0.0011 n=69/71;
Sites: All non-overlapping 2007-2012 CENPL_2015 RT_NLA12_2015=R
Set *hiiAg* to lowest value in the region (0)

Note: 2007-2012 NLA sites in CENPL with hiiAg=0 in NPL(>25%) SPL(>50%) TPL(75%)
LitRipCvrQc3x15=10**(***L_LitRipCvrQc3x15***)-0.01;

<u>Adjustment for reference distribution of O/E values</u>:
L_LitRipCvrQc3OE15= 0.04303 - (0.59485 ***hiiAg***);
Rsq= 0.1465 RMSE=0.22462 p=0.0012 n=69/71;

Ref O/E distribution based on Y-intercept and RMSE of adjustment regression.

**** Note: If remove sites East of approximately -95 degrees LON that removes all *hiiAg* so association with LON is largely assoc with hiiAg -- adopted conservative model without LON. See dirty models for all three indices with hiiAg alone (prk 3/13/15 SAS EnterpriseGuide projects) for all three of the above, they all have higher Rsq, similar RMSE, similar intercepts, similar slopes p<0.0001 n= 669/694 to 673/694.

WMT Expected PHab Condition Models:

L_RVegQc3x15= 0.53572-(0.00008953****ELEV_use***)-(0.25957****Reservoir***)+(0.07296****L_LkAreakm2***)
-(0.01939****LATdd_use***);
Note: *Reservoir* = 0 for natural lakes, 1 for man-made reservoirs.
Rsq=0.2825 RMSE=0.16743 p=0.0001 n=74/75;
Sites: All non-overlapping 2007-2012 WMT RT_NLA12_2015=R;
RVegQc3x15=10**(***L_RVegQc3x15***)-0.01;

Ref O/E distribution based on mean and SD of ref sites.

L_LitCvrQc3x15= -1.10550-(0.00004299****ELEV_use***)-(0.05083****L_LkAreakm2***)+(0.00407****LATdd_use***)
-(0.18384****Reservoir***);
Note: *Reservoir* = 0 for natural lakes, 1 for man-made reservoirs.
Rsq=0.1555 RMSE=0.24373 p=.0187 n=74/75;
Sites: All non-overlapping 2007-2012 WMT RT_NLA12_2015=R;
LitCvrQc3x15=10**(***L_LitCvrQc3x15***)-0.01;

Ref O/E distribution based on mean and SD of ref sites.

L_LitRipCvrQc3x15= -0.08802-(0.00006666***ELEV_use**)+(0.04200****L_LkAreakm2***)-(0.01015****LATdd_use***)-
(0.22650****Reservoir***);
Note: *Reservoir* = 0 for natural lakes, 1 for man-made reservoirs.
Rsq=0.2922 RMSE=0.14513 p<.0001 n=74/75;
Sites: All no-repeat 2007-2012 WMT RT_NLA12_2015=R;
LitRipCvrQc3x15=10**(***L_LitRipCvrQc3x15***)-0.01;

Ref O/E distribution based on mean and SD of ref sites.

<u>XER Expected PHab Condition Models</u>:

L_RVegQc3x15= 0.44708 -(0.02612 ****LATdd_use***) -(0.00013249****ELEV_use***) ;

Rsq=0.2365 RMSE=0.28355 p=0.1009 n=20/21 ;
Sites: All no-repeat 2007-2012 XER RT_NLA12_2015=R;
RVegQc3x15=10**(***L_RVegQc3x15***)-0.01;

Ref O/E distribution based on mean and SD of ref sites.

L_LitCvrQc3x15=0.08706-(0.02849****LATdd_use***)-(0.00003932****ELEV_use***) ;

Rsq=0.1578 RMSE=0.29004 p=0.2322 n=20/21;
Sites: All no-repeat 2007-2012 XER RT_NLA12_2015=R;
*** Note this was 8th best in All Subsets Regression models with <=2 predictors ranked by Cp;
*** Note this was 6th best in All Subsets ranked by Rsq;
*** Consistent model across all the indicators and across full set of sites;
LitCvrQc3x15=10**(***L_LitCvrQc3x15***)-0.01;

Ref O/E distribution based on mean and SD of ref sites.

L_LitRipCvrQc3x15=0.24931 - (0.02529****LATdd_use***)-(0.00010090****ELEV_use***) ;

Rsq=0.2115 RMSE= 0.26455 p=0.1327 n=20/21;
Sites: All no-repeat 2007-2012 XER RT_NLA12_2015=R;
LitRipCvrQc3x15=10**(***L_LitRipCvrQc3x15***)-0.01;

Ref O/E distribution based on mean and SD of ref sites.

NOTE 3/13/15 prk: Reexamined models. The p-values (and of course also r2 and RMSE) not improved by using
single predictors (*ELEV_use LATdd_use* and *ELEVxLatdd_use*). The mechanisms and univariate plots of these single predictors all convincing and support the 3 models above;

Chapter 6: Water Chemistry

6.1 Background information

The NLA report summarizes water quality stressor data collected at the deepest part of each study lake (up to 50 m). Field sampling included a depth profile and a 0-2 m depth integrated water sample. Variables analyzed for the NLA 2012 report include: total nitrogen (TN), total phosphorus (TP), chlorophyll-a (CHLA), turbidity, acidity, and dissolved oxygen. Acidity, dissolved oxygen and trophic state class thresholds were based on established criteria and applied consistently across the nation. Least, moderate, and most disturbed condition classes were established for TP, TN, CHLA, and turbidity using the same percentile of reference sites approach that was used in NLA 2007 (Herlihy and Sifneos, 2013). Thresholds, however, were recalculated to include additional nutrient reference sites sampled in 2012. This more than doubled the number of nutrient reference sites available in each ecoregion allowing for better estimation of the percentiles used to calculate the thresholds. Separate thresholds were established for each of the nine ecoregions reported on in NLA 2012. As a result of threshold refinement 2007 benchmark values were revised; therefore, direct comparisons should not be made between 2012 results and those reported in 2007.

6.2 Threshold development

6.2.1 *Acidity and Dissolved Oxygen*

For setting acidity classes, concentrations of acid neutralizing capacity (ANC) and dissolved organic carbon (DOC) were analyzed following the scheme developed by Herlihy et al. (1991). Sites with acid neutralizing capacity (ANC) > 50 ueq/L were considered to be non-acidic and least disturbed for acidification. Sites with ANC ≤ 50 µeq/L and DOC values ≥ 6 mg/L were classified as naturally acidic due to organic acids. Sites with ANC ≤ 0 µeq/L and DOC values < 6 mg/L were classified as acidic due to either acidic deposition or acid mine drainage and considered most disturbed. Sites with ANC between 0 and 50 µeq/L and DOC < 6 mg/L were considered acid-influenced but not currently acidic. These low ANC sites typically become acidic during high flow events (episodic acidity) and were considered moderately disturbed.

Depth profiles of dissolved oxygen were collected at the deepest of the lake. Surface water dissolved oxygen was calculated by removing all duplicate depth observations and taking the mean of all dissolved oxygen values between 0 and 2 meters depth, inclusive. If the lake was shallower than 2 m depth, the entire depth profile was used. Surface water dissolved oxygen was classified into three classes, least disturbed (≥5 mg/L), moderately disturbed (3-5 mg/L), and most disturbed (≤3 mg/L).

6.2.2 *Trophic State*

Lakes have long been classified according to their trophic state. By the dictionary, "trophic" is defined as of or relating to nutrition. A eutrophic lake has high nutrients and high algal and/or macrophyte plant growth. An oligotrophic lake has low nutrient concentrations and low plant growth. Mesotrophic lakes fall somewhere in between eutrophic and oligotrophic lakes and hypereutrophic lakes have very high nutrients and plant growth. Lake trophic state is typically determined by a wide variety of natural factors that control nutrient supply, climate, and basin morphometry. Trophic state can be defined based on a number of different nutrient or plant biomass variables. For NLA 2012, trophic state was defined using specific numeric criteria for concentrations CHLA (Table 6-1). The same trophic state classification was used for all ecoregions.

Table 6-1. Trophic State Classification used in NLA 2012.

Analyte	Oligotrophic	Mesotrophic	Eutrophic	Hypereutrophic
Chlorophyll-a (μg/L)	≤2	>2 and ≤7	>7 and ≤30	>30

6.2.3 *Total nitrogen, total phosphorus, chlorophyll-a, and turbidity*

TN, TP, CHLA, and turbidity were classified into least, moderate, or most, disturbed condition classes based on percentiles of the nutrient reference site distribution (Herlihy and Sifneos, 2008, 2013). See Section 3.4 for more information on selecting reference sites for nutrients. Once the nutrient reference lakes were selected, nutrient levels for separating least disturbed, moderately disturbed, and most disturbed were determine from the distribution of reference lake nutrient concentrations from each ecoregion (and for the Southern Plains for natural and manmade lakes separately). Nutrient levels were determined for both total phosphorus (TP) and total nitrogen (TN). The cutoff between least disturbed and moderately disturbed lakes was set at the 75^{th} percentile (Q3) of reference lakes, and the cutoff between moderately disturbed and most disturbed lakes was set at the 95^{th} percentile (P95) of reference lakes. If a nutrient ecoregion had < 20 lakes, then the cutoff between the moderately disturbed and most disturbed lakes was the maximum nutrient concentration (P95 = maximum) for reference lakes in that nutrient ecoregion.

In addition to developing thresholds for nutrients, we determined thresholds from population percentiles in the reference lakes in each of the nutrient ecoregion for chlorophyll-*a* and turbidity. Like the nutrient thresholds, these percentile-based thresholds were used to determine least disturbed, moderately disturbed, and most disturbed lake conditions for the NLA. With the cutoff between least disturbed and moderately disturbed lakes set at the 75^{th} percentile (Q3), and the cutoff between the moderately disturbed and most disturbed lakes set at 95^{th} percentile (P95).

There was a very large difference in the absolute concentrations of TP and TN among ecoregions in the nutrient reference sites (Figure 6-1 and Figure 6-2). Looking at the data, it is also evident why the natural lakes in the SPL need their own threshold versus man-made SPL lakes. Table 6-2 reports the 75th and 95th percentile-based thresholds used to define the least, moderately, and most, disturbed condition classes for TP, TN, CHLA, and turbidity for each of the ecoregions.

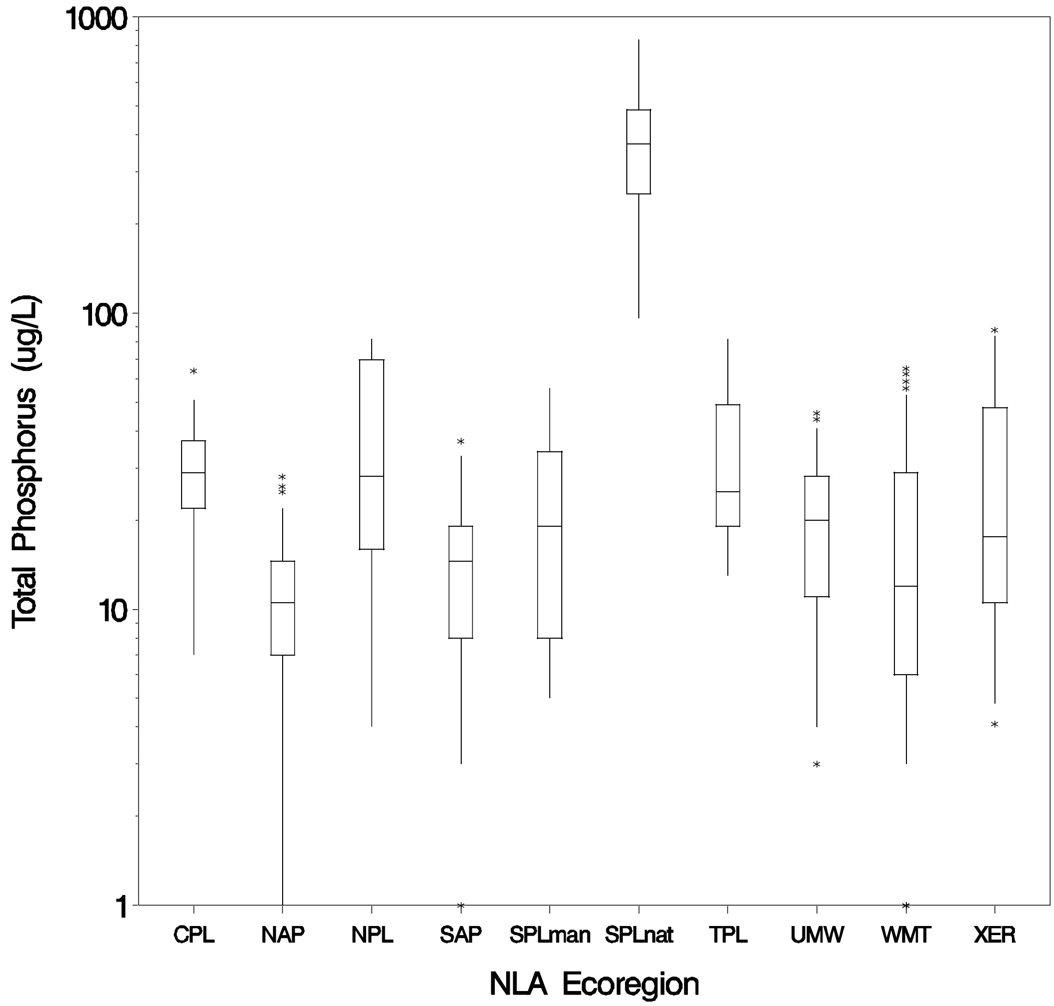


Figure 6-1. Box and whisker plot of Total Phosphorus in GIS screened, outlier removed, reference sites by ecoregion. . Boxes are interquartile range, whiskers are 5th/95th percentiles.

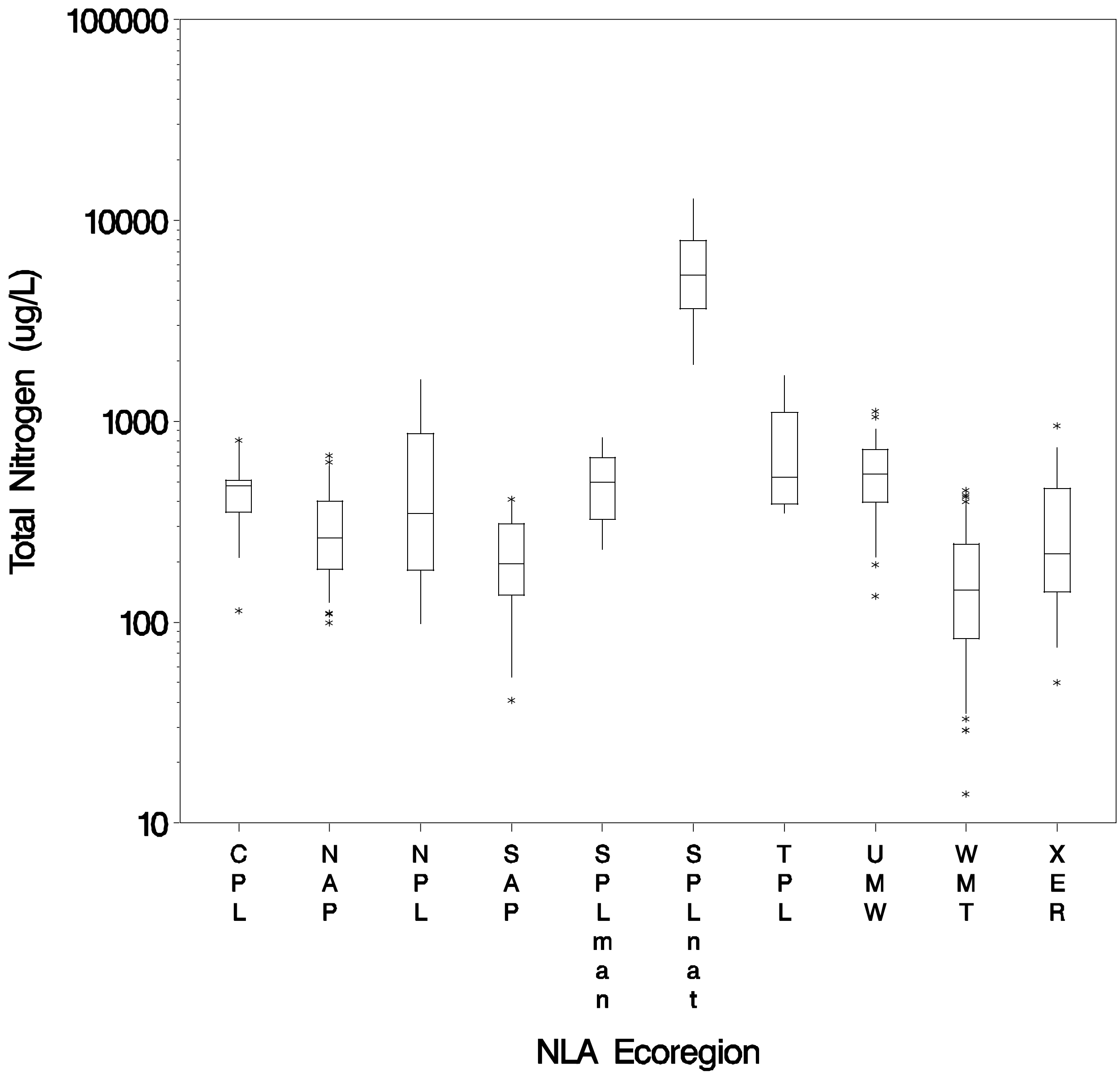


Figure 6-2. Box and whisker plot of Total Nitrogen in GIS screened, outlier removed, reference sites by ecoregion. Boxes are interquartile range, whiskers are 5th/95th percentiles.

Table 6-2. NLA2012 least, moderately, and most disturbed thresholds (75th/95th percentiles) for TP, TN, CHLA, and turbidity condition classes.

Ecoregion	TP (µg/L) 75th Least-moderately	TP (µg/L) 95th Moderately-Most	TN (µg/L) 75th Least-moderately	TN (µg/L) 95th Moderately-Most
CPL	37.0	51.0	510	801
NAP	14.5	22.0	400	600
NPL	69.5	82.0	866	1,620
SAP	19.0	33.0	309	407
SPL-manmade	34.0	56.0	657	830
SPL-natural	486	839	7,925	12,875
TPL	49.0	82.0	1,105	1,699
UMW	28.0	41.0	722	920
WMT	29.0	53.0	245	380
XER	48.0	84.0	465	746

Ecoregion	CHLA (µg/L) 75th Least-moderately	CHLA (µg/L) 95th Moderately-Most	Turbidity (NTU) 75th Least-moderately	Turbidity (NTU) 95th Moderately-Most
CPL	11.5	28.0	3.38	4.05
NAP	3.81	7.76	1.10	1.46
NPL	8.53	13.0	3.19	4.46
SAP	5.23	11.5	2.83	3.94
SPL-manmade	6.85	13.8	3.32	4.67
SPL-natural	118.4	218.7	73.5	172.0
TPL	13.9	22.7	3.70	5.38
UMW	6.70	9.60	2.13	2.89
WMT	1.83	3.04	0.760	1.43
XER	6.65	12.2	2.97	4.84

6.3 Literature cited

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Herlihy, A. T., N. C. Kamman, J. C. Sifneos, D. Charles, M. D. Enache, and R. J. Stevenson. 2013. Using multiple approaches to develop nutrient criteria for lakes in the conterminous USA. Freshwater Science **32**:367-384. doi: 10.1899/11-097.

Chapter 7: Zooplankton

7.1 Background information

Zooplankton assemblages have several attributes that make them potentially useful for assessing the ecological condition of lakes (Stemberger and Lazorchak 1994, Jeppesen et al. 2011). Zooplankton are typically the dominant pelagic consumer in lakes (in terms of both biomass and numbers (Larsen and Christie 1993). Taxa richness tends to be high in nearly all lakes. Zooplankton species or guild structure can respond to abiotic stressors such as eutrophication and acidification, and possibly climate change. Zooplankton occupy an intermediate level in the overall food web of lakes, and thus can respond to stress responses from within lower (e.g., phytoplankton) or higher trophic levels (e.g., fish). Zooplankton taxa demonstrate a range of life history strategies and patterns (e.g., parthenogenesis, resting eggs) that can be related to environmental stress, both natural and anthropogenic.

The use of zooplankton assemblages in the context of bioassessment appears to be limited, with many studies focused mainly on taxa richness and taxonomic composition changes in response to disturbance. Gannon and Stemberger (1978) discussed the potential of using zooplankton communities to help determine trophic state in lakes, primarily through the use of "indicator species" that were associated with either oligotrophic or eutrophic conditions. Sprules and Holtby (1979) and Sprules (1980) examined the utility of using metrics related to body size and feeding ecology of zooplankton to evaluate lake condition. Duggan et al. (2001, 2002) investigated the potential for developing bioindicators of trophic state using rotifer assemblages. Dodson et al. (2005) concluded that zooplankton assemblages are indirectly associated with land use through effects on riparian vegetation and lake characteristics such as typology and water chemistry. Dodson et al. (2009) examined changes in zooplankton community structure within a set of lakes in northern Wisconsin in relation to a variety of within-lake and watershed level characteristics (including human disturbance in the riparian zone). Stemberger and Lazorchak (1994) calculated 14 metrics based on taxonomy, body size, life history stage, and trophic guild in 19 lakes in the northeastern USA representing a gradient of human disturbance, lake type, and land use. Stemberger and Miller (1998) discussed expected changes in zooplankton assemblage trophic structure and species composition in response to changes in the N:P ratio that might result from increased anthropogenic disturbance.

More recently, there have been attempts to develop indices of biotic condition in lakes using plankton assemblages, following two approaches. The multimetric approach pioneered by Karr (e.g., Karr 1981, Karr 1991) has been implemented successfully for other assemblages (e.g., fish, benthic invertebrates) in streams. Kane et al. (2009) combined zooplankton and phytoplankton metrics from Lake Erie into a single multimetric index (MMI), the Planktonic Index of Biotic Integrity, to reflect the response of the plankton to eutrophication. The second approach (predictive model approach) compares the observed taxa collected at each site to the list of taxa expected at that site under least disturbed conditions by means of an Observed/Expected index (O/E, e.g., Wright 1995, Hawkins et al. 2000, Hawkins 2006, Hawkins et al. 2010). The predictive modelling approach has been used successfully for other assemblages, principally benthic invertebrates, but also fish, in streams. The National Lake Assessment 2007 (NLA 2007) used an O/E model that combined zooplankton and phytoplankton assemblages to assess ecological condition of lakes in the conterminous US (Yuan et al. 2008, USEPA 2009). Table 7-1 summarizes current knowledge regarding the hypothesized responses of zooplankton assemblages to different types of disturbance.

For the NLA 2012, we decided to develop a MMI for pelagic zooplankton assemblages to assess biological condition in lakes. We followed the approach described by Stoddard et al. (2008) to screen candidate metrics for possible inclusion in an MMI. We then computed a large number of MMIs based on all possible combinations of the metrics that passed the screening process, following Van Sickle (2010), and selected the MMI that showed the best combination of responsiveness to disturbance, repeatability, and low redundancy among component metrics.

7.2 Methods

7.2.1 *Field Methods*

Sample collection procedures for zooplankton are described in the NLA 2012 field operations manual (USEPA 2012a). Field crews collected two samples at the index site (deepest area of a lake or the midpoint of a reservoir) of each lake. The crew collected a “Coarse” sample (ZOCN) using a 1-m long, 30-cm diameter plankton net having a mesh size of 150 µm. The crew collected a “Fine” sample (ZOFN) using a 1-m long net with a reducing collar (20-cm diameter) with a mesh size of 50 µm. The total tow length for each net was 5 m, with the number of tows being dependent on the site depth. At lakes deeper than 6 m, a single 5 m tow was done. At lakes between 3 and 6 m deep, two 2.5-m tows were done. At lakes shallower than 3 m, five 1-m tows were done. Results from pilot studies suggested that a total tow length of 5 m would provide sufficient numbers of taxa and organisms to develop the MMI from nearly all lakes.

Table 7-1. Hypothesized-responses of zooplankton assemblages to disturbance

Assemblage component or metric	Type of disturbance	Hypothesized response	References
Species richness	Nutrients; Agricultural land use; riparian buffer presence	Decrease	Gannon and Stemberger (1978), Dodson et al. (2005)
Native species richness, abundance, or biomass	Invasive species	Decrease	Kane et al. (2009)
Large-sized species richness (e.g., Daphnia spp., calanoid copepods)	Nutrients, land use	Decrease	Stemberger and Lazorchak (1994)
Small-sized species richness (e.g., Ceriodaphnia, rotifers)	Nutrients, land use	Increase	Stemberger and Lazorchak (1994)
Proportion of calanoid copepod taxa	Nutrients	Decrease	Jeppesen et al. (2000), Du et al. (2015)
Proportion of cyclopoid copepod taxa	Nutrients	Increase	Jeppesen et al. (2000), Du et al. (2015)
Rotifer assemblage composition	Nutrients, chlorophyll a, Secchi transparency, temperature, dissolved oxygen	Change	Duggan et al. (2001), (2002)
Mean size	Nutrients	Decrease	Gannon and Stemberger (1978)
Total biomass	Nutrients	Increase	Gannon and Stemberger (1978)
Ratio of calanoid copepods to (cyclopoid copepods + cladocerans)	Nutrients	Decrease	Gannon and Stemberger (1978), Kane et al. (2009)_ENREF_11
Biomass of rotifers and cyclopoid copepods	Nutrients (total P)	Increase	Du et al. (2015)
Biomass of cladocerans and cyclopoid copepods	Nutrients (total P)	Decrease	Du et al. (2015)
Biomass of small cladocerans	Catchment development	increase	Gélinas and Pinel-Alloul (2008), Beaver et al. (2014)

Assemblage component or metric	Type of disturbance	Hypothesized response	References
Proportion of cladoceran biomass	Nutrients	Decrease	Jeppesen et al. (2000), Du et al. (2015)
Abundance of large-bodied zooplankton	Decrease in acid neutralization capacity/calcium concentrations	Decrease	Tessier and Horwitz (1990)
Abundance of small daphnids and cladocerans	Catchment development	Increase	Gélinas and Pinel-Alloul (2008), Dodson et al. (2009), Van Egeren et al. (2011), Beaver et al. (2014)
Relative abundance of calanoid copepods	Nutrients	Decrease	Brooks (1969), Gannon and Stemberger (1978)
Relative abundance of cyclopoid copepods and small-bodied cladocerans	Nutrients	Increase	Brooks (1969), Attayde and Bozelli (1998)
Omnivorous taxa richness, abundance, or biomass	Nutrients	Increase	Stemberger and Lazorchak (1994), Stemberger et al. (2001)

7.2.2 *Laboratory Methods*

Laboratory methods for zooplankton samples are described in the NLA 2012 laboratory operations manual (USEPA 2012b). For both the ZOCN and ZOFN samples, the objective was to subsample a sufficient volume to enumerate and identify at least 400 individuals. In the ZOCN samples, all taxa were enumerated. In the ZOFN samples, only “small” taxa were enumerated (Cladocera < 0.2 mm long, copepods < 0.6 mm long, rotifers, and nauplii). Veligers were not enumerated in the ZOFN sample. Individuals were identified to species where possible. A “Large/Rare” search of the entire subsample was done to identify larger taxa (e.g., *Chaoborus*, *Leptodora*, Mysidae, Ostracoda, and Hydracarina). Only the presence of these taxa in the subsample was noted (i.e., they were not enumerated).

Besides the number of individuals enumerated in the subsample (abundance), we estimated the volume of water sampled by the tow using the tow length and the radius of the net mouth for the sample. We used this tow volume to estimate density (no. individuals/L) of each taxon:

$$Density = \frac{\left(\dfrac{Sample\,Vol.\,(mL)}{Vol.\,Counted\ (mL)} \times Abundance\right)}{Tow\,Vol.\,(L)}$$

The biomass (mg dry mass/L) of each taxon in a sample was estimated by measuring the length of 20 individuals (if possible). Length was converted to a biomass factor (mg dry mass/individual) using proprietary equations developed by the laboratory that processed the majority of the zooplankton samples. Biomass was then calculated as:

$$Biomass = Density\ (Indiv./L) \times Biomass\ Factor\ \ (mg/Indiv.)$$

One state laboratory did not estimate biomass for their samples. For these samples, we estimated biomass as the mean biomass of a taxon from samples collected from surrounding states, or used a national mean (all samples collected that included the taxon) if the regional sample size was too small.

7.3 Data Preparation

7.3.1 *Data Quality Assurance*

We reviewed field data to correct recording errors and, when possible, to fill in missing values, especially for critical variable like tow length. We reviewed the raw count files from each laboratory to correct spelling errors in taxon names, and to make the taxonomy consistent across laboratories (using the national lab taxonomy as the standard for all labs). We used range checks on count, density, and biomass estimates to identify outliers, and corrected them if they were due to recording errors.

7.3.2 *Master Taxa List*

We developed a master taxa list that included all taxa identified in the ZOFN and ZOCN samples. The master taxa list included taxonomic information (e.g., phylum, class, order, suborder, family, subfamily, genus, species, and subspecies). Autecological information for each taxon included feeding guild (Predator, Omnivore, or Herbivore), Cladocera size class (LARGE vs. SMALL), based on data from Stemberger and Lazorchak (1994) and the Northeastern Lakes Survey (Whittier et al. 2002), and a size class variable (NET_SZECLS_NEW) based on whether a taxon was collected in the ZOCN samples vs. only in the ZOFN samples. Additional attributes for a limited number of taxa that are included in the list but were not used include trophic assignments from Sprules and Holtby (1979), and some trait information from Barnett et al. (2007, 2013).

The laboratory identified 535 unique taxa in the NLA 2012 ZOCN and ZOFN samples (variable=TAXANAME). We combined some of these unique taxa using a different variable (TARGET_TAXON), which resulted in 481 unique taxon names as used in metric calculations.

We also had some information regarding non-native zooplankton taxa based on the USGS Nonindigenous Aquatic Species (NAS) database (Fuller and Neilson 2015). *Bosmina coregoni* (or *Eubosmina coregoni*), *Daphnia lumholtzi*, and *Sinocalanus doerri* were considered to be introduced to North America. *Eutymora affinis* was considered to be introduced to inland waters of the US. *Pseudodiaptomus forbesi* has been introduced into San Francisco Bay, and so we considered it to be non-native if collected from nearby lakes. *Arctodiaptomus dorsalis* has been introduced into lakes in Arizona, Hawaii, and Indiana.

7.3.3 *Aggregations and Rarefaction of Count Data*

We aggregated some values of TARGET_TAXON within a given ZOCN or ZOCN sample. We combined copepodites and nauplii with adults of the same taxon if both were present in a sample. If a species and a lower level taxon (i.e., subspecies, variety, or form) were both present in a single sample, we aggregated the count data to the species level.

After aggregating at the sample level, we combined the results for each ZOCN and ZOFN sample to create a separate site-level count file. We assumed that individuals collected in the ZOCN samples that were also present in the ZOFN sample represented smaller individuals that passed through the coarse-mesh net, and so we added the counts from the two samples together.

Because not all zooplankton individuals in a sample can be confidently identified to species, there is a risk of overestimating taxa richness. For each sample, we reviewed the list of taxa to determine whether they were represented at more than one level of resolution. For example, if a "*Daphnia* sp." was collected, and it was the only representative of the genus in the sample (or at the site), we assigned it as distinct. If any other members of the genus were collected, then we considered the unknown as not distinct. We used only the number of distinct taxa in the sample to calculate any metrics based on species richness. We calculated distinct taxa for both the sample-level aggregated count file and the site-level count file. Taxa that were identified (but not enumerated) during the Large/Rare search were included in calculating richness metrics.

We created an additional count file to use for metric calculation by subjecting the sample-level aggregated count data to a rarefaction procedure to randomly select 300 individuals per sample (for those samples that had > 300 individuals enumerated and identified). We repeated the sample level aggregation of taxa on the 300-count file, thus the resultant site-level count file typically had a total count of 600 individuals. We did not calculate density on the 300-count files, but did calculate biomass.

7.4 Zooplankton MMI Development

7.4.1 *Regionalization*

We divided the conterminous US into five “bio-regions” based on nine aggregated Omernik Level III ecoregions (Omernik 1987, Stoddard 2004, Herlihy et al. 2008, Omernik and Griffith 2014) that were developed for use on NARS reporting (Figure 7-1). We combined the Northern and Southern Appalachian regions (NAP, SAP) into a single bio region (Eastern Highlands, EHIGH). We combined the three “plains” regions (Northern, Southern, and Temperate [NPL, SPL, and TPL]) into a single bio-region (PLAINS). In the western US, we combined the Xeric and Western Mountains regions (XER, WMT) into a single “Western Mountains” bio-region (WMTNS). Despite relatively small sample sizes of least disturbed sites, we kept the Coastal Plain (CPL) and Upper Midwest (UMW) as separate bio-regions.

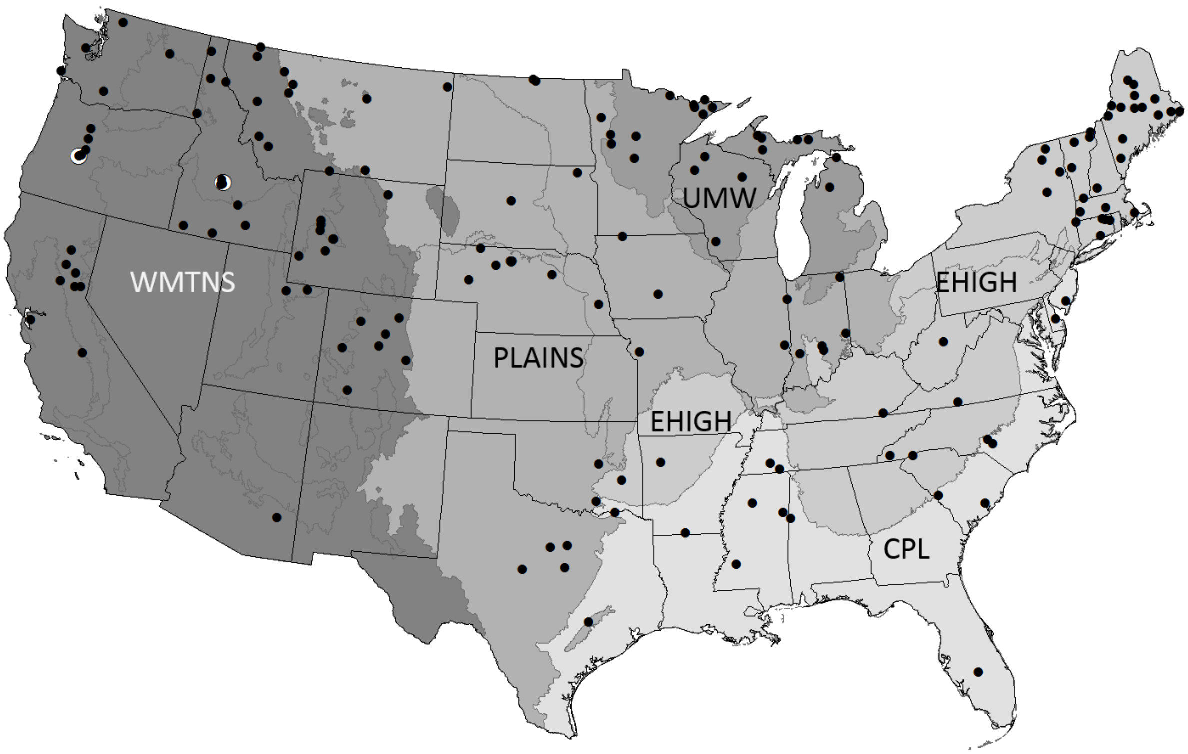

Figure 7-1. Five aggregated bio-regions used to develop zooplankton MMIs for the 2012 National Lake Assessment (CPL=Coastal Plains; EHIGH=Eastern Highlands, PLAINS= Plains, UMW=Upper Midwest, and WMTNS=Western Mountains). Solid dots indicate least disturbed sites used for developing the zooplankton MMI. White circles indicate least disturbed sites that we excluded because of atypical samples (too few taxa or number of individuals collected).

7.4.2 *Least and Most Disturbed Sites*

For the zooplankton MMI, we used the same list of sites as those selected for benthic macroinvertebrates (RT_NLA12; see Section 3.3). We identified two least disturbed sites that appeared to have abnormal zooplankton samples. The ZOCN sample collected from McDonald Lake, ID (NLA12ID-142) did not have any individuals in the ZOCN sample, and < 100 individuals enumerated from the ZOFN sample. For Waldo Lake, OR (NLA12_OR-109), only 6 individuals were collected in the ZOCN sample, and 53 individuals were collected in the ZOFN sample. We created a new variable (RT_ZOOP) to use for zooplankton, and these two sites were assigned a value of “B” for RT_ZOOP.

7.4.3 *Least Disturbed Sites: Calibration versus Validation*

As an independent check on the MMI developed for each bio-region, we set aside a small number of least disturbed sites as "validation" and did not include them in any MMI or metric evaluations or performance testing. We used revisit sites (typically VISIT_NO=2) as validation sites because they are not used in any metric or MMI testing. We then supplemented the list of revisit sites in each region by randomly selecting sites from the list of least disturbed sites. Where possible, we withheld ~10% of the least disturbed sites in each bio-region as validation sites, leaving at least 15 least disturbed sites available for developing and evaluating metrics and MMIs. For the CPL and UMW bio-regions, the small number of least disturbed sites prevented setting aside 10% of the site for validation. Numbers of validation sites were as follows: CPL (8), EHIGH (16), PLAINS (14), UMW (10), and WMTNS (18).

7.4.4 *Candidate Metrics*

We used the count data file and the master taxa list file to calculate candidate metrics. We assigned candidate metrics to one of six metric categories, with each category reflecting a different attribute of assemblage structure or ecological function.

The *Abundance* category included metrics based on abundance, density, or biomass. We calculated these metrics separately for the ZOFN samples, the ZOCN samples, and for the combined samples. Within the combined sample, we also calculated abundance metrics separately for the net-based size classes (COARSE vs. FINE).

The *Richness* category included metrics based on taxa richness and metrics related to taxa diversity or dominance. Richness metrics included total distinct taxa richness, number of genera, and number of families. We calculated these metrics separately for the ZOCN, ZOFN, and combined sample. We calculated diversity and dominance metrics for the combined sample based on abundance, density, and biomass. Diversity metrics included Shannon-Weiner and Simpson indices, and Hurlbert's Probability of Interspecific Encounter (PIE, Hurlbert 1971, Jeppesen et al. 2000). We developed dominance metrics for the most dominant taxon and for the three and five most dominant taxa in each sample.

We assigned separate categories for each of the three principal taxonomic components of the zooplankton assemblage: *Cladoceran, Copepod,* and *Rotifer.* Metrics in these three categories included abundance and richness metrics calculated separately for each taxonomic group. For copepods, we also calculate the ratio of calanoids to the sum of cladocerans and cyclopoids, following Gannon and Stemberger (1978) and Kane et al. (2009).

The sixth metric category was trophic guild. We identified three major guilds, herbivores, omnivores, and predators. Each taxon was assigned to a trophic guild based on information from the Northeast Lakes Survey (Stemberger and Lazorchak 1994, Stemberger et al. 2001).

We calculated metrics using both the entire sample and for the 300-count rarefied samples. Metrics derived from the rarefied sample have "300" in the variable name.

For many metrics, we could calculate six different variants: the number of distinct taxa (*metric*_NTAX), total biomass (*metric*_BIO), density (*metric*_DEN), percent of individuals (*metric*_PIND), percent of total biomass (*metric*_PBIO) and percent of total density (*metric*_PDEN). We did not calculate density-based metrics for the 300-count rarefied samples. Each variant was calculated based using all the individuals in the sample, and for just the native individuals in the sample. We calculated a total of 374 candidate metrics for the whole sample count data, and an additional 272 metrics from the 300-count rarefied sample data.

7.4.5 *Final Metric Selection*

We subjected all of the candidate metrics to five screening procedures, following Stoddard et al. (2008). The first was a range test. We excluded richness metrics (*metric*_NTAX) with a range of <4 from further consideration. We excluded metrics based on biomass (*metric*_BIO), density (*metric*_DEN), diversity metrics, and zooplankton ratio if the 90th percentile (P_{90}) was 0. We excluded percentage metrics (*metric*_PTAX, *metric*_PBIO, *metric*_PDEN) if the 75th percentile (P_{75}) was <10%.

The second screen was a signal to noise (S:N) test, following Kaufmann et al. (1999). We compared the total variance observed across all sites (signal) against the variance observed for sites that were sampled twice in the same index period (noise). We excluded metrics that had S:N values < 1.25.

The third screen was for responsiveness to disturbance. For each metric, we calculated the t-statistic for each metric comparing values for the set of least disturbed sites with those for the set of most disturbed sites. We considered metrics having $|t|$ values < 1.73 as non-responsive to disturbance.

The fourth screen was to determine if metrics required adjustment for lake size. We generated plots of linear regressions of each metric with lake area (AREA_HA) to determine if the metric response changed with increasing lake size. For all metrics, the upper 95% prediction interval at the minimum response value overlapped the lower 95% prediction interval at the maximum response value, indicating there was no significant effect of lake size on the metric response.

For each bio-region, we used the set of candidate metrics that had passed the four screens describe above to develop candidate MMIs. We constrained the MMIs to contain at least one metric from each of the six metric categories (abundance, richness, crustacean, copepod, rotifer, and trophic). If no metrics within a category passed all of the screens, we selected one or more metrics that had the highest *t* values and had S:N values near 1 (if possible). Values of S:N ≤ 1 indicate that that variation within a site is equal to or greater than the variation among sites, so the metric cannot discriminate among sites.

Finally, we evaluated the redundancy among candidate metrics using correlation analysis. Historically, we have evaluated redundancy based on the establishing a maximum allowable correlation coefficient (*r*) between two metrics (e.g., r >0.7; Stoddard et al. 2008). Van Sickle (2010) demonstrated that MMIs containing a suite of metrics that have a low average correlation among them perform better that simply using a maximum threshold value of *r* to reduce redundancy within the suite of metrics. We included correlations in the procedure below, computing correlations among metrics for each candidate MMI, rather that evaluating individual input metrics within a category and choosing only non-redundant metrics to include in a final MMI, as described by Stoddard et al. (2008).

Candidate metrics that we considered for inclusion into an MMI for each of the five bio-regions are listed in Section 7.10. For each bio-region, we computed MMIs from all possible combinations of candidate metrics from the six categories. We evaluated each MMI for responsiveness (*t* test of least disturbed vs. most disturbed sites) and repeatability (S:N). For each bio-region, we selected MMI that had a combination of high *t* value, a reasonable value for S:N, low mean *r* among the suite of metrics, and, when possible, a maximum value of *r* for the suite of metrics that was <0.7.

7.4.6 *Metric Scoring*

We followed the approach described by Stoddard et al. (2008) to transform metric responses into a metric score that ranged between 0 and 10 (Blocksom 2003). For positive metrics (i.e., *t* ≥0), we used the 5th percentile of all sites in the bio-region as the "floor" value, and the 95th percentile of the set of least disturbed sites as the "ceiling" value. For negative metrics (i.e., *t* <0), we used the 5th percentile of least disturbed sites in the bio-region as the "floor" value, and the 95th percentile of all sites as the "ceiling" value. When metric response values were less than the floor value, we assigned a score of 0. When metric response values were greater than the ceiling, we assigned a score of 10. We estimated scores for response values that were between the floor and ceiling values by linear interpolation.

We calculated the final MMI score for each bio-region by summing the six component metric scores, and then multiplying by 100/6. This resulted in an MMI score that ranged between 0 and 100.

7.5 Zooplankton MMI Metric Composition and Performance

7.5.1 *Coastal Plain MMI*

The component metrics for the Coastal Plain MMI are presented in Table 7-2. Information related to the performance of the Coastal Plain MMI are presented in Section 7.6. Figure 7-2 compares the distributions of the six metrics in least disturbed vs. most disturbed sites. Three metrics are "negative" metrics (*t* <0) values, indicating that the response is greater in most disturbed sites compared to least disturbed sites. No abundance or cladoceran metrics passed both the responsiveness and repeatability screens. The abundance metric (FINE_BIO [biomass of smaller-sized taxa]) had a *t* value and an S:N value that were just below the screening criterion. The cladoceran metric (SIDID_PIND [percent of individuals of the cladoceran family Sididae]) had an S:N value that was below the screening criterion.

The abundance metric (FINE_BIO), the cladoceran metric (SIDID_PIND), the richness metric (FAM300_NAT_NTAX), and the trophic metric (OMNI_PTAX) responded to disturbance as expected (Figure 7-2; Table 7-1). The copepod metric (DOM1_300_COPE_PBIO) and the rotifer metric (COLLO_PBIO) decreased in response to disturbance (Figure 7-2). Declines in the proportion of total biomass contributed by either dominant copepods or a subgroup of rotifers might be expected if the total richness, abundance, and total biomass of cyclopoid copepods and rotifers increased with disturbance (Table 7-1).

Table 7-2. Component metrics of the zooplankton MMI for the Coastal Plain bio-region.Evaluations for responsiveness (*t*-value) and signal:noise (S:N) based on index visits and do not include least disturbed “validation” sites. Negative values for *t* indicate response is greater in most disturbed sites vs. least disturbed sites. Metrics having values marked with an asterisk were among the best performing metric of that category, but failed one or more evaluation screens. Floor and ceiling values are used to derive a score for the metric. See Section 7.10 for metric descriptions.

Metric Type	Metric Variable Name (floor, ceiling)	*t* value	S:N (bio-region)
Abundance/Size	FINE_BIO (2.913623, 173.279784)	-1.67*	1.2*
Cladoceran	SIDID_PIND (0, 24.88)	-1.80	0.5*
Copepod	DOM1_300_COPE_PBIO (45.90, 100)	+1.82	1.9
Richness/Diversity	FAM300_NAT_NTAX (5, 15)	+2.66	1.8
Rotifer	COLLO_PBIO (0, 5.90)	+1.85	7.6
Trophic	OMNI_PTAX (10.53, 47.06)	-3.35	4.3

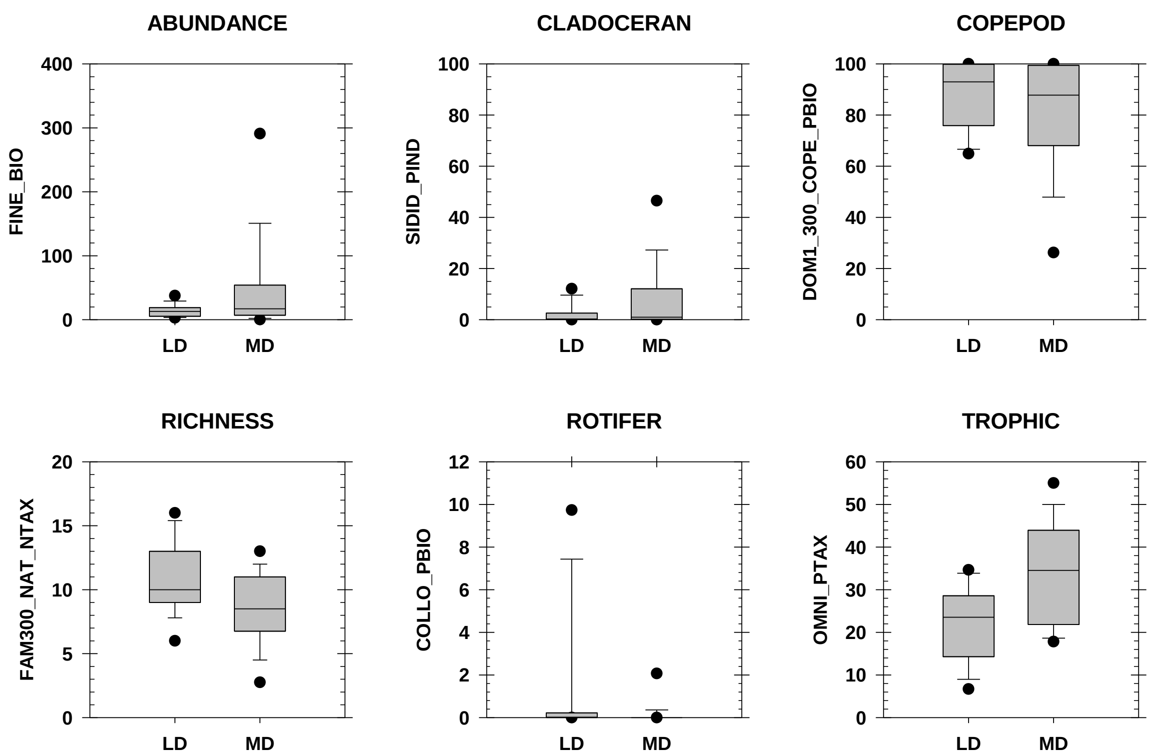

Figure 7-2. Distribution of six component metrics of the zooplankton MMI for the Coastal Plain bio-region in least disturbed versus most disturbed sites. Dots indicate the 5th and 95th percentiles.

7.5.2 *Eastern Highlands MMI*

The component metrics for the Eastern Highlands MMI are presented in Table 7-3. Information related to the performance of the Eastern Highlands MMI are presented in Section 7.6. Figure 7-3 compares the distributions of the six metrics in least disturbed vs. most disturbed sites. The suite of metrics includes both positive (2) and negative (4) metrics. No richness metrics passed the screens for responsiveness or repeatability. The richness metric (ZOCN300_FAM_NTAX) had a *t* value (1.64) just below the screening criterion, while the S:N value (0.3) was well below the screening criterion.

The cladoceran metric (SMCLAD_PBIO), the richness metric (COARSE_NAT_PTAX), the rotifer metric (ROT_PBIO), and the trophic metric (OMNI300_PTAX) responded as expected to increased disturbance (Figure 7-3; Table 7-1). The abundance metric (ZOCN_DEN) and the copepod metric (COPE_NAT_DEN) both increased in response to disturbance (Figure 7-3). An increase in cyclopoid copepods expected with increased disturbance (Table 7-1) would help to explain the observed response in both of these metrics.

7.5.3 *Plains MMI*

The component metrics for the Plains MMI are presented in Table 7-4. Information related to the performance of the Plains MMI are presented in Section 7.6. Figure 7-4 compares the distributions of the six metrics in least disturbed vs. most disturbed sites. The MMI was comprised of two negative and four positive metrics. All metrics passed the screening criteria for both responsiveness and repeatability.

The copepod (COPE_RATIO_300_BIO), richness (FAM300_NAT_TAX), and the trophic (COPE_HERB_PDEN) metrics responded as expected to increased disturbance (Figure 7-4; Table 7-1). The abundance (FINE300_NAT_PBIO), cladoceran (SMCLAD_NAT_PIND), and the rotifer (ROT_NTAX) metrics all decreased with response to increased disturbance. If herbivorous cyclopoid copepods are becoming more dominant in terms of richness, abundance, and biomass, that may result in a decline in the relative biomass of individuals collected in the fine-mesh net (principally rotifers), a decline in the relative abundance of smaller cladocerans, and a decline in rotifer taxa richness.

Table 7-3. Component metrics of the zooplankton MMI for the Eastern Highland bio-region. Evaluations for responsiveness (*t*-value) and signal:noise (S:N) based on index visits and do not include least disturbed "validation" sites. Negative values for *t* indicate response is greater in most disturbed sites vs. least disturbed sites. Floor and ceiling values are used to derive a score for the metric. See Section 7.10 for metric descriptions.

Metric Type	Metric Variable Name (floor, ceiling)	*t* value	S:N (bio-region)
Abundance/Size	ZOCN_DEN (0.096200402, 115.2464653)	-1.89	7.1
Cladoceran	SMCLAD_PBIO (0, 51.41)	-2.84	1.4
Copepod	COPE_NAT_DEN (7.5388,385.279)	-1.74	1.5
Richness/Diversity	COARSE_NAT_PTAX (22.22,57.14)	+1.64*	0.3*
Rotifer	ROT_PBIO (1.69, 89.89)	-1.89	1.3
Trophic	OMNI300_PTAX (12.50, 43.75)	-2.60	1.5

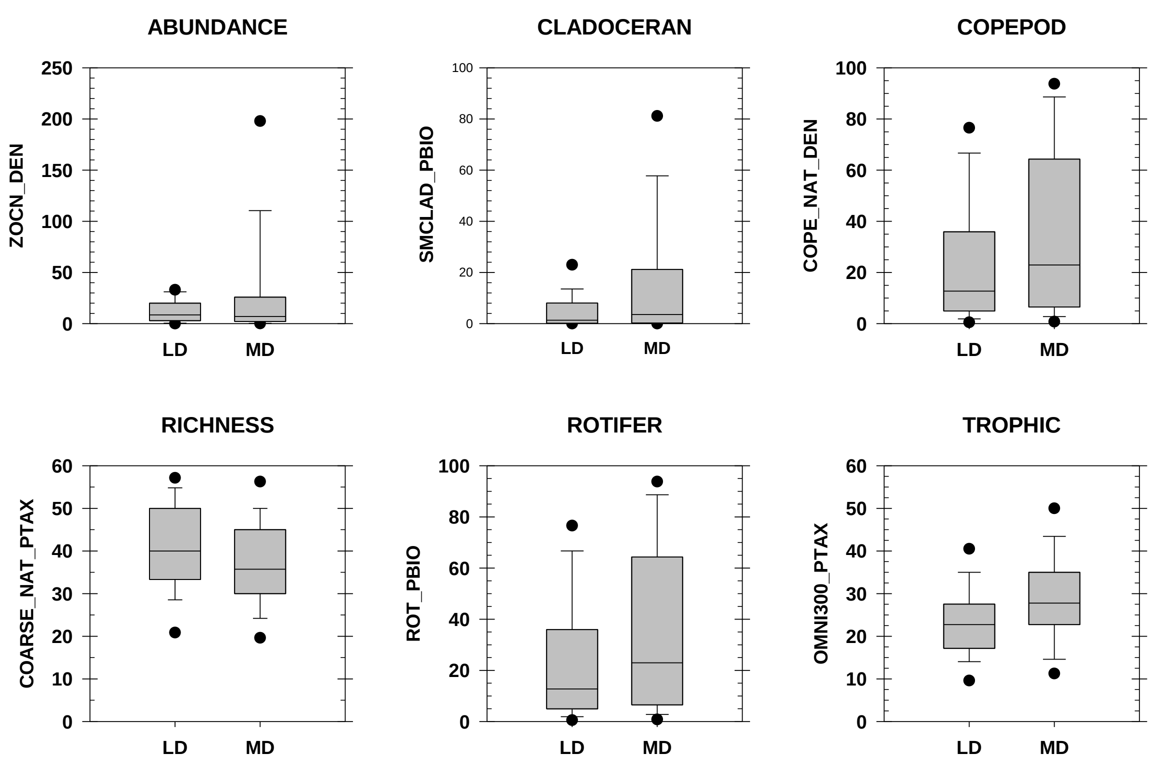

Figure 7-3. Distribution of six component metrics of the zooplankton MMI for the Eastern Highlands bio-region in least disturbed versus most disturbed sites. Dots indicate the 5th and 95th percentiles.

Table 7-4. Component metrics of the zooplankton MMI for the Plains bio-region. Evaluations for responsiveness (*t*-value) and signal:noise (S:N) based on index visits and do not include least disturbed "validation" sites. Negative values for *t* indicate response is greater in most disturbed sites vs. least disturbed sites. Floor and ceiling values are used to derive a score for the metric. See Section 7.10 for metric descriptions.

Metric Type	Metric Variable Name (floor, ceiling)	*t* value	S:N (bio-region)
Abundance/Size	FINE300_NAT_PBIO (0.66, 85.12)	+1.74	6.2
Cladoceran	SMCLAD_NAT_PIND (0, 49.03)	+3.11	1.8
Copepod	COPE_RATIO_300_BIO (0, 62.81)	+2.41	3.0
Richness/Diversity	FAM300_NAT_NTAX (5, 14)	+2.21	2.6
Rotifer	ROT_NTAX (3, 17)	+2.63	1.7
Trophic	COPE_HERB_PDEN (0, 21.07)	-2.13	13.0

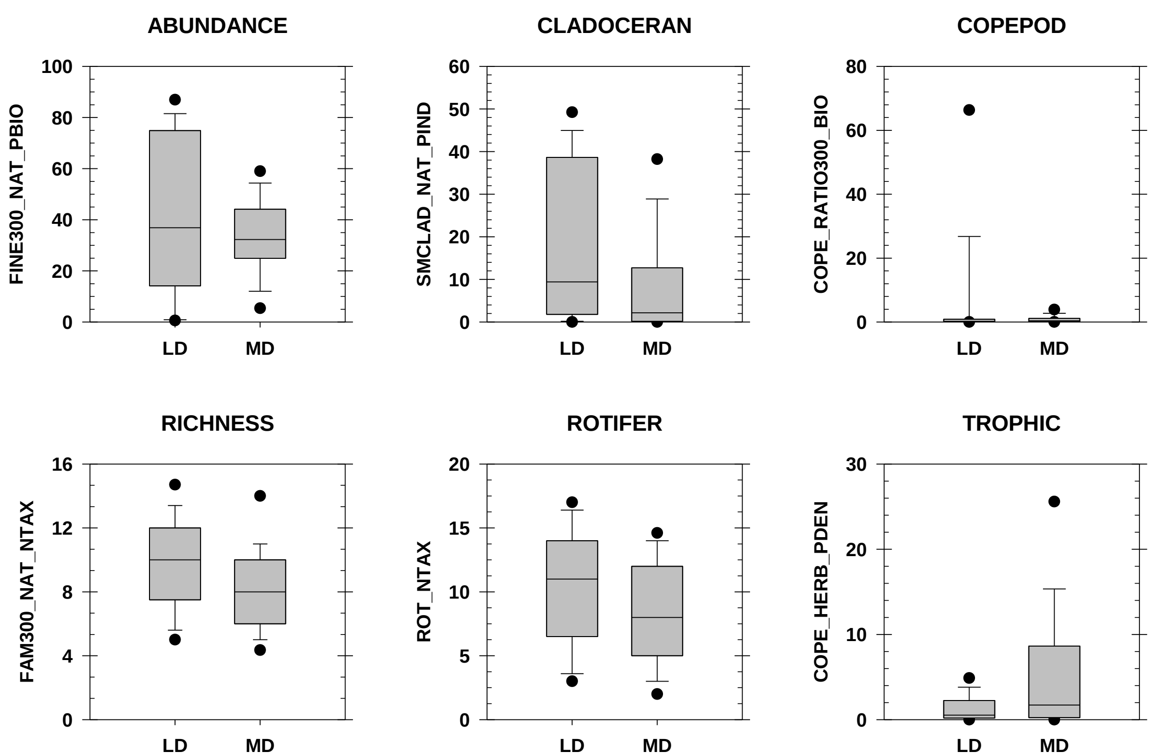

Figure 7-4. Distribution of six component metrics of the zooplankton MMI for the Plains bio-region in least disturbed versus most disturbed sites. Dots indicate the 5th and 95th percentiles.

7.5.4 *Upper Midwest MMI*

The component metrics for the Upper Midwest MMI are presented in Table 7-5. Information related to the performance of the Upper Midwest MMI are presented in Section 7.6. Figure 7-5 compares the distributions of the six metrics in least disturbed vs. most disturbed sites. The MMI is composed of four negative and two positive metrics. No abundance metrics passed the screen for responsiveness. The abundance metric (ZOCN_NAT_PDEN [the percent of total density represented by native individuals in the coarse net sample]) had a *t*-value that is below the screening criteria for responsiveness. Repeatability (S:N values) of the metrics in this bio-region are higher than in other bio-regions, but interpretation of the S:N values is constrained somewhat by a limited number of revisit samples (5).

Only three of the six metrics responded to disturbance as expected (Figure 7-5; Table 7-1). The abundance metric (TOTL_NAT_PIND) showed a slight decrease with disturbance, indicating the effect of non-native taxa in this bio-region. The rotifer metric (DOM1_ROT_PBIO) indicates a reduction in species richness (i.e., increased dominance by one or a few taxa) with increased disturbance. The trophic metric (COPE_HERB300_PBIO) indicates an increase in herbivorous taxa (possibly cyclopoid copepods) with increased disturbance. The cladoceran metric (BOSM300_NAT_PTAX) was expected to increase with increased disturbance, but the response may reflect a larger increase in the taxa richness of other forms of smaller zooplankton (e.g., cyclopoid copepods). The copepod metric (CALAN300_NAT_BIO) indicates an increase in larger forms of zooplankton. Such a response might occur if the least disturbed population of lakes is dominated by oligotrophic lakes that do not support large populations of zooplankton. The richness metric (FINE_PTAX) decreased in response to disturbance. This response may be similar to that observed for the cladoceran metric, where other forms of smaller zooplankton (e.g., cyclopoid copepods) increase in tax richness compared to rotifers, which are the dominant taxa collected in the fine-mesh net.

7.5.5 *Western Mountains MMI*

The component metrics for the Western Mountains MMI are presented in Table 7-6. Information related to the performance of the Western mountains MMI are presented in Section 7.6. Figure 7-6 compares the distributions of the six metrics in least disturbed vs. most disturbed sites. The MMI is composed of three negative and three positive metrics. No richness metrics passed the screen for responsiveness. The richness metric (ZOFN300_NTAX [Number of distinct taxa in the 300-count rarefied sample from the fine net sample]) had a *t* value that was below our acceptance criteria for responsiveness.

The abundance (COARSE300_NAT_PBIO), cladoceran (LGCLAD300_NAT_PTAX), richness (ZOFN300_NTAX), rotifer (PLOIMA_PTAX), and trophic (COPE_OMNI_PTAX) metrics responded as expected to increased disturbance (Figure 7-6, Table 7-1). The copepod metric (COPE300_BIO) would respond as expected to disturbance if the increase in biomass was due primarily to smaller forms (e.g., cyclopoid copepods).

Table 7-5. Component metrics of the zooplankton MMI for the Upper Midwest bio-region. Evaluations for responsiveness (*t*-value) and signal:noise (S:N) based on index visits and do not include least disturbed "validation" sites. Negative values for *t* indicate response is greater in most disturbed sites vs. least disturbed sites. Metrics having values marked with an asterisk were the best performing metric of that category, but failed one or more evaluation screens. Floor and ceiling values are used to derive a score for the metric. See Section 7.10 for metric descriptions.

Metric Type	Metric Variable Name (floor, ceiling)	*t* value	S:N (bio-region)
Abundance/Size	TOTL_NAT_PIND (96.75, 100)	+1.47*	Noise=0
Cladoceran	BOSM300_NAT_PTAX (0, 12.5)	+2.73	1.4
Copepod	CALAN300_NAT_BIO (0, 58.429968)	-2.17	9.2
Richness/Diversity	FINE_PTAX (37.50, 77.78	+1.87	1.4
Rotifer	DOM1_ROT_PBIO (25.03, 93.60)	-2.46	3.5
Trophic	COPE_HERB300_PBIO (0.19, 59.65)	-1.96	5.1

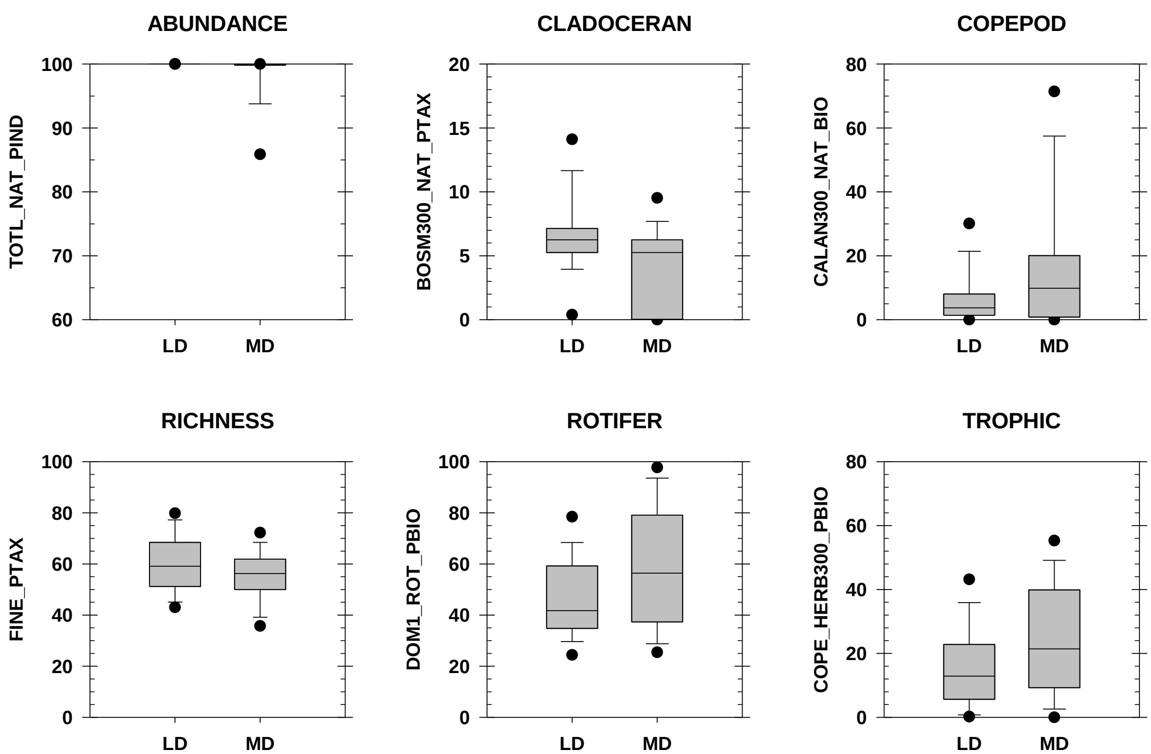

Figure 7-5. Distribution of six component metrics of the zooplankton MMI for the Upper Midwest bio-region in least disturbed versus most disturbed sites. Dots indicate the 5th and 95th percentiles.

Table 7-6. Component metrics of the zooplankton MMI for the Western Mountains bio-region. Evaluations for responsiveness (*t*-value) and signal:noise (S:N) based on index visits and do not include least disturbed "validation" sites. Negative values for *t* indicate response is greater in most disturbed sites vs. least disturbed sites. Metrics having values marked with an asterisk were the best performing metric of that category, but failed one or more evaluation screens. Floor and ceiling values are used to derive a score for the metric. See Section 7.10 for metric descriptions.

Metric Type	Metric Variable Name (floor, ceiling)	*t* value	S:N (bio-region)
Abundance/Size	COARSE300_NAT_PBIO (10.94, 99.26)	+1.88	5.7
Cladoceran	LGCLAD300_NAT_PTAX (0, 30.385)	+2.12	2.3
Copepod	COPE300_BIO (0.074, 150.462701)	-2.76	2.0
Richness/Diversity	ZOFN300_NTAX (3, 15)	-1.69*	1.9
Rotifer	PLOIMA_PTAX (20, 70.835)	+2.28	4.3
Trophic	COPE_OMNI_PTAX (0, 22.22)	-2.52	1.5

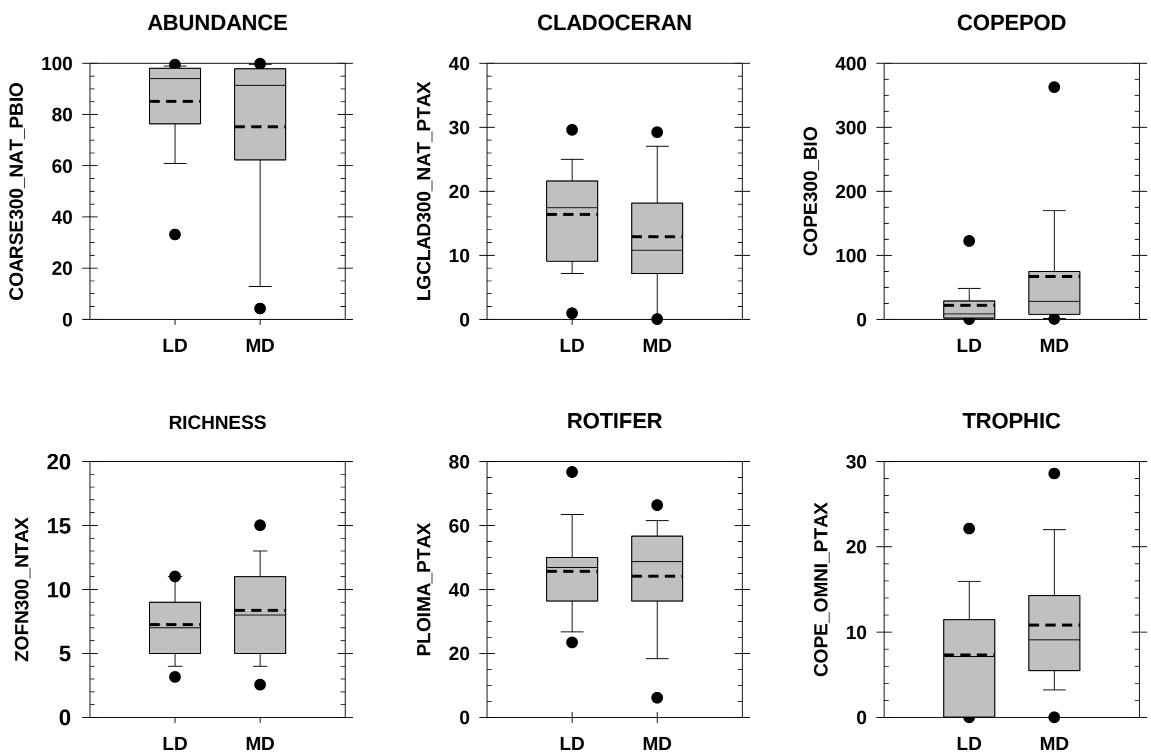

Figure 7-6. Distribution of six component metrics of the zooplankton MMI for the Western Mountains bio-region in least disturbed versus most disturbed sites. Dots indicate the 5th and 95th percentiles.

7.6 Zooplankton MMI Performance

We evaluated each of the five regional MMIs in several ways.

7.6.1 *Calibration versus Validation Sites*

To provide an independent assessment of MMI performance, we compared the distribution of MMI scores between the set of validation sites (which we did not use in MMI development) and the calibration sites using a *t*-test. The null hypothesis was that the mean values of the two groups would be equal. Mean values of the two groups were not significantly different ($p < 0.05$) for any bio-region (Table 7-7). Figure 7-7 shows the distribution of MMI scores between the calibration and validation sites in the five bio-regions.

7.6.2 *Precision of MMIs based on Least Disturbed Sites*

We evaluated the precision of the regional MMIs using the sets of least disturbed calibration sites, following Van Sickle (2010). We rescaled the MMI scores in each bio-region by dividing each site score by the mean MMI score, which resulted in a mean rescaled MMI score of 1. We calculated the standard deviation of the rescaled MMI scores (Table 7-7). The smaller the standard deviation, the more precise the index is, and the better the ability to detect sites that are not in least disturbed condition. Standard deviations were generally small except for the Plains, where site MT-104 had a large influence.

7.6.3 *Responsiveness, Redundancy, and Repeatability of Zooplankton MMIs*

We compared the MMI scores from the set of least disturbed sites to the set of most disturbed sites (excluding the validation sites) using a *t*-test. We calculated the S:N values using the set of revisit sites within each bio-region (again excluding the validation sites). Table 7-8 presents the results of these tests, along with the maximum and average correlations observed for the component metrics. The *t* values for responsiveness are comparable to MMIs developed for other resource types and assemblages (e.g., benthic invertebrates). Figure 7-8 shows the distribution of MMI scores between least- and most disturbed sites in the five bio-regions. Signal:Noise values are comparable to other MMIs that have been developed for other assemblages. The S:N value for the UMW bio-region is constrained by the small number of revisit sites (5) available. When MMI scores from all bio-regions are considered, the national-level estimate of S:N is 6.7.

7.6.4 *Responsiveness to a Generalized Stressor Gradient*

We performed an additional evaluation of the MMIs for responsiveness to disturbance. We performed principal components analysis (PCA) on the set of chemical, physical habitat, and visual assessment stressor variables used to screen for least disturbed and most disturbed sites. Chemical stressor variables included chloride, sulfate, turbidity, and acid neutralizing capacity

(CL, SO4, TURB, and ANC, respectively). Habitat stressor variables (Kaufmann et al. 2014; see Chapter 5 for descriptions and calculations) included shoreline disturbance due to non-agricultural activities (hiiNonAg), shoreline disturbance due to agricultural activities (hiiAg_Syn), and the proportion of shoreline stations with at least one type of disturbance present in either the littoral zone or shoreline plots (hifpAnyCirca_syn). Stressor variables from the visual assessment included the intensity of observed types of agricultural activities (AGR_SCORE), intensity of observed types of residential activities (RES_SCORE), and intensity of observed types of commercial and industrial activities, excluding evidence of fire (IND_NOFIRE). We transformed the chemical variables ($\log_{10}[x+1]$), and standardized all variables to mean=0 and variance=1. The first PCA axis explained 38% of the total variance, and the highest variable loadings were for the chemical and agricultural-related habitat variables. The second PCA axis explained an additional 18% of the total variance, and the highest variable loadings were for the non- agricultural habitat variables and the intensity of residential activities. Linear regression of the MMI score versus the PCA axis 1 scores yielded an r^2 of 0.32 (r=0.56) for PCA axis 1 (Figure 7-9), and 0.006 for PCA axis 2 scores. These results indicate the zooplankton MMI is principally responsive to nutrient conditions resulting from agricultural disturbance, and less responsive to other types of habitat disturbance.

Table 7-7. Results of independent assessment and precision tests of NLA 2012 zooplankton MMIs based on least disturbed sites. None of the *t*-values were significant at $p = 0.05$. Standard deviations were calculated using only calibration sites.

Regional MMI	Calibration vs. Validation Sites (t-value)	Standard Deviation of Standardized MMI scores
Coastal Plains (CPL)	0.73	0.164
Eastern Highlands (EHIGH)	-1.08	0.116
Plains (PLAINS)	1.87	0.332
Upper Midwest (UMW)	0.86	0.115
Western Mountains (WMTNS)	0.49	0.122

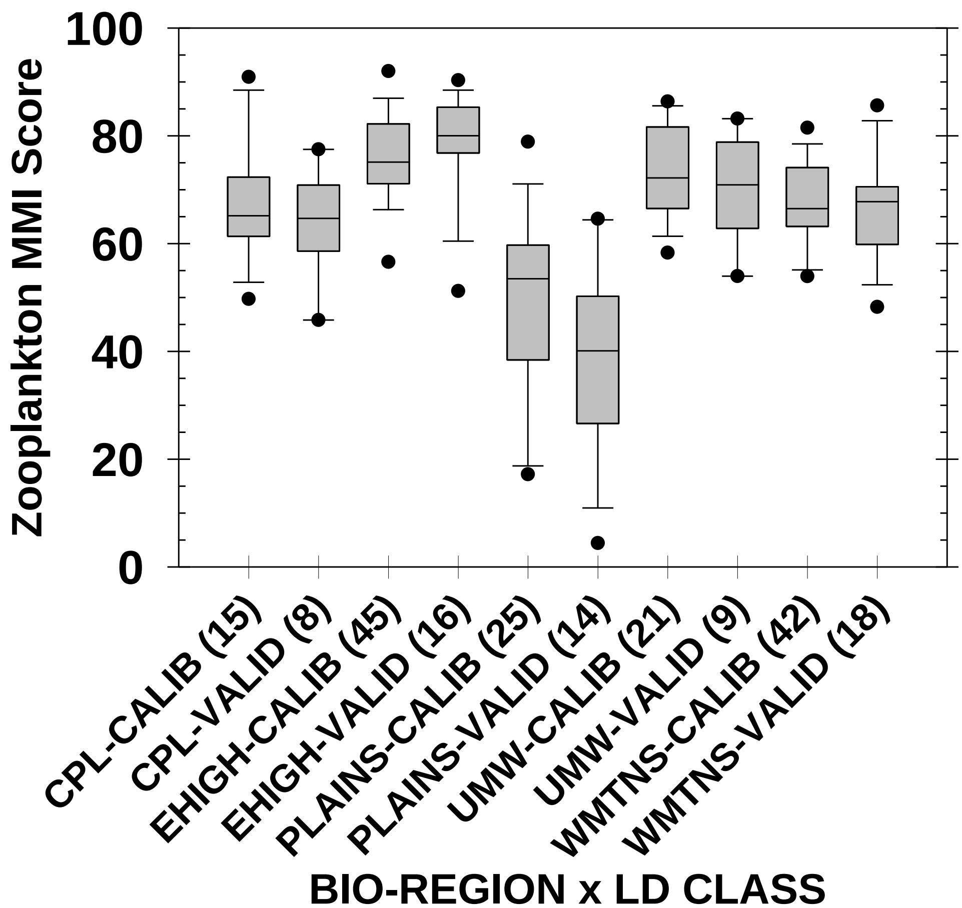

Figure 7-7. Distribution of zooplankton MMI scores in-calibration vs. validation sites for five bio-regions. Sample sizes are in parentheses. Dots indicate the 5th and 95th percentiles.

Table 7-8. Results of responsiveness, redundancy, and repeatability tests for NLA 2012 zooplankton MMIs. Metrics having values marked with an asterisk were the best performing metric of that category, but failed one or more evaluation screens.

Bio-Region	Responsiveness *t*-test of Least disturbed vs. Most disturbed Sites	Redundancy (Maximum pairwise correlation among component metrics)	Redundancy (Mean pairwise correlation among component metrics)	Repeatability Signal: Noise ratio based on revisit sites
Coastal Plains (CPL)	4.68	0.58	0.26	2.7
Eastern Highlands (EHIGH)	5.42	0.48	0.17	2.5
Plains (PLAINS)	4.47	0.72*	0.25	3.6
Upper Midwest (UMW)	5.84	0.48	0.26	19.0
Western Mountains (WMTNS)	6.30	0.63	0.24	3.1

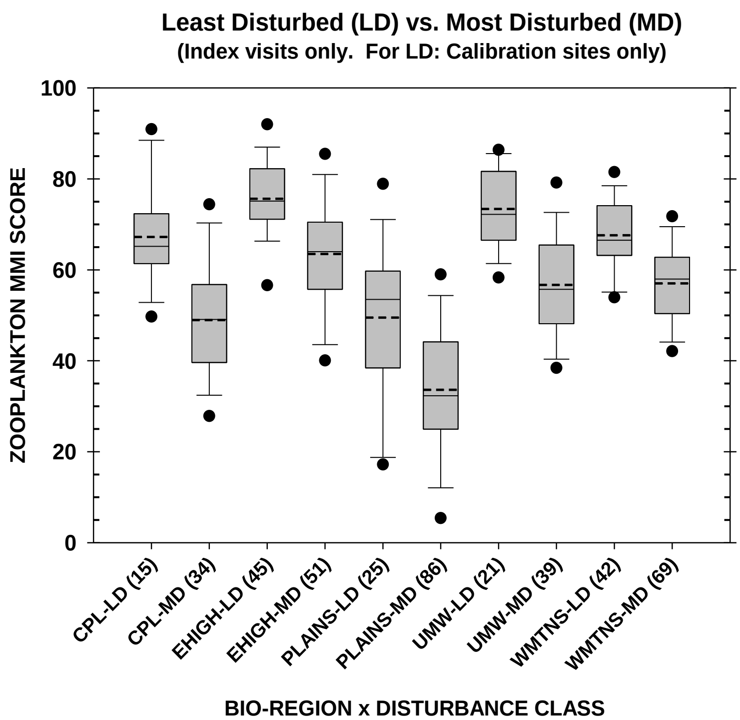

Figure 7-8. Distribution of zooplankton MMI scores in-least- vs. most disturbed sites for five bio-regions. Sample sizes are in parentheses. Dots indicate the 5th and 95th percentiles.

7.6.5 *Effect of Natural Drivers and Tow Length on MMI* Scores

The set of lakes sampled for the 2012 NLA included both natural and man-made lakes, and included a wide range of sizes (as estimated by lake area as represented in NHD). In addition, the sampling protocol did not include a vertical tow through the entire water column. Any one of these factors might produce a bias in the MMI scores that would require assessing ecological condition separately for one or more of these groups of lakes (natural vs. man-made, small vs. large lakes, or shallow versus deeper lakes). We use the set of least disturbed sites (calibration and validation) to evaluate the potential differences in MMI scores in these groups of lakes.

7.6.5.1 *Lake Origin*

We compared the distributions of MMI scores in least disturbed natural lakes vs. man-made reservoirs for each of the five bio-regions (Figure 7-10). The distributions are similar within each bio-region except the WMTNS, where man-made lakes appear to have much lower MMI scores than natural lakes. In the Coastal Plain, man-made lakes have higher MMI values than natural lakes, but interpretation is constrained by the small number of least disturbed natural lakes (n=3). In the WMTNS, the sample size for least disturbed man-made lakes is relatively small (n=16) and is influenced to some extent by the presence of outliers with low MMI scores (Figure 7-10). We did not feel the observed differences were large enough to treat MMI scores from lakes and reservoirs differently in terms of setting thresholds for condition.

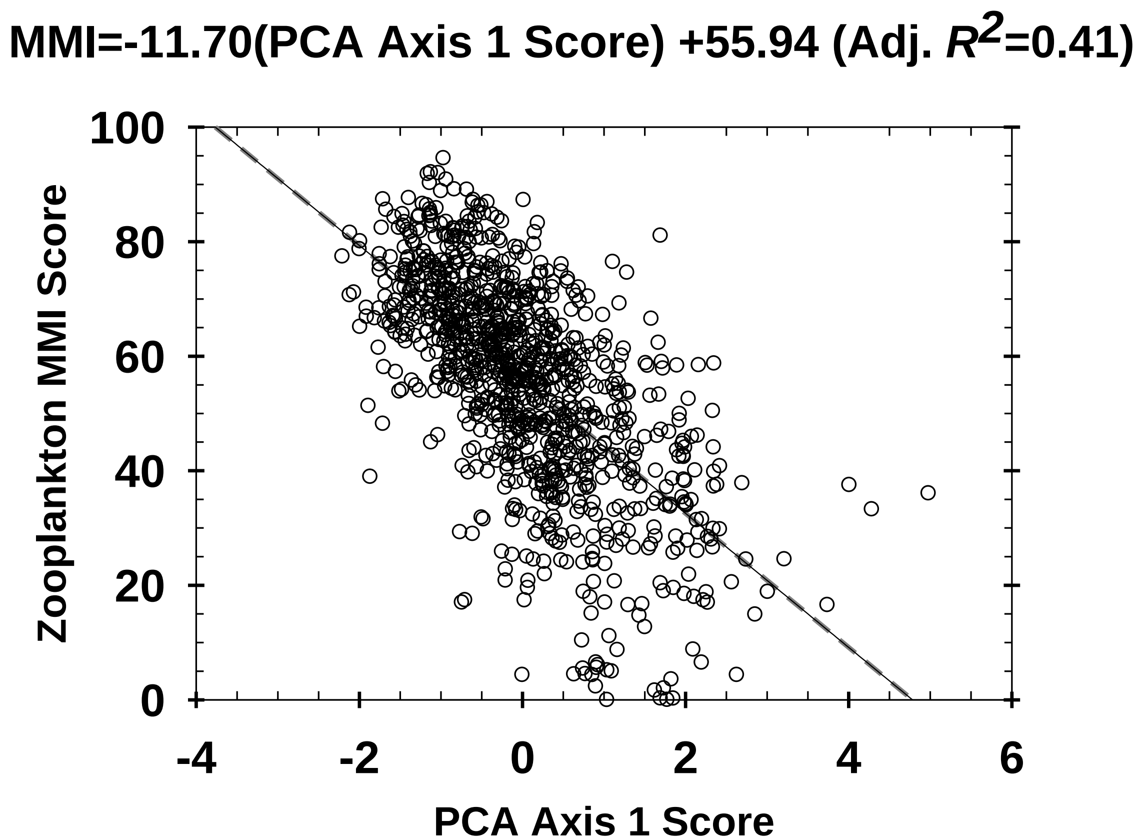

Figure 7-9. Linear regression of NLA 2012 Zooplankton MMI scores vs. first axis score from principal components analysis (PCA) based on chemical, habitat, and visual assessment stressor variables used to screen least- and most disturbed sites.

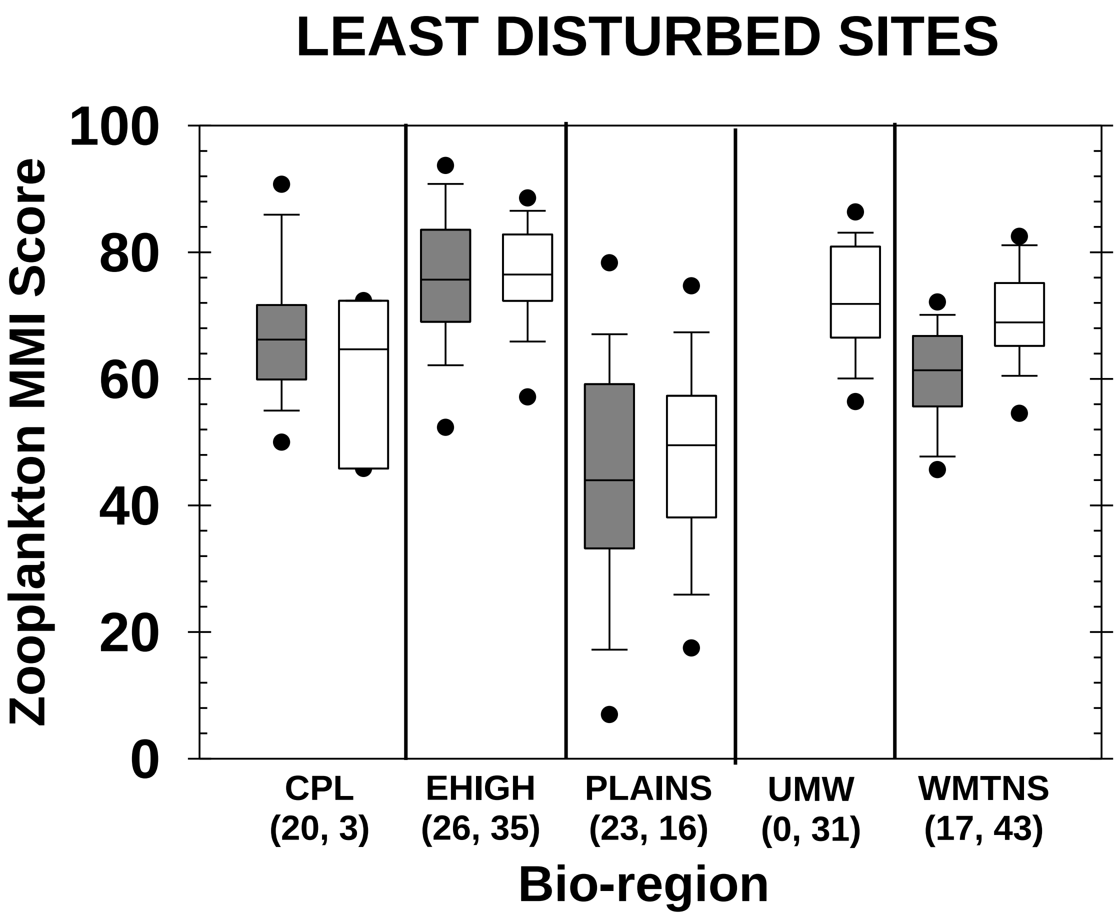

Figure 7-10. NLA 2012 Zooplankton MMI scores of man-made (shaded boxes) versus natural lakes (unshaded boxes) for least disturbed sites in five bio-regions. See Figure 7-1 for bio-region codes. Sample sizes for each type are in parentheses. Dots indicate 5th and 95th percentiles.

7.6.5.2 *Lake Size*

We examined the set of least disturbed sites for evidence of difference in MMI scores due to lake size (Figure 7-11). We noted earlier than we did not have to calibrate individual metrics for lake size (Section 7.4.5). Distributions of MMI scores were similar in median values and ranges for all size classes except for the largest (> 500 ha), which had a similar median but a wider range.

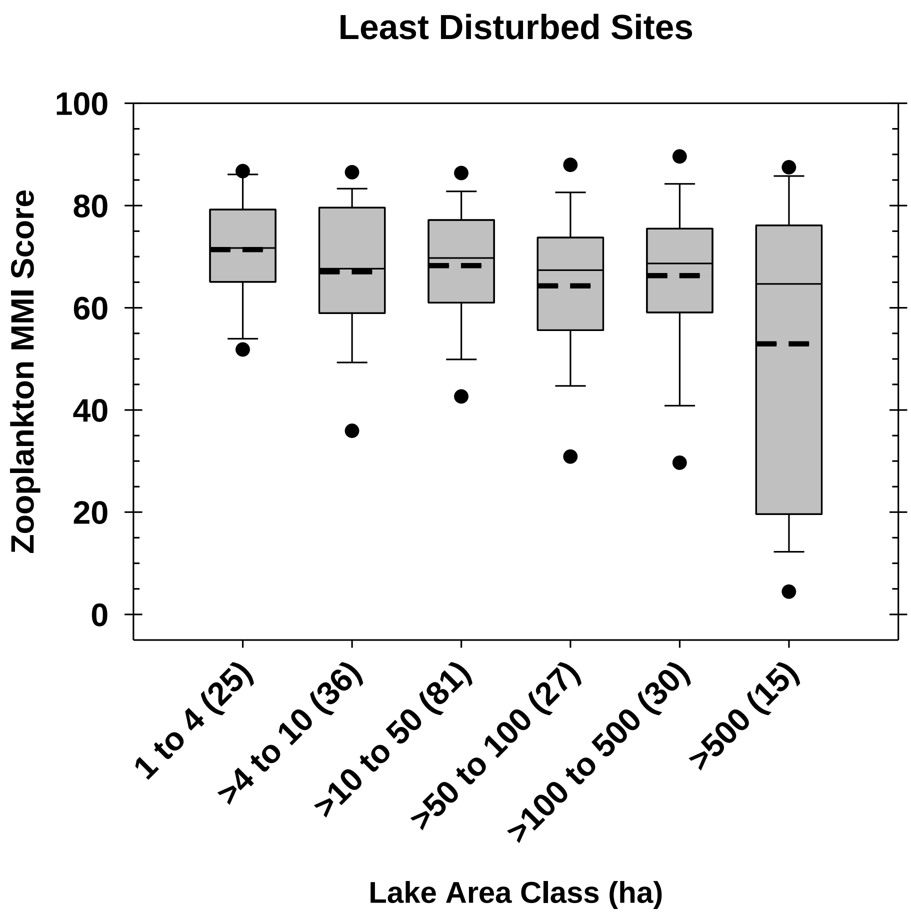

Figure 7-11. Zooplankton MMI scores versus lake size class within least disturbed lakes of the 2012 NLA. Sample sizes are in parentheses. Dashed lines are mean values. Dots indicate the 5th and 95th percentiles.

7.6.5.3 *Site Depth*

We had some concerns that the 5-m tow length used to collect zooplankton samples might be less effective in deeper lakes, where larger taxa may migrate to deeper waters during the day to avoid fish predation, and thus be underrepresented in the samples. We examined MMI scores in least disturbed sites as they related to the depth of the index site where samples were collected (Figure 7-12). There was no apparent pattern in relation to site depth, and the distribution of MMI scores was similar for least--disturbed lakes that were ≤ 6 m deep (the maximum depth where the tow length encompassed the entire water column), and for lakes > 6 m deep (where part of the water column would not be subject to sampling).

7.7 Thresholds for Assigning Ecological Condition

We followed Stoddard et al. (2008) in using the set of least disturbed sites (including calibration and validation sites) to set threshold values to assign ecological condition based on the zooplankton MMI. We used the 25th percentile value to distinguish sites in "good" condition (similar to least disturbed) from sites in "fair" condition (slightly deviant from least disturbed). We used the 5th percentile value to distinguish sites in "fair" condition from sites in "poor" condition (different from least disturbed).

Because of varying quality of least disturbed sites within each bio-region, we adjusted the percentiles using the same process as for the NLA 2012 benthic macroinvertebrate indicator (Herlihy et al. 2008; see Chapter 4). We performed principal components analysis (PCA) based on all variables used in the screening of least disturbed sites (TP, TN, Cl, SO4, Turbidity, physical habitat disturbance indices, and assessment indices). We transformed values ($\log_{10}[x]$ or $\log_{10}[x+1]$) before analysis. Initially, there were 214 least disturbed sites for zooplankton. We performed a linear regression of zooplankton MMI score versus the score for the first principal component. Before calculating thresholds, we performed a 1.5*IQR outlier analysis on the set of least disturbed site MMIs to remove outliers. We excluded three sites based on this test (one each in the CPL EHIGH, and WMTNS), leaving 211 least disturbed sites. Of the 211 least disturbed sites, 9 sites (8 in WMTNS and 1 in PLAINS) were missing data required for the PCA analysis, and so do not have principal component scores (mostly missing turbidity in CA). Thus, there were a total of 202 sites used for the threshold adjustment statistical analysis.

The best regression model had two different slopes and separate intercepts for each bio-region (Table 7-9). The pooled model RMSE was 10.86. We used a pooled RMSE (based on all sites) to provide an adequate sample size for estimating the distribution of MMI scores about the intercept value for each bio-region. The regression models for the CPL, EHIGH and UMW bio-regions had no relationship with disturbance and their slopes were set to zero. The slopes for the PLAINS and WMTNS bio-regions were similar enough that a single value (-6.113) was used for both. The intercepts were 74.16 in the CPL, 78.75 in the EHIGH, 74.10 in the UMW, 58.32 in

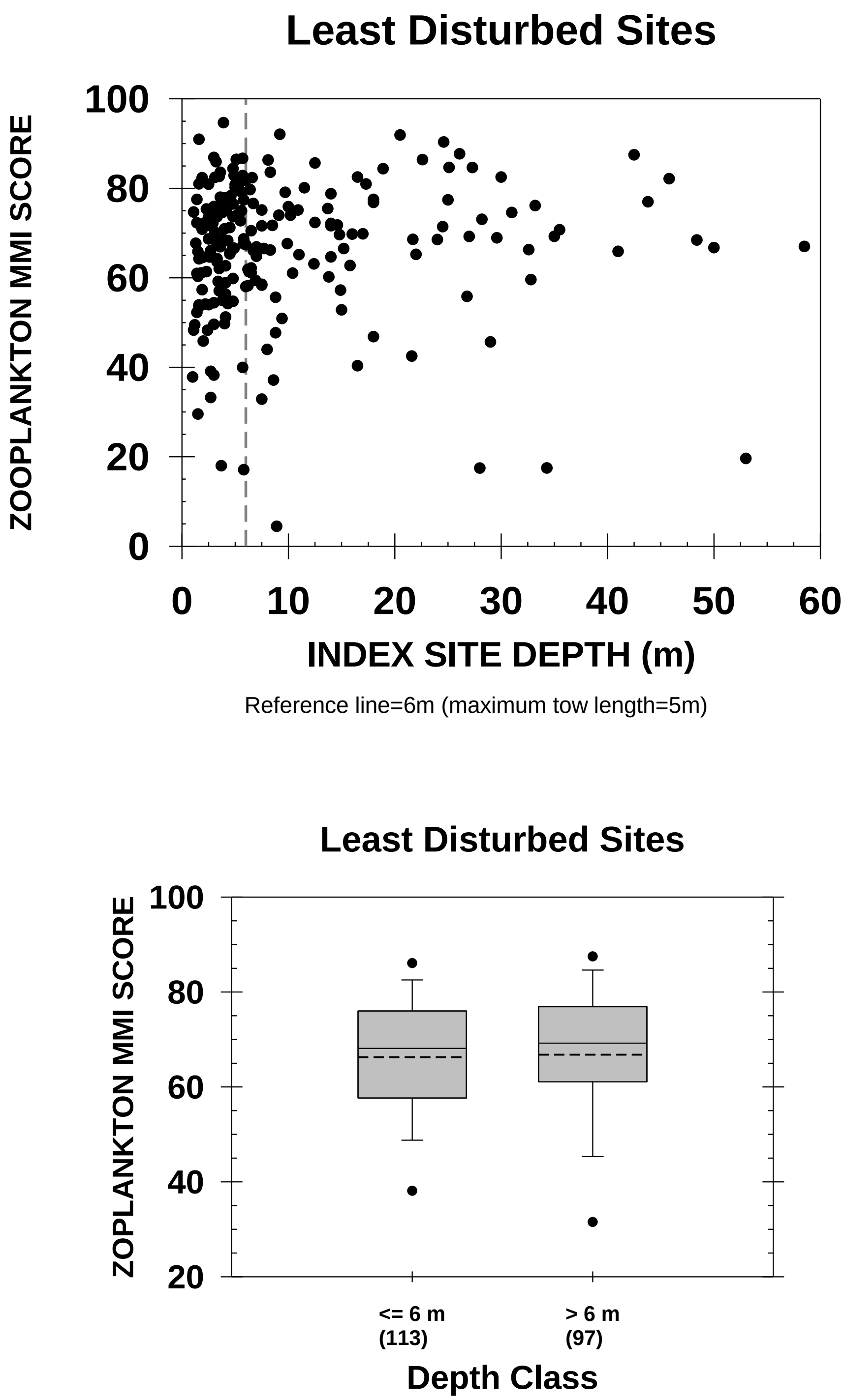

Figure 7-12. Zooplankton MMI scores versus site depth for least disturbed sites. Upper panel shows MMI scores versus actual site depth. The reference line of 6 m separates shallower lakes where the entire water column was sampled and deeper lakes where part of the water column was not sampled. The lower panel compares distribution of MMI scores in shallow lakes (≤6 m; *n*=113) versus deeper lakes (> 6 m, *n*=97). Dots indicate the 5th and 95th percentiles.

Table 7-9. Linear regression statistics of zooplankton MMI scores versus pca-based disturbance score for each bio-region.

Bio-Region	Slope	Intercept	RMSE (Pooled)
Coastal Plains (CPL	0	64.94	10.01
Eastern Highlands (EHIGH	0	76.50	10.01
Plains (PLAINS)	-6.143	54.55	10.01
Upper Midwest (UMW)	0	72.49	10.01
Western Mountains (WMTNS)	-6.143	63.48	10.01

Table 7-10. Thresholds for assigning ecological condition for zooplankton MMI scores based on the distribution of least disturbed sites in five bio-regions. Poor condition indicates a site is different from least disturbed condition. Fair condition indicates a site is somewhat deviant from least disturbed condition. Good condition indicates a site is similar to least disturbed condition. Values in bold (adjusted based on the regressions of MMI scores to PCA-based disturbance scores) are used to assign condition.

		Good/Fair Threshold (P_{25})		Fair/Poor Threshold (P_5)		Range of MMI scores in Least disturbed Sites
Bio-Region	***n*[a]**	**Adjusted**	**Unadjusted**	**Adjusted**	**Unadjusted**	
Coastal Plains (CPL)	22	**57.7**	59.4	**48.4**	49.7	38.80 to 94.47
Eastern Highlands (EHIGH)	59	**57.2**	58.0	**60.0**	57.3	46.37 to 92.62
Plains (PLAINS)	37	**42.4**	37.8	**33.2**	17.4	4.42 to 78.57
Upper Midwest (UMW	31	**73.3**	73.7	**56.0**	58.0	53.37 to 92.01
Western Mountains (WMTNS)	51	**69.2**	63.6	**54.6**	53.9	31.24 to 97.94

[a] Number of least disturbed sites remaining after excluding statistical outliers and sites with missing PCA –based disturbance scores.

the PLAINS, and 74.39 in the WMTNS. Table 7-10 shows both the raw (unadjusted sample) 5th and 25th percentiles and the regression model adjusted percentiles that we are using as the MMI thresholds. In three bio-regions (CPL, EHIGH, and UMW), the adjustment resulted in as slight lowering (< 2 points) of the Good/Fair threshold value. In the PLAINS and WMTNS bio-regions, the Good/Fair threshold values were increased (4.6 to 5.6 points). Adjustment lowered the Fair/Poor threshold values in the CPL, EHIGH, and UMW bio-regions by 2.7 to 6.7 points. The Fair/Poor threshold value was increased by 14.5 points in the PLAINS bio-region, and 3.9 points in the WMTNS bio-region.

7.8 Discussion

We were able to develop regional MMIs for pelagic zooplankton assemblages that were sufficiently responsive and repeatable to allow us to assess ecological condition for the 2012 NLA. The zooplankton assemblage appears to be responsive principally to disturbance resulting from increased nutrients and from increases in agricultural-related activity, which is consistent with previous studies (e.g., Gannon and Stemberger 1978, Stemberger and Lazorchak 1994). We did not observe a strong response of the zooplankton assemblage to shoreline habitat disturbance, as has been noted by others (e.g., Stemberger and Lazorchak 1994).

Based on our evaluations, the zooplankton MMIs we developed do not appear to be affected by lake origin (except possibly in the WMTNS), lake size, or by the use of a restricted tow length that does not collect individuals which might be occupying waters deeper than 6 m. Presence of these effects requires dealing with different types or sizes of lakes differently, either in terms of developing separate MMIs for them, or in setting different threshold values for them based on a very small number of least disturbed lakes.

The regional zooplankton MMIs we developed for the 2012 NLA do have some limitations. Samples must be collected using the same protocols and nets. Individuals were identified to the lowest practical taxon (with species being the target level). However, total richness metrics did not perform well in terms of responsiveness or repeatability, so coarser level identification may be possible in the future. However, coarser-level identification will constrain the development of predictive models based on taxa richness (O/E models; see Section 7.1), and would reduce the precision associated with biomass estimates due to lumping of taxa to coarser levels. While many richness metrics may not have performed well, many density- and biomass-based metrics did, thus laboratory analyses require determination of biomass, which increases costs and requires the use of conversion equations that may not be easily available to outside users.

In some bio-regions, our requirement for inclusion of at least one metric from each of the six categories resulted in using metrics that were either not very responsive to disturbance or were not very repeatable, and, in some bio-regions, including metrics that were highly correlated. Eliminating the poor-performing metrics from the suite of metrics did not appear to improve the MMI performance, so we retained them for consistency across bio-regions. Moreover, in those cases where we had a pair of highly correlated metrics, the mean correlation among all pairs of component metrics was low, so we did not feel the correlation unduly influenced the performance of the MMI (Van Sickle 2010). Future research might eliminate the requirement of metric categories and just include the best performing metrics regardless of metric category to determine if the resulting MMIs prove to be more responsive and repeatable than those developed for the 2012 NLA.

We observed that the responses of some metrics were contradictory to what we expected with increased disturbance (Table 7-1). However, little information is available, other than generalization about taxa richness and assemblage composition, and possibly feeding ecology, to support or refute the responses we observed in metrics related to density or biomass.

We also worked with a limited set of autecological information for the zooplankton taxa that were collected (essentially taxonomic and coarse-level feeding ecology). Additional information is available for a limited number of taxa (e.g., Sprules and Holtby 1979, Barnett et al. 2007, 2013, Vogt et al. 2013), but it is uncertain if this information can be assigned to related taxa. We did not have any information regarding the tolerance of zooplankton taxa either to specific stressors or to a generalized disturbance variable. These values have been developed for large numbers of fish taxa as well as benthic invertebrate taxa (Yuan 2004, Carlisle et al. 2007, Whittier et al. 2007, Meador et al. 2008, Whittier and Van Sickle 2010), and for rotifers in New Zealand (Duggan et al. 2001). Data are available from the 2007 NLA that would allow tolerance values to be developed and applied to the 2012 NLA, albeit at a coarser taxonomic level than species, and tolerance values derived from the 2012 NLA would be available for future assessments.

Finally, it is well known that predation by fish and larger invertebrate predators can affect zooplankton assemblages. Predation by planktivorous fish can result in smaller-sized taxa becoming more abundant. The 2012 NLA did not collect any detailed information about fish assemblages, so interpretations of response of metrics or the MMI to increased nutrients may be confounded with an increase in the number of fish species (including planktivorous species) that might accompany an increase in nutrients and a shift in the temperature regime from cold water to warm water.

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7.10 List of Candidate Metrics for Zooplankton

This section provides additional details for the candidate metrics we considered when developing the MMIs for each bio-region. Table 7-11 through Table 7-15 list each metric by its variable name, which of the six metric categories it was assigned to (see Section 7.4.4), and a description of the metric for the Coastal Plains, Eastern Highlands, Plains, Upper Midwest, and Western Mountains bio-regions, respectively. In addition, the responsiveness to disturbance and repeatability of each metric is provided (*t*-value for responsiveness, and S:N value for repeatability).

Table 7-11. List of candidate metrics used to develop the zooplankton MMI for the Coastal Plain bio-region.

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Abundance/ Biomass/ Density	FINE_BIO	Biomass of individuals of smaller-sized taxa (NET_SIZECLS_NEW=FINE; coarse and fine net samples combined)	14.73941733	50.21840118	4. -1,67	1.2
Abundance/ Biomass/ Density	ZOFN_BIO	Biomass represented by individuals collected in fine mesh net (50-um for 2012 samples, 80-um for 2007 resamples)	20.49135593	67.15372044	5. -1.79	1.2
Cladoceran	SIDID_PIND	Percent of total individuals that are within the cladoceran family Sididae (coarse and fine net samples combined)	2.10	8.18	-1.80	0.4
Copepod	CALAN_DEN	Total density of individuals within the copepod order Calanoida (coarse and fine net samples combined)	2.806313333	15.22849706	-1.46	2.2
Richness/Diversity	FAM_NAT_NTAX	Number of families represented by distinct native taxa (coarse and fine net samples combined)	11.9	9.3	2.62	1.9
Richness/Diversity	FAM_NTAX	Number of families represented by distinct taxa (coarse and fine net samples combined)	11.9	9.4	2.55	2.0
Richness/Diversity	GEN_NTAX	Number of genera represented by distinct taxa (coarse and fine net samples combined)	15.4	12.1	2.21	1.5
Richness/Diversity	GEN_NAT_NTAX	Number of genera represented by distinct native taxa (coarse and fine net samples combined)	15.4	12	2.27	1.3
Richness/Diversity	ZOFN_FAM_NAT_NTAX	Number of families represented by distinct native taxa in the fine mesh net (50-um)	7.4	5.4	2.32	1.4
Rotifer	COLLO_BIO	Total density of individuals within the rotifer order Collothecaceae (coarse and fine net samples combined)	0.198623267	0.021970559	1.79	3.3
Rotifer	COLLO_PIND	Percent of total individuals within the rotifer order Collothecaceae (coarse and fine net samples combined)	2.27	0.32	1.87	2.0
Rotifer	COLLO_PBIO	Percent of total biomass within the rotifer order Collothecaceae (coarse and fine net samples combined)	1.08	0.15	1.8	7.6
Trophic	PRED_NTAX	Number of distinct predator taxa (coarse and fine net samples combined)	2.5	1.3	2.56	4.6
Trophic	PRED_PTAX	Percent of distinct taxa that are predators (coarse and fine net samples combined)	12.01	6.59	2.71	2.2
Trophic	HERB_NTAX	Number of distinct herbivore taxa (coarse and fine net samples combined)	11.9	8.7	2.27	2.1
Trophic	OMNI_PTAX	Percent of distinct taxa that are omnivorous (coarse and fine net samples combined)	22.03	34.10	-3.35	4.3

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Trophic	OMNI_PDEN	Percent of total density represented by omnivorous individuals (coarse and fine net samples combined)	18.31	40.85	-2.42	1.6
Trophic	ROT_PRED_NTAX	Number of distinct rotifer taxa that are predators (coarse and fine net samples combined)	2.2	1.1	2.50	4.5
Trophic	ROT_PRED_PTAX	Percent of distinct rotifer taxa that are predators	10.78	5.64	2.70	1.9
Trophic	ROT_HERB_NTAX	Number of distinct rotifer taxa that are herbivores (coarse and fine net samples combined)	6.8	4.6	2.00	1.8
Trophic	ROT_OMNI_BIO	Biomass represented by rotifer individuals that are omnivores	4.7929874	35.027427794	-1.76	1.4
Trophic	ROT_OMNI_PIND	Percent of rotifer individuals represented by omnivores	13.41	26.55	-1.88	2.0
Trophic	ROT_OMNI_PTAX	Percent of distinct rotifer taxa that are omnivorous	17.26	27.95	-3.34	2.6
Trophic	ROT_OMNI_PDEN	Percent of rotifer density represented by omnivores	18.15	40.57	-2.42	1.6
Metrics Derived from 300-count Subsamples of Coarse and Fine Net Samples						
Abundance/ Biomass Density	ZOFN300_BIO	Total biomass in 300-count subsample of fine-mesh net sample (50-µm)	10.962325	35.92416574	-1.89	0.9
Cladoceran	BOSM300_PTAX	Percent of distinct taxa in the 300-count subsamples that are in the family Bosminidae (coarse and fine net samples combined)	7.357333333	3.916470588	2.77	0.3
Cladoceran	SIDID300_PIND	Percent of individuals within the cladoceran family Sididae in 300-count subsamples (coarse and fine net samples combined)	2.95	9.10	-1.68	0.7
Copepod	DOM1_300_COPE_PBIO	Percent of biomass in dominant copepod taxon in the 300 count subsamples (coarse and fine net samples combined)	90.00	76.87	1.82	1.9
Richness/Diversity	GEN300_NTAX	Number of genera represented by distinct taxa (coarse and fine net samples combined)	14	11.1	2.13	1.6
Richness/Diversity	GEN300_NAT_NTAX	Number of genera represented by distinct native taxa (coarse and fine net samples combined)	14	11.0	2.18	1.4
Richness/Diversity	FAM300_NTAX	Number of families represented in 300 count subsamples (coarse and fine net samples combined)	10.9	8.6	2.61	1.9
Richness/Diversity	FAM300_NAT_NTAX	Number of native families represented in 300 count subsamples (coarse and fine net samples combined)	10.9	8.5	2.66	1.8
Richness/Diversity	ZOFN300_FAM_NAT_NTAX	Number of distinct native families in 300-count subsample of fine-mesh net sample (50-µm)	6.7	4.8	2.49	1.3
Rotifer	COLLO300_BIO	Biomass represented by individuals of the rotifer order Collothecaceae in the 300-count subsamples (coarse and fine net samples combined)	0.0838373333	0.0125823235	1.76	3.4

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Rotifer	COLLO300_PBIO	Percent of biomass within the rotifer order Collothecaceae in the 300-count subsamples (coarse and fine net samples combined)	0.96	0.16	1.75	5.9
Trophic	PRED300_NTAX	Number of distinct taxa that are predators in 300 count subsamples (coarse and fine net samples combined)	1.7	1.0	1.94	2.7
Trophic	PRED300_BIO	Biomass of predator individuals in 300 count subsamples (coarse and fine net samples combined)	0.4595966	0.1407230588	2.45	1.5
Trophic	HERB300_NTAX	Number of distinct taxa that are herbivores in 300 count subsamples (coarse and fine net samples combined)	11.0	7.9	2.41	1.7
Trophic	OMNI300_PIND	Percent of omnivorous individuals in 300 count subsamples (coarse and fine net samples combined)	15.53	28.44	-1.86	1.4
Trophic	OMNI300_PTAX	Percent of distinct taxa that are omnivores in 300 count subsamples (coarse and fine net samples combined)	23.38	37.04	-3.27	4.9
Trophic	OMNI300_PBIO	Percent of biomass represented by omnivorous individuals in 300 count subsamples (coarse and fine net samples combined)	27.224	35.48058824	-2.96	4.7
Trophic	ROT_PRED300_NTAX	Number of distinct rotifer taxa that are predators in 300 count subsamples (coarse and fine net samples combined)	1.7	1.0	1.940	2.7
Trophic	ROT_PRED300_BIO	Biomass represented by rotifer individuals that are predators in 300 count subsamples (coarse and fine net samples combined)	0.4595966	0.1407230588	2.45	1.5
Trophic	ROT_HERB300_NTAX	Number of distinct rotifer taxa that are herbivores in 300 count subsamples (coarse and fine net samples combined)	6.1	4.0	2.24	1.4
Trophic	ROT_OMNI300_PIND	Percent of rotifer individuals that are omnivorous in 300 count subsamples (coarse and fine net samples combined)	12.24	25.10	-2.00	1.9
Trophic	ROT_OMNI300_PTAX	Percent of distinct rotifer taxa that are omnivorous in 300 count subsamples (coarse and fine net samples combined)	18.47	30.13	-3.00	4.3

Table 7-12. List of candidate metrics used to develop the zooplankton MMI for the Eastern Highlands bio-region

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Abundance/ Biomass/ Density	ZOCN_DEN	Density represented by individuals collected in coarse mesh net (150-um for 2012 samples, 243 um for 2007 resamples)	12.56848	34.33432549	-1.89	7.1
Abundance/ Biomass/ Density	ZOCN_NAT_DEN	Density represented by native individuals collected in coarse mesh net (150-um for 2012 samples, 243 um for 2007 resamples)	12.56848	34.33106863	-1.89	2.1
Abundance/ Biomass/ Density	COARSE_DEN	Density represented by individuals of taxa collected in coarse mesh net (150-um; coarse and fine net samples combined)	21.26666667	53.84573922	-2.13	2.4
Abundance/ Biomass/ Density	COARSE_PBIO	Biomass represented by individuals of taxa collected in coarse mesh net (150-um; coarse and fine net samples combined)	68.49155556	56.48058824	1.86	1.7
Abundance/ Biomass/ Density	COARSE_NAT_DEN	Density represented by individuals of native larger-sized taxa (NET_SIZECLS_NEW=COARSE; coarse and fine net samples combined)	21.266666667	53.80877451	-2-12	1.5
Abundance/ Biomass/ Density	COARSE_NAT_PBIO	Biomass represented by individuals of native larger-sized taxa (NET_SIZECLS_NEW=COARSE; coarse and fine net samples combined)	68.491555556	56.44254902	1.86	1.5
Abundance/ Biomass/ Density	FINE_PBIO	Biomass represented by individuals of smaller-sized taxa (NET_SIZECLS_NEW=FINE; coarse and fine net samples combined)	31.508444444	43.519411765	-1.86	1.7
Cladoceran	CLAD_DEN	Density of native individuals within the suborder Cladocera (coarse and fine net samples combined)	6.813766667	27.71694902	-1.94	1.9
Cladoceran	CLAD_NAT_DEN	Density of native individuals within the suborder Cladocera (coarse and fine net samples combined)	6.813766667	27.71382549	-1.94	1.8
Cladoceran	LGCLAD_BIO	Biomass represented by large cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=LARGE; coarse and fine net samples combined)	25.780533111	10.663794725	2.16	1.3
Cladoceran	LGCLAD_NAT_BIO	Biomass represented by native large cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=LARGE; coarse and fine net samples combined)	25.780533111	10.656975706	2.16	1.3
Cladoceran	SMCLAD_BIO	Biomass represented by small cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=SMALL; coarse and fine net samples combined)	2.985147667	31.80179637	-2.37	2.6
Cladoceran	SMCLAD_DEN	Density represented by small cladoceran individuals (SUBORDER=CLADOCERA and CLADOCERAN_SIZE=SMALL; coarse and fine net samples combined)	2.476364444	22.86743922	-1.99	2.4

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Cladoceran	SMCLAD_PIND	Percent of small cladoceran individuals (SUBORDER=CLADOCERA and CLAD-SIZE=SMALL; coarse and fine net samples combined)	9.58	17.42	-2.73	1.6
Cladoceran	SMCLAD_PDEN	Percent of total density represented by small cladoceran individuals (SUBORDER=CLADOCERA and CLADOCERAN_SIZE=SMALL; coarse and fine net samples combined)	1.03	3.34	-1.91	19.1
Cladoceran	SMCLAD_NAT_BIO	Biomass represented by native small cladoceran individuals (SUBORDER=CLADOCERA and CLADOCERAN_SIZE=SMALL; coarse and fine net samples combined)	2.985147667	31.79812541	-2.37	2.5
Cladoceran	SMCLAD_NAT_DEN	Density represented by native small cladoceran individuals (SUBORDER=CLADOCERA and CLADOCERA_SIZE=SMALL; coarse and fine net samples combined)	2.476364444	22.86662549	-1.99	2.2
Cladoceran	SMCLAD_NAT_PDEN	Percent of total density represented by native small cladoceran individuals (SUBORDER=CLADOCERA and CLADOCERAN_SIZE=SMALL; coarse and fine net samples combined)	1.03	3.33	-1.91	19.1
Cladoceran	DAPHNIID_DEN	Density of individuals within the family Daphniidae (coarse and fine net samples combined)	3.223097778	16.27482549	-2.09	2.5
Cladoceran	DAPHNIID_NAT_DEN	Density of native individuals within the family Daphniidae (coarse and fine net samples combined)	3.223097778	16.27251961	-2.09	2.5
Copepod	COPE_DEN	Density represented by individuals within the subclass Copepoda (coarse and fine net samples combined)	81.931315556	139.66798235	-1.74	1.5
Copepod	COPE_NAT_DEN	Density represented by native individuals within the subclass Copepoda (coarse and fine net samples combined)	81.931315556	139.66784314	-1.74	1.5
Copepod	CALAN_NTAX	Number of distinct taxa within the copepod order Calanoida (coarse and fine net samples combined)	1.3	1.1	2.10	2.4
Copepod	CALAN_PDEN	Percent of total density represented by taxa of the copepod order Calanoida (coarse and fine net samples combined)	3.82	1.64	1.80	35.0
Copepod	CALAN_NAT_NTAX	Number of distinct native taxa within the copepod order Calanoida (coarse and fine net samples combined)	1.3	1.0	2.22	1.3
Copepod	CALAN_NAT_PDEN	Percent of total density represented by individuals of native taxa within the copepod order Calanoida (coarse and fine net samples combined)	3.81	1.64	1,80	35.0
Richness/Diversity	COARSE_NAT_PTAX	Percent of distinct larger-sized native taxa (NET_SIZECLS_NEW=COARSE; coarse and fine net samples combined)	40.65	37.17	1.64	0.3

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Rotifer	ROT_PBIO	Percent total biomass from rotifers (coarse and fine net samples combined)	23.72	34.91	-1.88	1.3
Trophic	OMNI_PTAX	Percent of distinct taxa that are omnivorous (coarse and fine net samples combined)	23.38	27.56	-2.36	1.6
	CLAD_HERB_DEN	Density of herbivorous cladocerans (suborder=CLADOCERA; coarse and fine net samples combined)	6.8127244444	27.71694902	-1.94	1.9
	COPE_HERB_PDEN	Percent density represented by herbivorous copepods (order=COPEPODA; coarse and fine net samples combined)	4.22	1.92	1.86	20.0
Metrics Derived from 300-count Subsamples of Coarse and Fine Net Samples						
Abundance/ Biomass/ Density	COARSE300_PBIO	Percent of biomass represented by individuals of taxa collected in coarse mesh net (150-um; NET_SIZECLS_NEW=COARSE) in 300 count subsamples (coarse and fine net samples combined)	70.74	58.61	1.96	1.7
Abundance/ Biomass/ Density	COARSE300_NAT_PBIO	Percent of biomass represented by individuals of native taxa collected in coarse mesh net (150-um; NET_SIZECLS_NEW=COARSE) in 300 count subsamples (coarse and fine net samples combined)	70.738666667	58.570196078	1.96	1.5
Abundance/ Biomass/ Density	FINE300_PBIO	Percent biomass represented by individuals of smaller-sized taxa (NET_SIZECLS_NEW=FINE) in 300-count subsamples (coarse and fine net samples combined)	29.26	41.39	-1.96	1.7
Cladoceran	LGCLAD300_BIO	Biomass represented by large cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=LARGE) in 300-count subsamples (coarse and fine net samples combined)	15.692285844	7.0078742941	2.02	1.4
Cladoceran	LGCLAD300_NAT_BIO	Biomass represented by native large cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=LARGE) in 300-count subsamples (coarse and fine net samples combined)	15.692285844	7.0031208824	2.02	1.4
Cladoceran	SMCLAD300_BIO	Biomass represented by small cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=SMALL) in 300-count subsamples (coarse and fine net samples combined)	1.8545441111	21.410646353	-2.40	2.6
Cladoceran	SMCLAD300_PIND	Percent of small cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=SMALL) in 300-count subsamples (coarse and fine net samples combined)	10.90	19.03	-2.72	1.7

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Cladoceran	SMCLAD300_PBIO	Percent of biomass represented by small cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=SMALL) in 300-count subsamples (coarse and fine net samples combined)	5.50	16.12	-2.82	1.6
Cladoceran	SMCLAD300_NAT_BIO	Biomass represented by native small cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=SMALL) in 300-count subsamples (coarse and fine net samples combined)	1.8545441111	21.410646353	-2.40	2.5
Cladoceran	SMCLAD300_NAT_PIND	Percent of native small cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=SMALL) in 300-count subsamples (coarse and fine net samples combined)	10.90	19.03	-2.72	1.4
Copepod	CALAN300_NTAX	Number of distinct taxa within the copepod order Calanoida in 300-count subsamples (coarse and fine net samples combined)	1.3	1.0	1.94	2.8
Copepod	CALAN300_NAT_NTAX	Number of distinct native taxa within the copepod order Calanoida in 300-count subsamples (coarse and fine net samples combined)	1.3	1.0	2.08	1.4
Richness/Diversity	ZOCN300_NAT_PTAX	Percent distinct native taxa in 300-count subsample of coarse net sample (150-um)	100	98.55	1.88	0.1
Richness/Diversity	ZOCN300_FAM_NTAX	Number of distinct native taxa in coarse net samples (150-um) based on 300-count subsample	5.1	4.7	1.47	0.8
Rotifer	ROT300_PBIO	Percent biomass from rotifers in 300-count subsamples (coarse and fine net samples combined)	22.26	34.91	-1.89	1.3
Trophic	OMNI300_PTAX	Percent of distinct taxa that are omnivorous in 300-count subsamples (coarse and fine net samples combined)	23.31	28.29	-2.60	1.5

Table 7-13. List of candidate metrics used to develop the zooplankton MMI for the Plains bio-region

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Abundance/ Biomass/ Density	COARSE_PBIO	Percent of total biomass represented by individuals collected in coarse mesh net (150-um for 2012 samples, 243 um for 2007 resamples)	57.38	70.00	-1.75	6.3
Abundance/ Biomass/ Density	COARSE_NAT_PBIO	Percent of total biomass represented by native individuals collected in coarse mesh net (150-um for 2012 samples, 243 um for 2007 resamples)	57.38	69.94	-1.74	6.3
Abundance/ Biomass/ Density	FINE_PBIO	Percent of biomass represented by individuals of smaller-sized taxa (NET_SIZECLS_NEW=FINE; coarse and fine net samples combined)	42.62	30.00	1.75	6.3
Abundance/ Biomass/ Density	FINE_NAT_PBIO	Percent of biomass represented by native individuals of smaller-sized taxa (NET_SIZECLS_NEW=FINE; coarse and fine net samples combined)	42.62	29.99	1.75	6.2
Cladoceran	SMCLAD_PIND	Percent of total individuals within the suborder Cladocera that are "small" (CLADOCERA_SIZE=SMALL; coarse and fine net samples combined)	19.26	9.03	3.09	1.8
Cladoceran	SMCLAD_NAT_PIND	Percent of native individuals within the suborder Cladocera that are "small" (CLADOCERA_SIZE=SMALL; coarse and fine net samples combined)	19.26	8.94	3.11	1.8
Cladoceran	SMCLAD_NAT_PBIO	Percent of total biomass represented by native small cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=SMALL; coarse and fine net samples combined)	13.35	7.02	1.74	1.4
Copepod	COPE_PIND	Percent of total individuals within the subclass Copepoda (coarse and fine net samples combined)	29.45	41.97	-2.46	1.4
Copepod	COPE_NAT_PIND	Percent of native individuals within the subclass Copepoda (coarse and fine net samples combined)	29.45	41.97	-2.46	1.4
Copepod	CALAN_PTAX	Percent of distinct taxa that are within the copepod order Calanoida (coarse and fine net samples combined)	6.38	10.16	-2.32	2.0
Copepod	CALAN_PDEN	Percent of total density represented by individuals within the copepod order Calanoida (coarse and fine net samples combined)	1.20	6.52	-2.06	14.1
Copepod	CALAN_NAT_PDEN	Percent of total density represented by native individuals within the copepod order Calanoida (coarse and fine net samples combined)	1.20	6.52	-2.06	14.1

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Copepod	COPE_RATIO_NIND	Ratio of Calanoid to (Cladoccera+Cyclopoids) based on number of individuals (coarse and fine net samples combined). Adapted from Kane et al. (2009) Lake Erie plankton IBI. Calculated as CALANOID_NIND/(CLAD_NIND+CYCLOPOID_NIND)	17.435	0.812	1.84	38.9
Copepod	COPE_RATIO_BIO	Ratio of Calanoid to (Cladoccera+Cyclopoids) based on biomass (coarse and fine net samples combined). Adapted from Kane et al. (2009) Lake Erie plankton IBI. Calculated as CALANOID_BIO/(CLAD_BIO+CYCLOPOID_BIO)	7.325729723	1.327404241	2.31	4.6
Richness/Diversity	TOTL_NTAX	Total distinct taxa richness (coarse and fine net samples combined)	17.3	14..6	2.27	2.2
Richness/Diversity	TOTL_NAT_NTAX	Total distinct native taxa richness (coarse and fine net samples combined)	17.3	14.5	2.34	2.2
Richness/Diversity	GEN_NTAX	Number of genera represented by distinct taxa (coarse and fine net samples combined)	13.8	11.6	2.45	2.2
Richness/Diversity	GEN_NAT_NTAX	Number of genera represented by distinct native taxa (coarse and fine net samples combined)	13.8	11.5	2.56	2.2
Richness/Diversity	FAM_NTAX	Number of families represented by distinct taxa (coarse and fine net samples combined)	10.7	9.1	2.32	1.9
Richness/Diversity	FAM_NAT_NTAX	Number of families represented by distinct native taxa (coarse and fine net samples combined)	10.7	9.1	2.41	2.2
Richness/Diversity	ZOFN_NTAX	Number of distinct taxa in fine net sample (ZOFN; 80-um mesh)	12.4	9.8	2.69	1.7
Richness/Diversity	ZOFN_NAT_NTAX	Number of distinct native taxa in fine net sample (ZOFN; 80-um mesh)	12. 4	9.8	2.73	1.7
Richness/Diversity	ZOFN_GEN_NTAX	Number of genera represented by distinct taxa in fine net sample (ZOFN; 80-um mesh)	8.1	5.8	3.36	3.8
Richness/Diversity	ZOFN_GEN_NAT_NTAX	Number of genera represented by distinct native taxa in fine net sample (ZOFN; 80-um mesh)	8.1	5.8	3.42	3.8
Richness/Diversity	ZOFN_FAM_NTAX	Number of families represented by distinct taxa in fine net sample (ZOFN; 80-um mesh)	6.6	4.7	3.48	3.0
Richness/Diversity	ZOFN_FAM_NAT_NTAX	Number of families represented by distinct native taxa in fine net sample (ZOFN; 80-um mesh)	6.6	4.7	3.56	3.0
Richness/Diversity	FINE_NTAX	Number of distinct taxa collected only in the fine-mish net (80-um; NET_SIZECLS_NEW=FINE)	10.5	8.0	2.61	1.8
Richness/Diversity	FINE_NAT_NTAX	Number of distinct native taxa collected only in the fine-mish net (80-um; NET_SIZECLS_NEW=FINE)	10.5	8.0	2.63	1.7
Richness/Diversity	DOM5_PBIO	Percent of total biomass represented in top 5 taxa (coarse and fine net samples combined)	91.31	94.16	-1.77	2.5
Rotifer	ROT_NTAX	Number of distinct rotifer taxa (coarse and fine net samples combined)	10.5	8.0	2.63	1.7

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Trophic	COPE_HERB_PDEN	Percent of total density represented by herbivorous copepods (coarse and fine net samples combined)	1.23	6.58	-2.13	13.0
Metrics Derived from 300-count Subsamples of Coarse and Fine Net Samples						
Abundance/ Biomass/ Density	COARSE300_PBIO	Percent of biomass represented by individuals of taxa collected in coarse mesh net (150-um) in 300 count subsamples (coarse and fine net samples combined)	59.0316	71.48616279	-1.77	5.2
Abundance/ Biomass/ Density	COARSE300_NAT_PBIO	Percent of biomass represented by native individuals of taxa collected in coarse mesh net (150-um) in 300 count subsamples (coarse and fine net samples combined)	59.0316	71.42267442	-1.76	5.1
Abundance/ Biomass/ Density	FINE300_PBIO	Percent of biomass represented in individuals of smaller-sized taxa (NET_SIZECLS_NEW=FINE) in the 300-count subsample (coarse and fine mesh samples combined)	42.15	28.64	1.89	6.0
Abundance/ Biomass/ Density	FINE300_NAT_PBIO	Percent of biomass represented in native individuals of smaller-sized taxa (NET_SIZECLS_NEW=FINE) in the 300-count subsample (coarse and fine mesh samples combined)	42.15	28.63	1.90	5.8
Cladoceran	SMCLAD300_PIND	Percent of small cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=SMALL) in 300-count subsamples (coarse and fine net samples combined)	19.788	9.848139535	2.97	2.0
Cladoceran	SMCLAD300_PBIO	Percent of biomass represented by small cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=SMALL) in 300-count subsamples (coarse and fine net samples combined)	14.17	7.52	1.74	1.4
Cladoceran	SMCLAD300_NAT_PIND	Percent of native small cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=SMALL) in 300-count subsamples (coarse and fine net samples combined)	19.788	9.760930233	2.99	2.0
Cladoceran	SMCLAD300_NAT_PBIO	Percent of biomass represented by native small cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=SMALL) in 300-count subsamples (coarse and fine net samples combined)	14.17	7.47	1.76	1.4
Copepod	COPE300_PIND	Percent of individuals within the subclass Copepoda in 300-count subsamples (coarse and fine net samples combined)	30.94	43.16	2.42	1.3

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Copepod	COPE300_NAT_PIND	Percent of native individuals within the subclass Copepoda in 300-count subsamples (coarse and fine net samples combined)	30.94	43.16	30.93	1.3
Copepod	CALAN300_PTAX	Percent of distinct taxa within the copepod order Calanoida in 300-count subsamples (coarse and fine net samples combined)	7.51	11.20	-2.07	4.6
Copepod	COPE_RATIO_300_NIND	Ratio of Calanoid to (Cladoccera+Cyclopoids) based on number of individuals in 300-count subsamples (coarse and fine net samples combined). Adapted from Kane et al. (2009) Lake Erie plankton IBI. Calculated as CALANOID_NIND/(CLAD_NIND+CYCLOPOID_NIND)	12.675	0.800	1.83	19.6
Copepod	COPE_RATIO_300_BIO	Ratio of Calanoid to (Cladoccera+Cyclopoids) based on biomass in 300-count subsamples (coarse and fine net samples combined). Adapted from Kane et al. (2009) Lake Erie plankton IBI. Calculated as CALANOID_BIO/(CLAD_BIO+CYCLOPOID_BIO)	5.712	1.003	2.41	3.0
Richness/Diversity	TOTL300_NAT_NTAX	Total distinct native taxa richness in 300-count subsamples (coarse and fine net samples combined)	14.8	12.9	1.76	1.4
Richness/Diversity	GEN300_NTAX	Total distinct generic richness in 300-count subsamples (coarse and fine net samples combined)	12.3	10.6	2.03	2.7
Richness/Diversity	GEN300_NAT_NTAX	Total distinct native generic richness in 300-count subsamples (coarse and fine net samples combined)	12.3	10.5	2.13	2.9
Richness/Diversity	FAM300_NTAX	Total distinct family richness in 300-count subsamples (coarse and fine net samples combined)	9.8	8.4	2.11	2.3
Richness/Diversity	FAM300_NAT_NTAX	Total distinct native family richness in 300-count subsamples (coarse and fine net samples combined)	9.8	8.4	2.22	2.6
Richness/Diversity	ZOFN300_GEN_NTAX	Number of distinct genera in 300-count subsample of fine-mesh net sample (50-µm)	6.8	5.3	2.45	2.7
Richness/Diversity	ZOFN300_GEN_NAT_NTAX	Number of distinct native genera in 300-count subsample of fine-mesh net sample (50-µm)	6.8	5.2	2.48	2.9
Richness/Diversity	ZOFN300_FAM_NTAX	Number of distinct families in 300-count subsample of fine-mesh net sample (50-µm)	5.6	4.3	2.74	3.1
Richness/Diversity	ZOFN300_FAM_NAT_NTAX	Number of distinct native families in 300-count subsample of fine-mesh net sample (50-µm)	5.6	4.3	2.79	3.1
Richness/Diversity	DOM5_300_PBIO	Percent of biomass represented in top 5 taxa in 300-count subsamples (coarse and fine net samples combined)	91.38	94.27	-1.78	1.9

Table 7-14. List of candidate metrics used to develop the zooplankton MMI for the Upper Midwest bio-region

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Abundance/ Biomass/ Density	TOTL_NAT_PIND	Percent of native individuals (coarse and fine net samples combined)	100	98.02	1.47	2348
Abundance/ Biomass/ Density	ZOCN_NAT_PDEN	Percent of density represented by native individuals in coarse net sample (150-um)	100	95.90	1.52	Noise=0
Cladoceran	DAPHNIID_NTAX	Number of distinct taxa within the cladoceran family Daphniidae (coarse and fine net samples combined)	1.4	1.8	-1.91	3.1
Cladoceran	BOSM_DEN	Density of individuals within the cladoceran family Bosminidae (coarse and fine net samples combined)	28.20401905	6.857369231	1.85	2.8
Cladoceran	BOSM_PIND	Percent of individuals within the cladoceran family Bosminidae (coarse and fine net samples combined)	15.31	8.35	1.85	19.5
Cladoceran	BOSM_NAT_BIO	Biomass of native individuals within the cladoceran family Bosminidae (coarse and fine net samples combined)	16.33606357	3.165346051	1.89	1.8
Cladoceran	BOSM_NAT_DEN	Density of native individuals within the cladoceran family Bosminidae (coarse and fine net samples combined)	28.204019048	5.0981051282	2.01	4.9
Cladoceran	BOSM_NAT_PIND	Percent of native individuals within the cladoceran family Bosminidae (coarse and fine net samples combined)	15.31	6.71	2.29	9.6
Cladoceran	BOSM_NAT_PTAX	Percent of distinct native taxa within the cladoceran family Bosminidae (coarse and fine net samples combined)	5.59	3.96	2.16	1.6
Cladoceran	BOSM_NAT_PBIO	Percent of biomass represented by native individuals within the cladoceran family Bosminidae (coarse and fine net samples combined)	10.01	2.57	2.07	4.9
Cladoceran	HPRIME_CLAD	Shannon Diversity based on the number of cladoceran individuals (coarse and fine net samples combined). Calculated as SUM{p(i)*Log[p(i)]}, where p(i) is proportion of individuals of taxon i , and Log= natural logarithm.	0.579	0.772	-1.91	1.3
Copepod	CALAN_BIO	Biomass of individuals within the copepod order Calanoida (coarse and fine net samples combined)	12.010544048	27.035772872	-1.73	12.7
Copepod	CALAN_NAT_BIO	Biomass of native individuals within the copepod order Calanoida (coarse and fine net samples combined)	12.010544048	27.025444897	-1.73	12.8

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Richness/Diversity	TOTL_NAT_PTAX	Percent of distinct native taxa (coarse and fine net samples combined)	100	98.05	2.65	21.7
Richness/Diversity	ZOCN_NAT_PTAX	Percent of distinct taxa represented by native individuals in coarse net sample (150-um)	100	95.84	2.59	8.9
Richness/Diversity	COARSE_PTAX	Percent of distinct larger-sized taxa (NET_SIZECLS_NEW=COARSE; coarse and fine net samples combined)	39.74	45.09	-1.89	1.4
Richness/Diversity	FINE_PTAX	Percent of distinct smaller-sized taxa (NET_SIZECLS_NEW=FINE; coarse and fine net samples combined)	60.26	54.91	-1.89	1.4
Rotifer	ROT_PTAX	Percent of distinct taxa within the phylum Rotifera (coarse and fine net samples combined)	60.26	54.91	1.87	1.4
Rotifer	FLOS_DEN	Density of individuals within the rotifer order Flosculariaceae (coarse and fine net samples combined)	290.0439619	115.22284872	1.82	7.6
Rotifer	HPRIME_ROT	Shannon Diversity based on the number of rotifer individuals (coarse and fine net samples combined). Calculated as SUM{p(i)*Log[p(i)]}, where p(i) is proportion of individuals of taxon i , and Log= natural logarithm.	1.524	1.264	2.12	1.4
Rotifer	SIMPSON_ROT	Simpson Diversity based on the number of rotifer individuals (coarse and fine net samples combined). Calculated as SUM{p(i)*p(i)} where p(i) is the proportion of taxon I in the sample.	0.325	0.414	-1.79	2.4
Rotifer	PIE_ROT	Hurlbert's Probability of Interspecific Encounter (PIE) based on the number of rotifer individuals (coarse and fine net samples combined). Calculated as SUM{p(i)*[N-n(i)/N-1]} where p(i) is the proportion of taxon I in the sample, N is the total number of rotifer individuals in the sample, and n(i) is the number of rotifer individuals of taxon i in the sample.	0.678	0.590	1.76	2.5
Rotifer	DOM3_ROT_PIND	Percent of rotifer individuals in top 3 Rotifer taxa (coarse and fine net samples combined)	78.89	86.34	-2.35	1.6
Rotifer	DOM5_ROT_PIND	Percent of rotifer individuals in top 5 Rotifer taxa (coarse and fine net samples combined)	91.39	94.46	-1.81	2.6
Rotifer	DOM1_ROT_PBIO	Percent of rotifer biomass in dominant rotifer taxon (coarse and fine net samples combined)	45.30	59.27	-2.46	3.5
Rotifer	DOM3_ROT_PDEN	Percent of rotifer density in top 3 Rotifer taxa (coarse and fine net samples combined)	78.89	86.34	-2.35	1.6
Rotifer	DOM5_ROT_PDEN	Percent of density in top 5 rotifer taxa (coarse and fine net samples combined)	91.39	94.46	-1.81	2.6

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
		Metrics Derived from 300-count Subsamples of Coarse and Fine Net Samples				
Cladoceran	DAPHNIID300_NTAX	Number of distinct taxa within the cladoceran family Daphniidae in 300-count subsamples (coarse and fine net samples combined)	1.2	1.7	-2.3	3.1
Cladoceran	DAPHNIID300_NAT_NTAX	Number of distinct native taxa within the cladoceran family Daphniidae in 300-count subsamples (coarse and fine net samples combined)	1.4	1.7	-2.3	3.1
Cladoceran	BOSM300_PIND	Biomass of native individuals within the cladoceran family Bosminidae in 300-count subsamples (coarse and fine net samples combined)	16.74	9.15	1.87	15.4
Cladoceran	BOSM300_NAT_BIO	Density of native individuals within the cladoceran family Bosminidae in 300-count subsamples (coarse and fine net samples combined)	9.9940477143	2.211484641	1.84	2.1
Cladoceran	BOSM300_NAT_PIND	Percent of native individuals within the cladoceran family Bosminidae in 300-count subsamples (coarse and fine net samples combined)	16.74	7.12	2.42	15.3
Cladoceran	BOSM300_NAT_PTAX	Percent of distinct native taxa that are within the cladoceran family Bosminidae in 300-count subsamples (coarse and fine net samples combined)	6.48	4.08	2.73	1.4
Cladoceran	BOSM300_NAT_PBIO	Biomass of biomass represented by native individuals within the cladoceran family Bosminidae in 300-count subsamples (coarse and fine net samples combined)	10.56	2.78	211	4.7
Copepod	CALAN300_BIO	Biomass of individuals within the copepod order Calanoida in 300-count subsamples (coarse and fine net samples combined)	6.3444415238	17.540568538	-2.17	9.2
Richness/Diversity	TOTL300_NAT_PTAX	Percent of distinct native taxa in 300-count subsamples (coarse and fine net samples combined)	100	97.87	2.66	8.2
Richness/Diversity	ZOCN300_NAT_PTAX	Percent of distinct native taxa in the coarse net sample (150-um) based on the 300-individual subsamples	100	95.92	2.76	Noise=0
Rotifer	PLOIMA300_PTAX	Percent of distinct taxa represented by the rotifer order Ploima in 300-count subsamples (coarse and fine net samples combined)	48.72	42.16	2.05	9.8
Rotifer	HPRIME_ROT300	Shannon Diversity based on the number of rotifer individuals in 300-count subsamples (coarse and fine net samples combined). Calculated as SUM{p(i)*Log[p(i)]}, where p(i) is proportion of individuals of taxon i , and Log= natural logarithm.	1.515	1.254	2.12	1.4

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Rotifer	SIMPSON_ROT300	Simpson Diversity based on the number of rotifer individuals in 300-count subsamples (coarse and fine net samples combined). Calculated as SUM{p(i)*p(i)} where p(i) is the proportion of taxon I in the sample.	0.324	0.416	-1.86	2.1
Rotifer	PIE_ROT300	Hurlbert's Probability of Interspecific Encounter (PIE) based on the number of rotifer individuals in 300-count subsamples (coarse and fine net samples combined). Calculated as SUM{p(i)*[N-n(i)/N-1]} where p(i) is the proportion of rotifer taxon I in the sample, N is the total number of rotifer individuals in the sample, and n(i) is the number of individuals of taxon i in the sample.	0.680	0.590	1,78	2.2
Rotifer	DOM1_300_ROT_PIND	Percent of rotifer individuals in dominant rotifer taxon in 300-count subsamples (coarse and fine net samples combined)	45.70	54.61	-1.74	2.1
Rotifer	DOM3_300_ROT_PIND	Percent of rotifer individuals in top 3 Rotifer taxa in 300-count subsamples (coarse and fine net samples combined)	78.91	86.25	-2.26	1.4
Rotifer	DOM5_300_ROT_PIND	Percent of rotifer individuals in top 5 Rotifer taxa in 300-count subsamples (coarse and fine net samples combined)	91.50	94.71	-1.91	3.7
Rotifer	DOM1_300_ROT_PBIO	Percent of rotifer biomass in dominant Rotifer taxon in 300-count subsamples (coarse and fine net samples combined)	47.97	58.94	-1.95	2.0
Trophic	PRED300_PBIO	Percent of biomass represented by predator individuals in 300-count subsamples (coarse and fine net samples combined)	2.06	0.93	1.86	95.5
Trophic	ROT_PRED300_PBIO	Percent of biomass represented by predaceous rotifer individuals in 300-count subsamples (coarse and fine net samples combined)	2.06	0.93	1.86	95.5
Trophic	COPE_HERB_PBIO	Percent of biomass represented by herbivorous copepods (coarse and fine net samples combined)	16.04	24.53	-1.96	5.0

Table 7-15. List of candidate metrics used to develop the zooplankton MMI for the Western Mountains bio-region

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Cladoceran	BOSM_NAT_PTAX	Percent of distinct native taxa within the cladoceran family Bosminidae (coarse and fine net samples combined)	5.59	3.96	2.16	1.3
Copepod	COPE_NTAX	Number of distinct taxa within the subclass Copepoda (coarse and fine net samples combined)	2.6	3.3	-2.15	1.7
Copepod	COPE_PTAX	Percent of distinct taxa within the subclass Copepoda (coarse and fine net samples combined)	14.33	18.08	-2.29	1.9
Copepod	COPE_NAT_NTAX	Number of distinct native taxa within the subclass Copepoda (coarse and fine net samples combined)	2.6	3.3	-2.07	1.7
Copepod	COPE_NAT_PTAX	Percent of distinct native taxa within the subclass Copepoda (coarse and fine net samples combined)	14.33	18.00	-2.21	1.9
Copepod	COPE_DEN	Total density of individuals within the subclass Copepoda (coarse and fine net samples combined)	177.8479619	156.08843077	0.3	1.6
Copepod	CALAN_BIO	Total biomass of individuals within the copepod order Calanoida (coarse and fine net samples combined)	12.010544048	27.035772872	-1.73	4.4
Copepod	CALAN_NAT_BIO	Total biomass of native individuals within the copepod order Calanoida (coarse and fine net samples combined)	12.010544048	27.025444897	-1.73	4.4
Richness/Diversity	COARSE_PTAX	Percent of distinct larger-sized taxa (NET_SIZECLS_NEW=COARSE; coarse and fine net samples combined)	39.75	45.09	-1.87	2.3
Richness/Diversity	FINE_PTAX	Percent of distinct taxa collected only in the fine-mesh net (50-um; NET_SIZECLS_NEW=FINE; coarse and fine net samples combined)	60.25	54.91	1.87	2.3
Richness/Diversity	SIMPSON_DEN	Simpson Diversity based on the total density individuals (coarse and fine net samples combined). Calculated as SUM{p(i)*p(i)} where p(i) is the proportion of density of taxon i in the sample.	0.288	0.353	-1.46	1.25
Rotifer	ROT_PTAX	Percent distinct rotifer taxa (coarse and fine net samples combined)	60.26	54.91	1.87	2.5
Rotifer	PLOIMA_PTAX	Percent distinct taxa that are within the rotifer order Ploima (coarse and fine net samples combined)	48.72	42.00	2.28	4.3
Rotifer	SIMPSON_ROT	Simpson Diversity based on the number of rotifer individuals (coarse and fine net samples combined). Calculated as SUM{p(i)*p(i)} where p(i) is the proportion of taxon I in the sample.	0.325	0.414	-1.79	1.4
Trophic	COPE_OMNI_PTAX	Percent of distinct taxa that are omnivorous copepods (coarse and fine net samples combined)	5.44	8.65	-2.526	1.5

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Metrics Derived from 300-count Subsamples of Coarse and Fine Net Samples						
Abundance/ Biomass/ Density	TOTL300_BIO	Total biomass of individuals in 300-count subsamples (coarse and fine net samples combined)	90.072878905	270.55043706	-3.09	1.4
Abundance/ Biomass/ Density	TOTL300_NAT_BIO	Total biomass of native individuals in 300-count subsamples (coarse and fine net samples combined)	90.072878905	269.19077886	-3.07	1.4
Abundance/ Biomass/ Density	ZOCN300_BIO	Biomass of individuals in 300-count subsample of coarse net sample (150 um)	81.538501524	226.56640233	-2.68	2.2
Abundance/ Biomass/ Density	ZOCN300_NAT_BIO	Biomass of native individuals in 300-count subsample of coarse net sample (150 um)	81.538501524	225.20674414	-2.65	2.2
Abundance/ Biomass/ Density	COARSE300_BIO	Biomass represented by individuals of large-sized taxa in 300-count subsamples (NET_SIZE_CLS=COARSE; coarse and fine net samples combined)	83.550340952	235.93896061	-2.77	3.0
Abundance/ Biomass/ Density	COARSE300_NAT_BIO	Biomass represented by native individuals of large-sized taxa in 300-count subsamples (NET_SIZE_CLS=COARSE; coarse and fine net samples combined)	62.150708119	234.5793024	-2.74	3.1
Abundance/ Biomass/ Density	COARSE300_NAT_PBIO	Percent biomass of native individuals of large-sized taxa in 300-count subsamples (NET_SIZE_CLS=COARSE; coarse and fine net samples combined)	85.15	75.20	1.88	5.7
Cladoceran	CLAD300_BIO	Biomass of individuals within the suborder Cladocera in 300-count subsamples (coarse and fine net samples combined)	62.150708119	173.03849657	-2.301	2.2
Cladoceran	CLAD300_NAT_BIO	Biomass of native individuals within the suborder Cladocera in 300-count subsamples (coarse and fine net samples combined)	61.59444164	171.73934691	-2.28	2.2
Cladoceran	LGCLAD300_BIO	Biomass represented by large cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=LARGE) in 300-count subsamples (coarse and fine net samples combined)	54.826014262	142.47459983	-1.92	2.2
Cladoceran	LGCLAD300_PIND	Percent of large cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=LARGE) in 300-count subsamples (coarse and fine net samples combined)	20.42	14.14	2.22	1.8
Cladoceran	LGCLAD300_NAT_BIO	Biomass represented by native large cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=LARGE) in 300-count subsamples (coarse and fine net samples combined)	54.826014262	142.37664379	-1.91	2.2

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Cladoceran	LGCLAD300_NAT_PIND	Percent of native large cladoceran individuals (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=LARGE) in 300-count subsamples (coarse and fine net samples combined)	20.41	13.47	2.49	1.8
Cladoceran	LGCLAD300_NAT_PTAX	Percent of distinct native taxa that are large cladocerans (SUBORDER=CLADOCERA and CLADOCEAN_SIZE=LARGE) in 300-count subsamples (coarse and fine net samples combined)	16.37	12.90	2.12	2.3
Cladoceran	DAPHNIID300_BIO	Biomass of individuals within the family Daphniidae in 300-count subsamples (coarse and fine net samples combined)	54.749187071	150.72825063	-2.08	3.0
Cladoceran	DAPHNIID300_NAT_BIO	Biomass of native individuals within the family Daphniidae in 300-count subsamples (coarse and fine net samples combined)	54.749187071	150.63029459	-2.08	3.0
Copepod	COPE300_BIO	Total biomass of individuals within the subclass Copepoda in 300-count subsamples (coarse and fine net samples combined)	22.109055071	66.786813029	-2.76	2.0
Copepod	COPE300_NAT_BIO	Total biomass of native individuals within the subclass Copepoda in 300-count subsamples (coarse and fine net samples combined)	22.109055071	66.726304529	-2.75	2.0
Copepod	CALAN300_BIO	Total biomass of individuals within the copepod order Calanoida in 300-count subsamples (coarse and fine net samples combined)	14.414470595	36.214300186	-2.00	3.2
Copepod	CALAN300_NAT_BIO	Total biomass of native individuals within the copepod order Calanoida in 300-count subsamples (coarse and fine net samples combined)	14.414470595	36.153791686	-1.99	3.2
Richness/Diversity	ZOFN300_NTAX	Number of distinct taxa in the 300-count subsample from the fine net sample (50-um)	7.3	8.4	-1.69	1.9
Richness/Diversity	SIMPSON300_NIND	Simpson diversity based on number of individuals (coarse and fine net samples combined)	0.307	0.306	0.08	0
Rotifer	ASPLAN300_PTAX	Percent of distinct taxa that are within the rotifer family Asplanchnidae in 300-count subsamples (coarse and fine net samples combined)	0.88	2.25	-2.04	1.3
Trophic	HERB300_BIO	Biomass of herbivorous individuals in 300-count subsamples (coarse and fine net samples combined)	75.625607619	201.15711961	-2.56	3.1
Trophic	HERB300_PBIO	Percent biomass of herbivorous individuals in 300-count subsamples (coarse and fine net samples combined)	76.31	65.36	2.06	3.6
Trophic	OMNI300_NTAX	Number of distinct taxa that are omnivorous in 300-count subsamples (coarse and fine net samples combined)	3.0	3.6	-1.94	1.8

Metric Category	Metric Name	Description	Mean Value for Least disturbed Sites	Mean Value for Most disturbed Sites	*t* value (Least disturbed vs. Most disturbed Sites)	Signal:Noise Value
Trophic	CLAD_PRED300_PTAX	Percent of distinct taxa that are predaceous cladocerans in 300-count subsamples (coarse and fine net samples combined)	0.87	0	2.67	Noise=0
Trophic	CLAD_HERB300_BIO	Percent biomass of herbivorous cladoceran individuals in 300-count subsamples (coarse and fine net samples combined)	62.140336143	173.03849657	-2.30	2.2
Trophic	COPE_OMNI300_BIO	Biomass of omnivorous copepod individuals in 300-count subsamples (coarse and fine net samples combined)	4.7491737381	24.176607243	-2.38	2.0
Trophic	COPE_OMNI300_PTAX	Percent of distinct taxa represented by omnivorous copepod individuals in 300-count subsamples (coarse and fine net samples combined)	8.16	11.5	-2.15	2.1

Chapter 8: From Analysis to Results

8.1 Background information

In the NLA 2012 report, lake condition estimates based on chemical, physical and biological information are expressed as percent of lakes or number of lakes; therefore, site weights from the probability design must be used to generate population estimates along with the data from the probability sites sampled (1038). Extent estimates for biological indicators and other measures are used to calculate relative and attributable risk.

8.2 Population Estimates

The survey design for the NLA, discussed in Chapter 2 of this report, produces a spatially-balanced sample using the NHD+ as the sample frame. Each lake has a known probability of being sampled (Stevens and Olsen 1999, Stevens and Olsen 2000, Stevens and Olsen 2004), and a sample weight is assigned to each individual site as the inverse of the probability of that lake being sampled. Sample weights are expressed in units of lakes.

The probability of a site being sampled was stratified by state and other factors. Site weights for the survey were adjusted to account for additional sites (i.e., oversample lakes) that were evaluated when the primary sites were not sampled (e.g., due to denial of access, being non-target). These site weights are explicitly used in the calculation of lake condition and extent estimates, so results can be expressed as estimates of lakes (i.e., numbers of lakes or percent of the entire resource) in a particular condition class for the entire conterminous U.S. For examples of how this has been done for other National Aquatic Resource Survey (NARS) assessments, see USEPA (2006), Olsen and Peck (2008), and USEPA (2009). It is important to note that the NLA was not designed to report on individual lakes or states, but to report at national and regional scales.

8.3 Lake Extent Estimates

Each NLA probability site is designated as least disturbed, moderately disturbed or most disturbed based on the appropriate indicator values and the thresholds established for that indicator and ecoregion. Next, the site weights from the probability design are summed across all sites in each condition class to estimate the percent of lakes nationally or in other sub populations (ecoregions, natural vs. manmade lakes, etc) in each condition category for the inference population. The survey design allows calculation of confidence intervals around these condition estimates and allows for estimates of the whole resource not just those lakes sampled. Note that only Visit 1 (i.e., the index visit) data and only probability sites are used in the calculation of extent. Hand-selected sites have a weight of zero. Using this method, the lakes in a particular condition class is estimated and reported in percent of lakes or number of lakes.

8.4 Relative Extent, Relative Risk and Attributable Risk

A major goal of the national aquatic surveys is to assess the relative importance of key stressors that impact aquatic biota on a national basis. EPA assesses the influence of stressors in three ways: relative extent (using the process described in 11.3), relative risk, and attributable risk. The following discussion describes the condition class assignments and calculations used in EPA's assessments. This discussion has been adapted from a journal article by Van Sickle and Paulsen (2008).

8.4.1 *Data preparation*

The NLA database contained the field and laboratory data for all sampled sites, whether selected as potential reference sites or from the statistical design. Within each region, least-disturbed sites (*i.e.*, reference sites described in Chapter 3) provide a benchmark against which all other sites were compared and classified. The condition classes for each stressor and biological response were determined from data and observations from the least-disturbed sites in each ecoregion and the continuous gradient of observed values at all sites.

The resulting three condition classes were defined as follows:

- Good (least disturbed): Not different from the reference sites;
- Fair (moderately disturbed): Somewhat different from the reference sites; and
- Poor (most disturbed): Markedly different from reference.

The condition classes were then used to estimate the extent, relative extent, relative risk, and attributable risk as described in the following sections.

8.4.2 *Methods*

8.4.2.1 *Estimating the Extent for Each Condition*

The estimated extent $\hat{E}$ measures the prevalence of a particular condition k (good, fair, or poor). For each *Y,* either a stressor or biological response, $\hat{E}$ provides an estimate of the number of lakes in that condition. For example, $\hat{E}$ could be the estimated number of lakes having excess phosphorus concentrations (*i.e.*, poor condition) in the lower 48 states.

The extent is estimated in two steps for each condition. The first step classifies each statistically selected site into one of the three conditions for each *Y*. The second step estimates the number of lakes using the estimated survey weights $\hat{w}_i$ for each site *i*, classified into condition *k.* Applying weights to the data allows inferences to be made about all lakes in the target population, not just the lakes from which physical samples were collected. Each sampled site is assigned an estimated weight for the number of lakes that it represents. For example, one site might represent 5,000 lakes in the entire target population, and thus, its sample weight would be $\hat{w}_{Yki}$ = 5,000. The following equation shows the estimation of extent ($\hat{E}_{X_k}$) for condition class *k* for each Y.

$$\hat{E}_{Y_k} = \sum_i \hat{w}_{Y_{k_i}} \quad (11.1)$$

8.4.2.2 *Relative Extent*

For each particular Y (*i.e.*, stressor or biological response), *Relative Extent* (RE_X) is the *proportion* of "poor" lakes in the target population. RE_X can also be interpreted as the probability that a lake *i* chosen at random from the population will have poor conditions for Y_i. In statistical terms where *k*=poor, this probability can be written as:

$$RE_{Y_{poor_i}} = \Pr(Y_i = Poor) \quad (11.2)$$

RE is estimated as the ratio of the sums of the sampling weights for the probability selected sites *i* assessed as: (1) poor condition and (2) all sites regardless of condition. Where n_k is the number of sites in each condition, $\widehat{RE}$ can be expressed in statistical terms as follows:

$$\widehat{RE}_{Y_{poor}} = \frac{\hat{E}_{Y_{poor}}}{\hat{E}_Y} = \frac{\sum_{i=1}^{n_{poor}} \hat{w}_{Y_{poor}i}}{\sum_{i=1}^{n_{poor}} \hat{w}_{Y_{poor}i} + \sum_{i=1}^{n_{fair}} \hat{w}_{Y_{fair}i} + \sum_{i=1}^{n_{good}} \hat{w}_{Y_{good}i}} \quad (11.3)$$

8.4.2.3 *Relative Risk*

Relative risk (*RR*) measures the likelihood (that is, the "risk" or probability) of finding poor (*P*) biological response *B* in a lake when the condition of a specific stressor *S* is also poor. For relative risk, the good and fair sites are combined into a single non-poor (*NP*) category. *RR*'s likelihood is expressed relative to the likelihood of poor biological response condition *B* in lakes that have non-poor stressor conditions *S*. That is,

$$RR = \frac{Pr(B=P|S=P)}{Pr(B=P|S=NP)} \quad (11.4)$$

To simplify the calculations, consider the notation in Table 8-1.

Table 8-1. Simplified Notation.

	Stressor (*S*)	
Biological Response (*B*)	Not-Poor (*NP*)	Poor (*P*)
Not-Poor (*NP*)	$Pr(B = NP \mid S = NP) = a$	$Pr(B = NP \mid S = P) = b$
Poor (*P*)	$Pr(B = P \mid S = NP) = c$	$Pr(B = P \mid S = P) = d$

Using the simplified notation, RR is estimated as follows:

$$\widehat{RR} = \frac{\frac{d}{b+d}}{\frac{c}{a+c}} \qquad (11.5)$$

RR = 1.0 indicates "No association" between stressor and response, that is, poor biological condition in a lake is equally likely to occur whether or not the stressor condition is poor. *RR* < 1.0 indicates that poor response condition is actually less likely to occur when the stressor is poor.

As a side note, using the simplified notation of Table 8-1, $\widehat{RE}_{S_{poor}}$ from the previous section (Equation 11.3) can be more simply written as:

$$\widehat{RE}_{S_{poor}} = \frac{b+d}{a+b+c+d} \qquad (11.6)$$

for a stressor *S* in poor condition.

8.4.2.4 *Attributable Risk*

Attributable risk (*AR*) estimates the change in ecological conditions when a stressor or biological response is reduced or removed. *AR* is based on a scenario in which the stressor would be restored through restoration activities to Not-Poor condition. For simplicity in terminology, this discussion refers to the stressor as being "eliminated." *AR* is then defined *as the proportional decrease in the extent of poor biological response condition that would occur if the stressor was eliminated from the poor category (only existed in good or fair) from lakes.*

Attributable risk is derived by combining relative extent and relative risk from the proceeding sections into a single estimate of the expected improvement in biological conditions if a particular stressor is eliminated from poor condition on a national or regional basis. Mathematically, *AR* is defined as:

$$AR = \frac{\Pr(Y = P) - \Pr(Y = P \mid S = NP)}{\Pr(Y = P)} \qquad (11.7)$$

We first calculated $RE_{Y,est}$ as shown in Equation 11.6 which is an estimate of Pr(Y = P). Then, using the notation in Table 8-1,

$$AR_{est} = [RE_{Y,est} - c/(a+c)] \,/\, RE_{Y,est} \qquad (11.8)$$

We calculated confidence intervals following the methodology described in Van Sickle and Paulsen (2008).

8.4.3 *Considerations When Calculating and Interpreting Relative Risk and Attributable Risk*

It is important to understand that contingency tables are created using a categorical, two-by-two matrix; therefore, only two condition classes / stress levels can be used. There are three ways in which condition classes / stress levels can be used for contingency tables:

- Good vs. Poor
- Good vs. Not-Good
- Not-Poor vs. Poor

where, “Not Good” combines fair and poor condition classes, and “Not Poor” combines good and fair condition classes. In the first bulleted method, “Good vs. Poor” data associated with the fair condition class is excluded from the analysis. Therefore, the results of the associated calculation of relative risk are affected by which one of the above combinations is used to make the contingency tables, and it is crucial that the objectives of the analysis are carefully considered to help guide this decision. For the NLA, for non-biological condition indicators (e.g., nutrients, physical habitat, etc.), a condition / stressor-level contingency table was created, comparing the Not Poor condition class (i.e., a combination of good condition and fair condition) to Poor condition class. This decision was made to indicate which stressors policy makers and managers may want to prioritize for management efforts to improve poor condition. After creating contingency tables, relative risk for each indicator was calculated.

A second consideration is that relative risk does not model joint effects of correlated stressors. In other words, each stressor is modeled individually, when in reality, stressors may interact with one another potentially increasing or decreasing impact on condition. This is an important consideration when interpreting the results associated with relative risk.

To appropriately interpret attributable risk, it is important to understand that attributable risk is associated with the following three major assumptions:

• ***Causality***, or that the stressor causes an increased probability of poor condition;
• ***Reversibility***, or that if the stressor is eliminated, causal effects will also be eliminated; and,
• ***Independence***, or that stressors are independent of each other, so that individual stressor effects can be estimated in isolation from other stressors.

These assumptions should be kept in mind when applying these results to management decisions.

Attributable risk provides much needed insight into how to prioritize management for the improvement of our aquatic ecosystems – lakes, in the case of the NLA. While the results of attributable risk estimates are presented as percent area in poor condition that could be reduced if the effects of a particular stressor were eliminated, these estimates are meant to serve as general guidance as to what stressors are affecting condition and to what degree (relative to the other stressors evaluated).

8.5 NLA 2007 versus NLA 2012 Change Analysis

8.5.1 *Background information*

One of the objectives of the National Lakes Assessment (NLA) is to track changes over time. The NLA conducted in 2012 was the second statistically valid survey of the nation's lakes and reservoirs. Previously, EPA and partners reported on the condition of the nation's natural and man-made lakes in the 2007 National Lakes Assessment. In NLA 2007, lakes 4 hectares and larger were sampled. As discussed earlier in the technical report, the NLA 2012 expanded the target population to include lakes within a smaller size class category (1-4 hectares). Because of this change in design between the two surveys, the change analysis can only assess lakes equal to or greater than 4 hectares. As with other NLA 2012 analyses, differences in the population condition estimates between surveys included both natural and man-made lakes.

8.5.2 *Data preparation*

All sites from NLA 2007 and all but 87 lakes (those from 1-4 hectares in size) from NLA 2012 were used in the change analysis. Due to changes in methodologies between NLA 2007 and NLA 2012, change estimates could not be made for some indicators, including zooplankton, total mercury, and methyl mercury. Additionally, change analysis was not conducted for acidification due to the relatively small percentage of lakes in condition classes other than least disturbed. Additionally, no changes analysis was conducted for atrazine since this indicator was not included in NLA 2007. All other indicators reported on in the NLA 2012 report were included in the change analysis.

8.5.3 *Methods*

Change analysis was conducted through the use of the spsurvey 3.3 package in R (Kincaid and Olsen, 2016). Within the GRTS (Generalized Random Tessellation Stratified) survey design, change analysis can be conducted on continuous or categorical response variables (e.g. least disturbed, moderately disturbed, and most disturbed). The analysis measures the difference between response variables of two separate surveys. For NLA 2012, the categorical response variables were used to compare changes between NLA 2007 and NLA 2012. When using categorical response variables, change is estimated by the difference in category estimates from the two surveys. Category estimates are defined as the estimated proportion of values in each category, for example least disturbed, moderately disturbed, and most disturbed categories. Change between the two years is statistically significant when the resulting error bars around the change estimate do not cross zero.

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Chapter 9: Quality Assurance Summary

The NLA has been designed as a statistically valid report on the condition of the Nation's lakes at multiple scales, i.e., ecoregion (Level II), and national, employing a randomized site selection process. The NLA is an extension of the EMAP methods for assessing lakes, similar to the 1997 Northeastern Lakes Assessment; therefore, it uses similar EMAP-documented and tested field methods for site assessment and sample collection as the Northeast Lakes Assessment.

Key elements of the NLA Quality Assurance (QA) program include:

Quality Assurance Project Plan – A Quality Assurance Project Plan (QAPP) was developed and approved by a QA team consisting of staff from EPA's Office and Wetlands, Oceans and Watersheds (OWOW) and Office of Environmental Information (OEI) and a Project QA Officer. All participants in the program signed an agreement to follow the QAPP standards. Compliance with the QAPP was assessed through standardized field training, site visits, and audits. The QAPP addresses all levels of the program, from collection of field data and samples and the laboratory processing of samples to standardized/centralized data management.

Field training and sample collection – EPA provided training sessions throughout the study area (with at least one instructor in each session) for all field crew members of each field crew team. All field teams were audited on site within the first few weeks of fieldwork. Adjustments and corrections were made on the spot for any field team problems. To assure consistency, EPA supplied standard sample/data collection equipment and site container packages for all random site, reference site, and repeat site sample collections.

Water chemistry laboratory QA procedures – NLA used the same single lab for all water chemistry samples. The Western Ecology Division (WED) was responsible for QA oversight in implementing the NLA QAPP and lab standard operating procedures (SOPs) for sample processing.

Zooplankton laboratory QA procedures – NLA used four labs, all four were audited for adherence to the NLA QAPP/SOP for benthic sample processing. This included internal quality control (QC) checks on sorting and identification of zooplankton and the use of the Integrated Taxonomic Information System for correctly naming species collected, as well as the use of a standardized data management system. Independent taxonomists were contracted to perform QC analysis of 10% of each labs samples (audit samples).

Benthic macroinvertebrate laboratory QA procedures – NLA used one lab, this lab was audited for adherence to the NLA QAPP/SOP for benthic macroinvertebrate sample processing. This included internal quality control (QC) checks on sorting and identification of benthic macroinvertebrates and the use of the Integrated Taxonomic Information System for correctly naming species collected, as well as the use of a standardized data management system. Independent taxonomists were contracted to perform QC analysis of 10% of each labs samples (audit samples).

Entry of field data – NLA used a standardized data management structure, i.e., the same standard field forms for data collected in the field, with centralized data entry through scanning in to electronic data files. Internal error checks were used to confirm data sheets were filled out properly.

Records management – These records include (1) planning documents, such as the QAPP, SOPs, and assistance agreements and (2) field and laboratory documents, such as data sheets, lab notebooks, and audit records. These documents are ultimately to be maintained at EPA. All data will eventually be archived in the STORET data warehouse at www.epa.gov/STORET.