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National Lakes Assessment 2022:

Technical Support Document

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Chapter 1: Project Overview

1.1 Overview

This document, the *National Lakes Assessment 2022: Technical Support Document*, accompanies the *National Lakes Assessment: The Fourth 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 2022 provides condition assessment results 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: The Fourth 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. It provides national-scale assessments and compares the condition of lakes to those from the earlier NLAs (2007, 2012, 2017) conducted by EPA and its partners. You can find results for regional scales and comparisons between natural lakes and reservoirs using the NLA 2022 interactive dashboard. This document 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 poor 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.

- National Lakes Assessment 2022: Quality Assurance Project Plan (EPA 841-B-21-009)(hereafter referred to as the NLA 2022 QAPP)
- National Lakes Assessment 2022: Site Evaluation Guidelines (EPA 841-B-21-008) (hereafter referred to as the NLA 2022 SEG)
- National Lakes Assessment 2022: Field Operations Manual (EPA 841-B-21-011) (hereafter referred to as the NLA 2022 FOM)
- National Lakes Assessment 2022: Laboratory Operations Manual (EPA 841-B-21-010) (hereafter referred to as the NLA 2022 LOM)

1.3 Considerations for the NLA 2022 TSD and public report

The EPA is working to stabilize benchmarks and data analyses across the NARS program to facilitate change and trend analyses. In NLA 2022, most aspects of the survey remained the same including the field methods, laboratory analyses, target population, benchmark selection process and data analyses. Changes since the NLA 2017 that are discussed in this document include:

- Updated sampling frame that uses NHDPlus High Resolution for all new lakes (see Chapter 2);
- The lake drawdown calculations and results are presented as small, medium and large conditions categories, in 2017 the categories included not large and large drawdown (see Chapter 5);
- The addition of enterococci and cylindrospermopsin (see Chapter 8); and
- The addition of Human Health Fish Tissue Indicator (see Chapter 9).

For purposes of identifying change and trends, prior survey results were recalculated based on updated 2022 benchmarks (see Appendix C) as needed. Given the above modifications, direct comparisons should not be made between the NLA 2022 results and those reported in earlier surveys as this will produce erroneous information.

Finally, the NLA 2022 public report and this document use the good/fair/poor terminology for condition class estimates consistent with the public report. Least, moderate, and most disturbed condition classes are also used in this document to describe anthropogenic disturbance pressure categories for index and model development (see Chapters 4, 5, and 7).

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.

This chapter provides details on the NLA survey design, sampling frame, analyses and estimated extent of the NLA lake population. Modifications to the survey design in 2022 are noted throughout the chapter and are summarized in *Appendix B: Survey Design Summary and Population Estimates for NLA 2007, 2012, 2017, and 2022*.

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, at least 1 meter deep, and have a minimum 0.1 ha of open water. In addition, lakes are required to have a minimum residence time of one week. The word "lake" in the remainder of this document includes lakes, reservoirs and ponds. The Great Lakes, Great Salt Lake and lakes that are tidally influenced are excluded; as are those used for aquaculture, disposal-tailings, sewage treatment, evaporation, or other unspecified disposal use.

NLA 2022 uses a spatially balanced survey design where lakes are viewed as a finite population (i.e., each lake is viewed as a point identified by the centroid of the lake polygon). To select sites for the NLA, EPA statisticians used a Generalized Random Tessellation Stratified (GRTS) (Stevens and Olsen 2004; Olsen et al. 2012) survey design for a finite resource with stratification and unequal probability of selection.

2.1.1 *Stratification*

The design is stratified by state. Within each state, lakes are selected using unequal probability categories based on lake area.

2.1.2 *Unequal probability categories*

Unequal probability categories used for the NLA 2017 subsample 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. For new NLA 2022 lakes, the unequal probability categories included 1 to 4 ha, 4 to 10 ha, 10 to 50 ha and greater than 50 ha. The collapsing to four lake area categories reflects that no differences in percent of non-target lakes nor in landowner access were found. Given that weight adjustment on all

evaluated sites is likely to use lake area categories, having fewer categories will result in more stable weight adjustments since they will be based on more evaluated lakes within a category.

2.1.3 *Fish Tissue Study*

A subset of the lakes selected using the above survey design will have fish sampled for the analysis of fish tissue contaminants. The subsample is approximately 2/3 of the base lakes selected for the main NLA 2022 survey. Approximately 50% of the lakes will be from the subsample of NLA 2017 lakes and 50% from new lakes selected for 2022. These lakes will be assigned to panels that will identify them.

2.1.4 *Panels*

The survey design incorporates lakes sampled in in prior NLAs as well as selecting new lakes. This improves the ability of the survey design to estimate change in condition in NLA 2022 from the condition in prior surveys. In addition, the survey design includes 96 lakes that are sampled twice in NLA 2022, providing information on measurement variability. These requirements result in five base and two oversample panels:

- **NLA22_17RVT2FT** – Panel of lakes originally sampled in NLA 2017. These lakes will be sampled twice in NLA 2022 for all indicators except for fish tissue which will be sampled for only one of the two visits.
- **NLA22_17BaseFT** – Panel of lakes originally sampled in NLA 2017 and will be sampled once in NLA 2022 for all indicators as well as fish tissue.
- **NLA22_17Base** – Panel of lakes originally sampled in NLA 2017 and will be sampled once in NLA 2022 for all indicators except fish tissue.
- **NLA22_22BaseFT** – Panel of new lakes to be sampled once in NLA 2022 for all indicators including fish tissue.
- **NLA22_22Base** – Panel of new lakes to be sampled once in NLA 2022 for all indicators except fish tissue.
- **NLA22_17Over** – Over sample lakes to be used as replacements for NLA22_17RVT2FT or NLA22_17BaseFT or NLA22_17Base lakes when they cannot be sampled for any reason. If the lake being replaced was scheduled to be sampled for fish tissue, then the replacement lake will be sampled for fish tissue.
- **NLA22_22Over** – Over sample lakes to be used as replacements for NLA22_22BaseFT or NLA22_22Base lakes when they cannot be sampled for any reason. If the lake being replaced was scheduled to be sampled for fish tissue, then the replacement lake will be sampled for fish tissue.

2.1.5 *Expected sample size*

For NLA 2022, 904 lakes will be sampled with 96 of the lakes sampled twice for a total of 1000 lake visits. Consequently, 904 unique sites will be sampled with 808 sampled only once and 96 sites being sampled twice during 2022 resulting in 1000 (808 + 2*96) total site visits. Reporting will be nationally as well as for nine aggregated ecoregions (CPL, NAP, SAP, UMW, NPL, SPL, TPL, WMT and XER). Approximately, 100 lakes will be sampled in each aggregated ecoregion. For each aggregated ecoregion, the number of lakes assigned to each state within the ecoregion will be proportional to the number of lakes in the sampling frame within the state. The total lakes for a state will be the sum across all ecoregions in the state. In addition, the minimum number of lakes for a state will be 8 and the maximum will be 50. With these constraints and with proportional allocation, two states (TX and MN) are allocated more than 50 lakes and 13 states (AZ, CT, DE, IA, MD, NH, NJ, NM, NV, RI, TN, VT, WV) have 8 or fewer. For these states, lakes in the sampling frame are allocated by ecoregion within each state to get minimum of 8 and maximum of 50. Then the remaining states are re-allocated lakes by ecoregion to satisfy the total sample size. The final allocation by state and aggregated ecoregion is given in Table 2-1. Approximately 50% of the lakes will be lakes sampled in NLA 2017 that were sampled as new lakes in 2017.

The survey design does not select lakes based on aggregated ecoregions; only the total number of lakes for a state is specified in the survey design. For new lakes, approximately an equal number of lakes by the four lake area categories are selected with unequal probability within each state. For lakes sampled as new lakes in 2017, the lakes selected for 2022 are the first lakes evaluated in 2017 to meet the sample size requirement for 2017 lakes to be resampled in 2022. Note that these are the expected number of lakes and not the final number of lakes selected by the survey design (see section “Final Survey Design Summary”).

Table 2-1. National Lakes Assessment 2022 Initial Design. The number of lakes to be sampled by state and the final design by aggregated ecoregion.

State	CPL	NAP	NPL	SAP	SPL	TPL	UMW	WMT	XER	Total
AL	11	0	0	3	0	0	0	0	0	14
AR	7	0	0	3	0	0	0	0	0	10
AZ	0	0	0	0	0	0	0	4	4	8
CA	0	0	0	0	0	0	0	25	23	48
CO	0	0	0	0	9	0	0	8	2	19
CT	0	8	0	0	0	0	0	0	0	8
DE	7	0	0	1	0	0	0	0	0	8
FL	11	0	0	0	0	0	0	0	0	11
GA	22	0	0	11	0	0	0	0	0	33
IA	0	0	0	0	0	7	2	0	0	9
ID	0	0	0	0	0	0	0	10	6	16
IL	0	0	0	1	0	15	1	0	0	17
IN	0	0	0	2	0	16	5	0	0	23
KS	0	0	0	0	5	14	0	0	0	19

State	CPL	NAP	NPL	SAP	SPL	TPL	UMW	WMT	XER	Total
KY	0	0	0	8	0	1	0	0	0	9
LA	13	0	0	0	0	0	0	0	0	13
MA	1	7	0	0	0	0	0	0	0	8
MD	6	0	0	2	0	0	0	0	0	8
ME	0	15	0	0	0	0	0	0	0	15
MI	0	0	0	0	0	1	28	0	0	29
MN	0	0	0	0	0	3	48	0	0	51
MO	2	0	0	5	0	7	0	0	0	14
MS	11	0	0	0	0	0	0	0	0	11
MT	0	0	28	0	0	0	0	17	0	45
NC	5	0	0	7	0	0	0	0	0	12
ND	0	0	23	0	0	15	0	0	0	38
NE	0	0	0	0	24	5	0	0	0	29
NH	0	8	0	0	0	0	0	0	0	8
NJ	4	0	0	4	0	0	0	0	0	8
NM	0	0	0	0	2	0	0	2	4	8
NV	0	0	0	0	0	0	0	0	8	8
NY	1	28	0	1	0	0	0	0	0	30
OH	0	4	0	3	0	6	0	0	0	13
OK	2	0	0	5	23	5	0	0	0	35
OR	0	0	0	0	0	0	0	15	6	21
PA	0	7	0	6	0	0	0	0	0	13
RI	1	8	0	0	0	0	0	0	0	9
SC	8	0	0	0	0	0	0	0	0	8
SD	0	0	18	0	0	21	0	1	0	40
TN	4	0	0	4	0	0	0	0	0	8
TX	26	0	0	0	23	0	0	0	1	50
UT	0	0	0	0	0	0	0	6	8	14
VA	4	0	0	7	0	0	0	0	0	11
VT	0	8	0	0	0	0	0	0	0	8
WA	0	0	0	0	0	0	0	20	7	27
WI	0	0	0	0	0	4	22	0	0	26
WV	0	0	0	8	0	0	0	0	0	8
WY	0	0	5	0	2	0	0	11	8	26
Sum	**146**	**93**	**74**	**81**	**88**	**120**	**106**	**119**	**77**	**904**

Table 2-2. Actual number of sites sampled for NLA 2022 by design categories, including state intensification sites that were used in the national condition estimate analyses. Two sampled sites were determined to be non-target and removed from the national analyses.

State	CPL	NAP	NPL	SAP	SPL	TPL	UMW	WMT	XER	Total
AL	10			4						14
AR	8			2						10
AZ								4	4	8
CA								20	26	46
CO					6			13		19
CT		8								8
DE	7			1						8
FL	11									11
GA	15			18						33
IA						8				8
ID								10	6	16
IL				1		16				17
IN				7		33	10			50
KS					4	16				20
KY	1			7		1				9
LA	13									13
MA	3	6								9
MD	2			6						8
ME		15								15
MI						4	46			50
MN						10	40			50
MO				4		10				14
MS	11									11
MT			23					22		45
NC	3			9						12
ND			28			12				40
NE			1		17	11				29
NH		8								8
NJ	4	1		3						8
NM					2			3	3	8
NV								1	7	8
NY	1	29								30
OH		7		2		4				13
OK	1			4	25	5				35
OR								18	3	21
PA		9		4						13
RI	1	7								8
SC	3			5						8
SD			12			33		1		46

State	CPL	NAP	NPL	SAP	SPL	TPL	UMW	WMT	XER	Total
TN	4			4						8
TX	21				27				2	50
UT								13	1	14
VA	5			6						11
VT		8								8
WA								16	11	27
WI						4	46			50
WV				8						8
WY			3		1			17	5	26
Sum	**124**	**98**	**67**	**95**	**82**	**167**	**142**	**138**	**68**	**981**

2.2 Sampling frame summary

The sampling frame was derived from the National Hydrography Dataset Plus High Resolution (NHDPlus HR) data layer. The total number of waterbody polygons in NHDPlus HR is 6,512,454, which includes several non-target waterbody types (e.g., swamp/marsh, estuary, etc). Attributes were created to identify the polygons that are lakes to included in the sampling frame and those to exclude from the sampling frame. First, polygons that were less than or equal to 1 hectare were excluded. Next polygons were included or excluded based on the NHD FTYPE.

Lakes included were FTYPEs:

- Lake/Pond
- Lake/Pond: Hydrographic Category = Perennial
- Lake/Pond: Hydrographic Category = Perennial; Stage = Average Water Elevation
- Lake/Pond: Hydrographic Category = Perennial; Stage = Date of Photography
- Lake/Pond: Hydrographic Category = Perennial; Stage = Normal Pool
- Lake/Pond: Hydrographic Category = Perennial; Stage = Spillway Elevation
- Stream/River: Hydrographic Category = Perennial

Lakes excluded were FTYPEs:

- Estuary
- Playa
- Inundation Area: Inundation Control Status = Not Controlled
- Lake/Pond: Hydrographic Category = Intermittent
- Lake/Pond: Hydrographic Category = Intermittent; Stage = Date of Photography
- Lake/Pond: Hydrographic Category = Intermittent; Stage = High Water Elevation
- Lake/Pond: Hydrographic Category = Perennial; Stage = Normal Pool
- Reservoir
- Reservoir: Construction Material = Earthen
- Reservoir: Construction Material = Nonearthen

- Reservoir: Reservoir Type = Aquaculture
- Reservoir: Reservoir Type = Cooling Pond
- Reservoir: Reservoir Type = Decorative Pool
- Reservoir: Reservoir Type = Disposal
- Reservoir: Reservoir Type = Disposal; Construction Material = Earthen
- Reservoir: Reservoir Type = Disposal; Construction Material = Nonearthen
- Reservoir: Reservoir Type = Evaporator
- Reservoir: Reservoir Type = Evaporator; Construction Material = Earthen
- Reservoir: Reservoir Type = Filtration Pond
- Reservoir: Reservoir Type = Settling Pond
- Reservoir: Reservoir Type = Sewage Treatment Pond
- Reservoir: Reservoir Type = Tailings Pond
- Reservoir: Reservoir Type = Tailings Pond; Construction Material = Earthen
- Reservoir: Reservoir Type = Water Storage
- Reservoir: Reservoir Type = Water Storage; Construction Material = Earthen; Hyd*
- Reservoir: Reservoir Type = Water Storage; Construction Material = Earthen;
- Hydrographic Category = Intermittent
- Reservoir: Reservoir Type = Water Storage; Construction Material = Earthen;
- Hydrographic Category = Perennial
- Reservoir: Reservoir Type = Water Storage; Construction Material = Nonearthen
- Reservoir: Reservoir Type = Water Storage; Hydrographic Category = Perennial
- Reservoir; Reservoir Type = Treatment
- Swamp/Marsh
- Swamp/Marsh: Hydrographic Category = Intermittent
- Swamp/Marsh: Hydrographic Category = Perennial"

Note that excluding lake objects that are coded “Reservoir” by NHD does not exclude run-of-the-river reservoirs or constructed ponds.

This review identified 497,840 lake objects to be included in the NLA 2022 sampling frame (Table 2-3). The number of lake objects in the sampling frame by aggregated ecoregions and lake are presented in Table 2-4.

Table 2-3 Number of waterbody objects in NHDPlusHR by type and sampling frame inclusion.

FTYPE	Exclude	Include	Total
LakePond	4,838,144	466,697	5,304,841
Reservoir	249,155	31,143	280,298
Estuary	8,592	0	8,592
Ice Mass	7,802	0	7,802
Playa	17,768	0	17,768
SwampMarsh	893,153	0	893,153
Total	6,014,614	497,840	6,512,454

Table 2-4 Number of lake objects in the sampling frame by aggregated ecoregion and lake area category.

Aggregated Ecoregion	1-4ha	4-10ha	10-50ha	>50ha	Total
Coastal Plains	122,756	25,436	13,004	2,965	164,153
Northern Appalachians	20,929	6,147	4,748	1,973	33,797
Northern Plains	24,520	4,436	2,339	668	31,963
Southern Applalachians	39,413	5,201	2,346	755	47,714
Temperate Plains	54,274	10,853	6,410	1,859	73,406
Upper Midwest	30,928	10,963	9824	4,052	55,767
Western Mountains	17,319	4,963	2,880	993	26,155
Xeric	11,330	2,775	1,907	825	16,837
Total	359,553	77,086	46,430	14,771	497,840

2.3 Survey design implementation and analysis

Field crews evaluated lakes from the NLA survey design using a variety of techniques including aerial photo interpretations, GIS analyses, local knowledge, etc. to identify lakes selected from the sampling frame that did not meet the definition of a lake for NLA. Crews also dropped lakes from sampling during field reconnaissance if they were a non-target type or could not be assessed due to accessibility issues (landowner permission, too dangerous to access, etc.). Dropped lakes were systematically replaced from a pool of replacement ("over sample") lakes from the survey design. This process is implemented to maintain the integrity of the survey design and to sample lakes consistent with the original number planned in different categories. In 2022, 3,636 lakes were evaluated by field crews.

Any statistical analysis of NLA data must incorporate information about its survey design and implementation. The statistical analysis accounts for the stratification and unequal probability selection by using the survey design weights. The initial survey design weights are adjusted to account for the change in sample size due to the use of over sample lakes within the strata and unequal probability categories, i.e., the design-as-implemented weights. The adjusted weight represents the number of lakes that each evaluated lake represents. The sum of all adjusted weights for lakes evaluated equals the number of lakes in the sampling frame. The subset of the lakes that are evaluated as target lakes and sampled is used to estimate the "sampled population" of lakes by using the design-as-implemented adjusted weights. Not all lakes evaluated as target lakes could be sampled. To account for these lakes, a second weight adjustment, non-response weight adjustment, is completed that enables the lakes that are target lakes and sampled to be used to estimate the "target population" of lakes.

The statistical estimates for the NLA population estimates were completed using lake weights (see the NLA 2022 Site Information - Data file) and the R package 'spsurvey' (Dumelle et. al. 2023). Population estimates were determined at the national level and for several subpopulations described in Chapter 10 .

2.4 Estimated number of the NLA lakes and implications for reporting

The number of lakes in the NLA 2022 target population is not known and must be estimated based on the lake evaluation conducted during the implementation of the survey design. The survey design identifies lakes for evaluation from the sampling frame, which is a subset of lake objects in NHDPlus HR described in Section 2.2. The NLA 2022 survey design identified 6,707 lake polygons for further evaluation. The NHD information may be termed the source of the sampling frame. Note that the subset is selected such that all lake objects in the sampling frame is expected to include all lake objects that are in the target population and may include lake objects that the lake evaluation determines are not in the target population. An assumption is that the sampling frame does include all lakes in the target population.

The lake evaluation categorizes the lake objects in the sample as non-target, target-not-sampled, target-sampled and unknown. The target-not-sampled and target-sampled categories are used to estimate the extent of the target population. Since not all lakes that are target lakes can be sampled, the target-sampled lakes are used to estimate the extent of the sampled population. The sampled population conceptually is all the target lakes that could have been sampled if they were selected. The difference between the target population and sampled population is due to “non-response” for target lakes that could not be sampled.

The initial survey design results in a survey weight for each lake that assumes that only lakes selected to be sampled are evaluated and sampled. Since some lakes selected to be sampled turn out to be non-target lakes or target lakes that cannot be sampled, additional lakes must be evaluated to achieve the sample size required for each state. The initial weights are adjusted for the survey design as implemented, i.e., the additional lakes evaluated. This initial lake weight adjustment results in weights that may be used to estimate the extent of the lake population and the characteristics of the sampled population. In 2007 and 2012, these weights for the design as implemented were used for population estimates. The sampled population estimates lead to inappropriate assumptions about the survey results (e.g., the assumption that target lakes that could not be sampled are missing completely at random). For 2017 and 2022, EPA determined it was more appropriate to do a second weight adjustment so that the weights reflect the complete estimated target population. This weight adjustment accounts for the “non-response”, i.e., target lakes that could not be sampled. The weight adjustment assumes that target lakes that could not be sampled are missing at random within the weight adjustment categories based on the combination of state and lake area categories. See Appendix B for a summary of the NLA survey design characteristics and estimated extent for all four surveys.

Figure 2.1 shows the known number of lake objects in the sampling frame, the number of lakes evaluated, and the number of lakes sampled to represent the estimated target population of 268,018. Note that to estimate the target population requires assumptions to be made about the target lakes in the sample that could not be sampled. It is assumed that within a state, that lakes in the same aggregated ecoregion and lake area category that could not be sampled would have characteristics similar to those lakes that could be sampled.

Figure 2.1. The number of lake objects in the NLA 2022 sampling frame, evaluated lakes, sampled lakes and lakes in the NLA target population.

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Chapter 3: Defining Reference Sites and Condition

3.1 Background information

NLA analysts used two types of benchmarks for determining condition estimates (good, fair, poor; above/below benchmark, etc) in the NLA public report. For trophic status, recreational indicator microcystin, dissolved oxygen, and atrazine, analysts used fixed, nationally consistent benchmarks that are discussed in Chapter 6 of this document. The second approach was to establish regionally consistent reference-based benchmarks.

Reference sites are those locations that display the best available (or least-disturbed) chemical, physical, and biological habitat condition given the current state of the landscape. To identify these sites, data from proposed sites were compared to a definition of what is least disturbed by human activities. To reflect the natural variability of the U.S., the definition of what is least disturbed varies by ecological region (ecoregion). The approach used in the NLA for developing benchmarks using reference conditions is consistent with current science, EPA guidance, state practice, and established protocols for ecological assessment (Bailey et al., 2004; Barbour et.al., 1999; Carter and Resh, 2013; Hughes, 1995; Reynoldson et.al., 1997; Stoddard et.al., 2006; and USEPA, 2011).

The EPA's approach for establishing reference conditions in the NLA is a well-documented, systematic process that screens sites using chemical and physical data to identify the least disturbed sites within each ecological region. The application of percentiles for selecting benchmarks is also consistent with established guidance and practice within the scientific community and state programs (Arizona DEQ, 2012; Vermont DEC, 2016; USEPA *Case Studies*).

The specific approaches used in the NLA have been used in various water quality surveys since the early 1990s and in the scientific literature since the mid-1990s (US EPA, 1998; Barbour et al., 1999; Gerritsen, 1995; Stoddard et al., 2006; and Herlihy et al., 2008). The reference-based approach is used by many organizations for defining benchmarks for assessing water quality. Related to nutrients, EPA's guidance for development of nutrient criteria includes identification of reference reaches considered to be the least impacted systems of the ecological region and recommends the 75th percentile of the nutrient reference condition distribution for selecting a criterion (USEPA 2000). Detailed information on the regionally consistent approach is presented below. A summary of all benchmarks used to generate the condition estimates in the public report can be found in Appendix C.

In refining benchmarks for NLA 2017, some 2012 benchmark values were updated; therefore, direct comparisons should not be made between 2017 and 2022 reported results and the results in 2007 and 2012 reports, as this will produce erroneous results. For purposes of identifying change in this document and the public report, prior results were recalculated based on new benchmarks as needed.

To assess ecological condition, it is standard scientific practice to compare measurements to reference condition. The NLA approach for identifying reference sites is more inclusive than

some approaches that restrict reference sites to only those with no or minimal human modification; or historical, pre-industrial or pre-Columbian conditions. Because of this, reference sites for this analysis are more accurately described as "least disturbed sites." Least disturbed sites contain 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). Benchmarks were based on the distribution or range of values found for each indicator at the reference sites (or sites with the best available conditions given today's state of the landscape) in each of nine major ecoregions. A total of four sets of reference sites were developed for use in establishing reference condition for the NLA results: one for the benthic macroinvertebrate 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 2017 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. Reference site classification and screening was done using the nine aggregate NARS ecoregions (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, 2012 or 2017 were considered potential reference lakes. 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 NARS ecoregions were used for the ecoregion classification although in some cases these ecoregions were further combined or lake types (natural vs. human-made) within an ecoregion were 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.

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 criterion was relaxed for lakes with elevated levels of lakeshore disturbance, as indexed by HiiALL_syn > 0.75. A step by step key to reference screening NLA lakes impacted by drawdown is provided in Table 3-3.

Table 3-1. Least disturbed reference screening filter thresholds for NLA 2017.
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.

Note that additional screening and refinement for macroinvertebrates, zooplankton, physical habitat, and nutrient reference sites are described subsequently in their respective chapters.

Table 3-2. Most disturbed site screening thresholds for NLA 2017.
If a lake exceeded any one of the thresholds it was considered a most disturbed site for that ecoregion. One screen for acidification was applied universally across all ecoregions, lakes with ANC ≤ 0 ueq/L and DOC < 5 mg/L were considered most disturbed.

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) ≥ 5%
   Yes – **Lake Drawdown, Not Reference**
   No – go to 6
6. Lake Disturbed, HiiAll_syn > 0.75
   Yes – **Lake Drawdown, Not Reference**
   No - **LAKE OK**

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Chapter 4: Benthic Macroinvertebrates

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 using 2007 and 2012 NLA data as described in Section 4.3. This NLA 2012 MMI and its condition class benchmarks (Table 4-3) were used for the 2017 and 2022 macroinvertebrate assessments.

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.

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, from the dataset, 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.

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 , 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 for tolerance values, functional feeding group and habitat preferences, and taxonomic resolution.

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; and
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 important 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-level 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 *Dataset*

The NLA macroinvertebrate 300 fixed count data were 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. The NLA macroinvertebrate MMI was developed using the combined the NLA 2007 and 2012 benthic metric files which were both calculated with common autecology and taxonomic resolution. All reference sites were defined using the NLA definitions described in Section 3 based on nine aggregate ecoregion criteria. Reference sites that had less than 250 individuals were not used as reference for MMI development. Altogether, there were 2330 site visits (samples) in the data used to develop the MMI; 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*

Many 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 for them will be set to missing values. These are identified as “not assessed” for macroinvertebrates in the NLA. In the NLA 2017 data, 60 of the 1191 samples had < 100 individuals. In NLA 2022, 55 of the 1071 samples had <100 individuals.

4.3.3 *Ecoregion classification*

For the NLA macroinvertebrate MMI development, the nine national aggregate ecoregions (Figure 3-1) were consolidated 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.

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 (L) versus the most disturbed (M) 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 10/6 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)); and
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 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	CHIRDOM5PIND	Negative	55.71	100.0
Coastal Plains	Feeding Group	PREDNTAX	Positive	6.00	23.0
Coastal Plains	Habit	SPWLNTAX	Positive	5.00	15.0
Coastal Plains	Richness	EPT_NTAX	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	CHIRDOM5PIND	Negative	57.46	95.24
E. Highlands	Feeding Group	COGANTAX	Positive	8.00	27.0
E. Highlands	Habit	CLNGNTAX	Positive	3.00	12.0
E. Highlands	Richness	EPOTNTAX	Positive	2.00	14.0
E. Highlands	Tolerance	TL23NTAX	Positive	1.00	9.00
Plains	Composition	DIPTPTAX	Negative	16.67	60.00
Plains	Diversity	CHIRDOM5PIND	Negative	50.44	100.0
Plains	Feeding Group	PREDNTAX	Positive	2.00	19.0
Plains	Habit	CLMBPTAX	Positive	10.0	33.33
Plains	Richness	EPOTNTAX	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	89.29

Ecoregion	Metric Class	Metric name*	Direction	Floor Value	Ceiling Value
Upper Midwest	Feeding Group	SHRDPIND	Negative	2.67	50.67
Upper Midwest	Habit	CLNGNTAX	Positive	3.00	14.0
Upper Midwest	Richness	CRUSNTAX	Negative	0	3.00
Upper Midwest	Tolerance	TL23PTAX	Positive	2.17	23.81
Western Mts.	Composition	DIPTPIND	Positive	5.97	84.33
Western Mts.	Diversity	HPRIME	Positive	1.09	2.87
Western Mts.	Feeding Group	SCRPNTAX	Negative	0	5.00
Western Mts.	Habit	CLNGNTAX	Positive	1.00	8.00
Western Mts.	Richness	EPT_NTAX	Positive	0	7.00
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

PREDNTAX = Predator Taxa Richness

COGANTAX = Collector-Gatherer Taxa Richness

SHRDPIND = % Shredder Individuals

SCRPNTAX = Scraper Taxa Richness

SPWLNTAX = Sprawler Taxa Richness

CLNGNTAX = Clinger Taxa Richness

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

EPT_NTAX = Ephemeroptera + Plecoptera + Trichoptera Taxa Richness

EPOTNTAX = Ephemeroptera + Plecoptera + Trichoptera + Odonata Taxa Richness

CRUSNTAX = Crustacean Taxa Richness

TRICNTAX = Trichoptera Taxa Richness

NTOLPIND = % Individuals with pollutant tolerance values < 6

TL23NTAX= 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. Benthic MMI statistics for the NLA 2007-2012 data used to develop the MMI.

Ecoregion	F-test	Box Delta	Max Corr.	Mean Corr.	S:N
Coastal Plains	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 least disturbed (reference) and most disturbed 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.

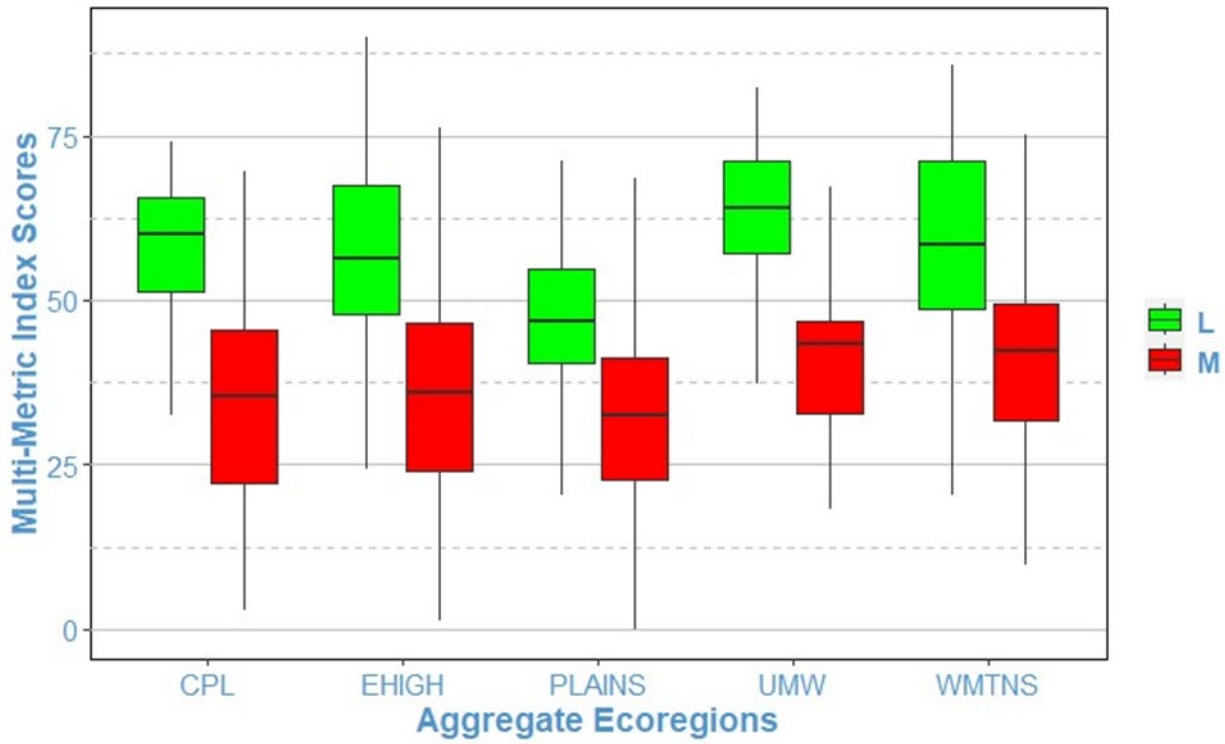

Figure 4.1. Box and whisker plots showing discrimination between least disturbed reference (L) and most disturbed (M) sites by biological ecoregion in the NLA 2007-2012 data used to develop the MMI. Boxes show the interquartile range and the whiskers show the 5th and 95th percentiles. Outliers are not presented.

4.3.6 *Setting MMI benchmarks*

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.

For calculating the benchmarks used in the NLA 2022 public report, we used all NLA reference sites sampled from 2007-2017 to maximize sample sizes used to calculate percentiles. When a site was sampled multiple times, only the first visit to the most recent year of sampling was used to calculate percentiles so sites were not double-counted. Also, only reference sites with at least 250 individuals were used. Before calculating benchmarks, a 1.5*IQR outlier analysis was done on the reference site MMIs to remove outliers. No sites were dropped as outliers in this process leaving 416 reference sites for calculating reference site percentiles to use as benchmarks. The resulting adjusted MMI benchmark values for the condition classes in each ecoregion are given in Table 4-3.

Table 4-3. Macroinvertebrate MMI benchmarks using 2007-2017 reference site data

Ecoregion	# of Ref Sites	Least Disturbed 25th Percentile Benchmark	Most Disturbed 5th Percentile Benchmark
Coastal Plains	29	≥ 51.8	< 40.4
East. Highlands	105	≥ 44.5	< 31.4
Plains	84	≥ 39.5	< 26.6
Upper Midwest	76	≥ 51.4	< 37.2
Western Mountains	122	≥ 47.6	< 32.6

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 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. For the

NLA 2017 physical habitat condition we used the same expected condition models that we used for the 2012 Assessment, with the exception of lake drawdown that is discussed in more detail below.

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 benchmarks 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, USEPA 2017). 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 2017 national probability sample of lakes and reservoirs. For the NLA 2017 physical habitat condition we used exactly the same expected condition models that we used for the 2012 Assessment, which were derived using combined NLA 2007 and NLA 2012 data, including reference sites defined based on NLA 2012 screening criteria. [But see notes on accommodating missing horizontal and vertical lake drawdown measurements.]

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 most disturbed lakes.

5.3 Methods

5.3.1 *NLA sites used for expected condition modeling and precision estimates*

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 data were collected between May and October of each survey year. See Chapter 2 of this document for additional details on the study area and site selection. To model expected condition for all four NLA surveys (’07, ’12, ’17, ‘22), we used physical habitat data collected in the 2007 and 2012 survey years. These data included data from 2268 lakes and reservoirs, 1156 in 2007, and 1112 in 2012. 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, USEPA 2017, 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 $\times$ 15m) abutting the shoreline, a riparian plot (15m $\times$ 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) including 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 grouping included 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 to be exceeded 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 most 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 most 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 2007,2012 and 2017 datasets released by the EPA on the NARS Data webpage. 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 $\boldsymbol{Rc_{syn}}$, 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:

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

where:

$\boldsymbol{Rc_{syn}}$ = 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.

$\boldsymbol{Rp_{draw}}$ and $\boldsymbol{Rp_{rip}}$ 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. [NOTE for NLA-2017 ONLY: There were a large number of missing measurements of horizontal and vertical drawdown in the 2017 survey. The field protocol directs field crews to NOT establish a drawdown plot when horizontal drawdown is <=1m. For 2017 we assumed drawdown was <1m where no Drawdown Plot was established and set horizontal drawdown to zero meters for the calculation of Eq 1. Specifically, that means setting $\boldsymbol{Rp_{draw}}$=0 and $\boldsymbol{Rp_{rip}}$=1.0.]

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 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:

Rp_{draw} = 10/15 = 0.67

Rp_{rip} = (1.0 - 0.67) = 0.33

Drawdown Canopy cover: Rc_{draw} = 0%

Riparian Canopy cover: Rc_{rip} = 100%

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 (Rc_{rip}) and that within 15 m of the lakeshore (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 was zero:

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

where:

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.

Lp_{draw} **and** 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). [NOTE for NLA-2017 ONLY: There were a large number of missing measurements of horizontal and vertical drawdown in the 2017 survey. The field protocol directs field crews to NOT establish a drawdown plot when horizontal drawdown is <=1m. For 2017 we assumed drawdown was <1m where no Drawdown Plot was established, and set horizontal drawdown to zero meters for the calculation of Eq .2. Specifically, that means setting Lp_{draw}=0 and Lp_{lit}=1.0.]

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

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

Lc_{lit} and 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 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:

Lp_{draw} = 10/10 = 1.00

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 document:*

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 document, 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 documnet, 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 human-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; (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.

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

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 boulder*s (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* ≥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 complexity index *LitRipCvrQ*:

$$\boldsymbol{LitRipCvrQ} = \frac{\left(\boldsymbol{RVegQ_n} + \boldsymbol{LitCvrQ_x}\right)}{2}; \qquad \text{(Eq 12)}$$

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 human-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 NLA 2007 and 2012 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 (human-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* $\leq$0.20
Fair (Medium Disturbance):	*RDis_IX* >0.20 but $\leq$ 0.75
Poor (High Disturbance):	*RDis_IX* >0.75

Lakes with *RDis_IX* $\leq$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 NLA 2007 and 2012 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 $\pm$ 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 $\geq 25^{th}$ 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 $<5^{th}$ 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 5^{th} and 25^{th} 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 most 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 human-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 human-made reservoirs. Vertical and horizontal drawdown were considered small if they were $\leq 75^{th}$ percentile of their respective reference distributions; large if $>95^{th}$ 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.

NOTE for NLA 2017 ONLY:
In several hundred NLA-2017 sample lakes, field crews did not measure horizontal or vertical drawdown in cases where they did not establish drawdown zone cover plots. In these cases, we assumed that missing horizontal drawdown values were <1m when no drawdown cover plots were established. Because the criteria for small drawdown in many regions are smaller than 1m, we could not evaluate drawdown in this least-altered condition class for all regions and lake origin classes (natural and human-made). We could not distinguish between medium and small drawdown classes for all regions and lake origin classes when horizontal drawdown values were quantified only as <1m. Consequently, we defined only two drawdown condition classes that could be nationally applied for the 2017 Assessment: “Large” and “Not Large”. We defined overall lake drawdown condition as Large if either vertical or horizontal drawdown or both were large, and “Not Large” if both vertical and horizontal drawdown were medium or small.

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 human-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, which is a good representation of precision for NLA 2017, based on Kaufmann et al. (2014a) and the NLA 2012 Technical Support Document (USEPA 2017b).

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 most-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 most disturbed sites. Note that a site with very low *RDis_IX* could be classified as most-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 most-disturbed lakes (Table 5-8, Figure 5-5). Except for lake drawdown, contrasts were very similar for the NLA 2007 and 2012 surveys (Figure 5-6). Although the t test between reference and most 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 most 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 most-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.

Fergus et al. (2020) examined differences in lake hydrologic variables between the 2007 and 2012 surveys, providing insight on the sensitivity of lake levels and water balance parameters to inter-annual climate conditions. Between-year variation in water-level decline was greater on natural lakes than human-made lakes, suggesting that natural lakes are more responsive to

changes in weather. They reported less vertical drawdown in natural lakes in 2012 (a cooler, wetter weather year) compared to 2007, whereas large drawdown persisted on human-made lakes, particularly in western regions. Dam and outlet structures can significantly alter lake and stream hydrology and potentially mask effects from climate or weather. Fergus et al. (2020, 2021) suggested, based on the 2007– 2012 changes in evaporative concentration and water levels and an index of the potential for anthropogenic hydro-alteration, that water levels in natural lakes levels may be more responsive to temperature and precipitation in a given year, whereas water levels in human-made lakes may be more strongly influenced by water management and indirectly by weather conditions, particularly in western U.S. regions. Fergus et al. (2021) also showed evidence that in the wetter eastern regions of the U.S., water management infrastructure is used to stabilize lake and reservoir water levels, whereas water management for irrigation, hydropower, and water supply in the drier regions leads to greater level fluctuation and drawdown.

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 most 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 public 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.

5.8 Literature cited

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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
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 Model	*RVegQ_OE*	(-0.02799)	**(0.3165)**	(0.84)	**(2.07)**	-------	-------
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	**0.2983**	0.98	**1.99**	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)	-------	-------
CENPL OE Yint	" "	+0.04303	0.2246	1.00	1.68	0.3703	0.6808
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**	**Human-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**
" "	Human-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**
" "	Human-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.

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)

Precision is expressed as: 1) the pooled standard deviation of repeat visits (σrep), 2) precision relative to potential or observed range (σrep/Rgpot and σrep/Rgpot), 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 = σ2lake/σ2rep). 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, RVegQc is the Riparian vegetation cover & structure index, Log(RVegQc3OE) is the log-transformed O/E index for Riparian vegetation cover & structure, LitCvrQc is the Littoral cover complexity index, Log(LitCvrQc3OE is the log-transformed O/E index for Littoral cover complexity , LitRipCvrQc is the Littoral-riparian habitat complexity index, Log(LitRipCvrQc3OE) is the log-transformed O/E index for Littoral-riparian habitat complexity, L_VertDD = Log10(Vertical drawdown +0.1m), and L_HorizDD = Log10(Horizontal drawdown + 1m).

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}	$p_{RT} > \lvert t_{RT} \rvert$
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 most 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 = L and M), 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***
Human-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

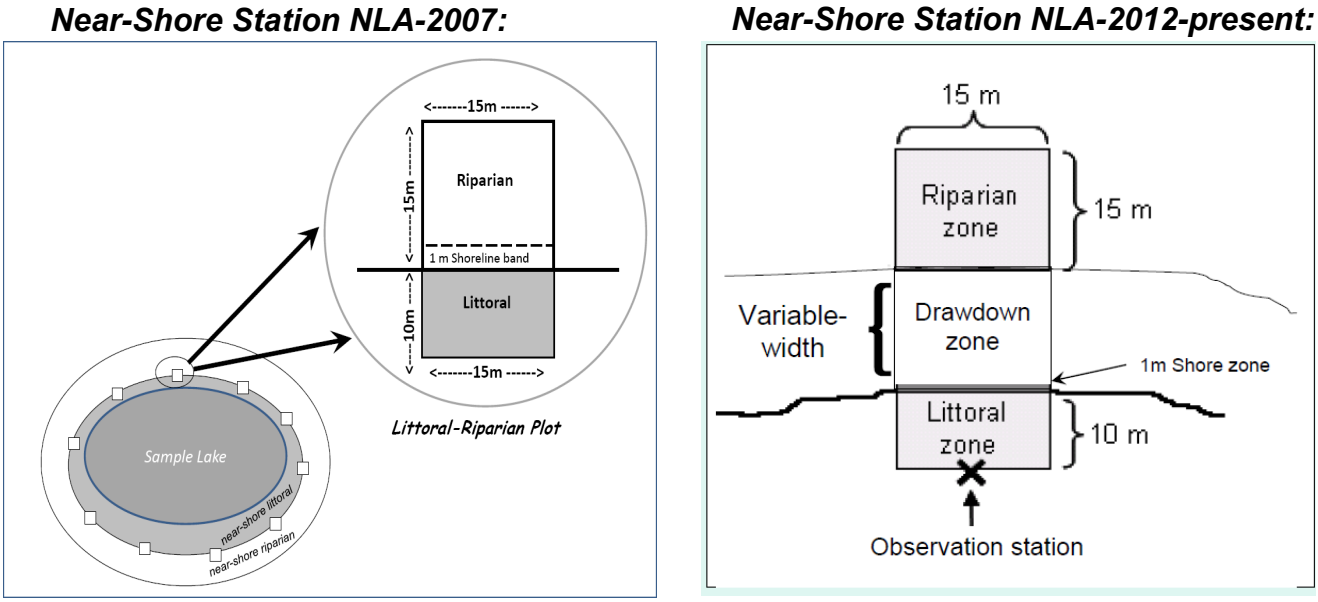


Figure 5.1. Field sampling design with 10 near-shore stations at which data were collected to characterize near shore lake riparian and littoral physical habitat in the 2007 and 2012 National Lakes Assessment (NLA) surveys. The 10 stations were systematically spaced around the shore of the lake from random starting point. Insert shows riparian plot, shoreline band, littoral plot, and (for NLA 2012 and later) drawdown zone plot located at each station.

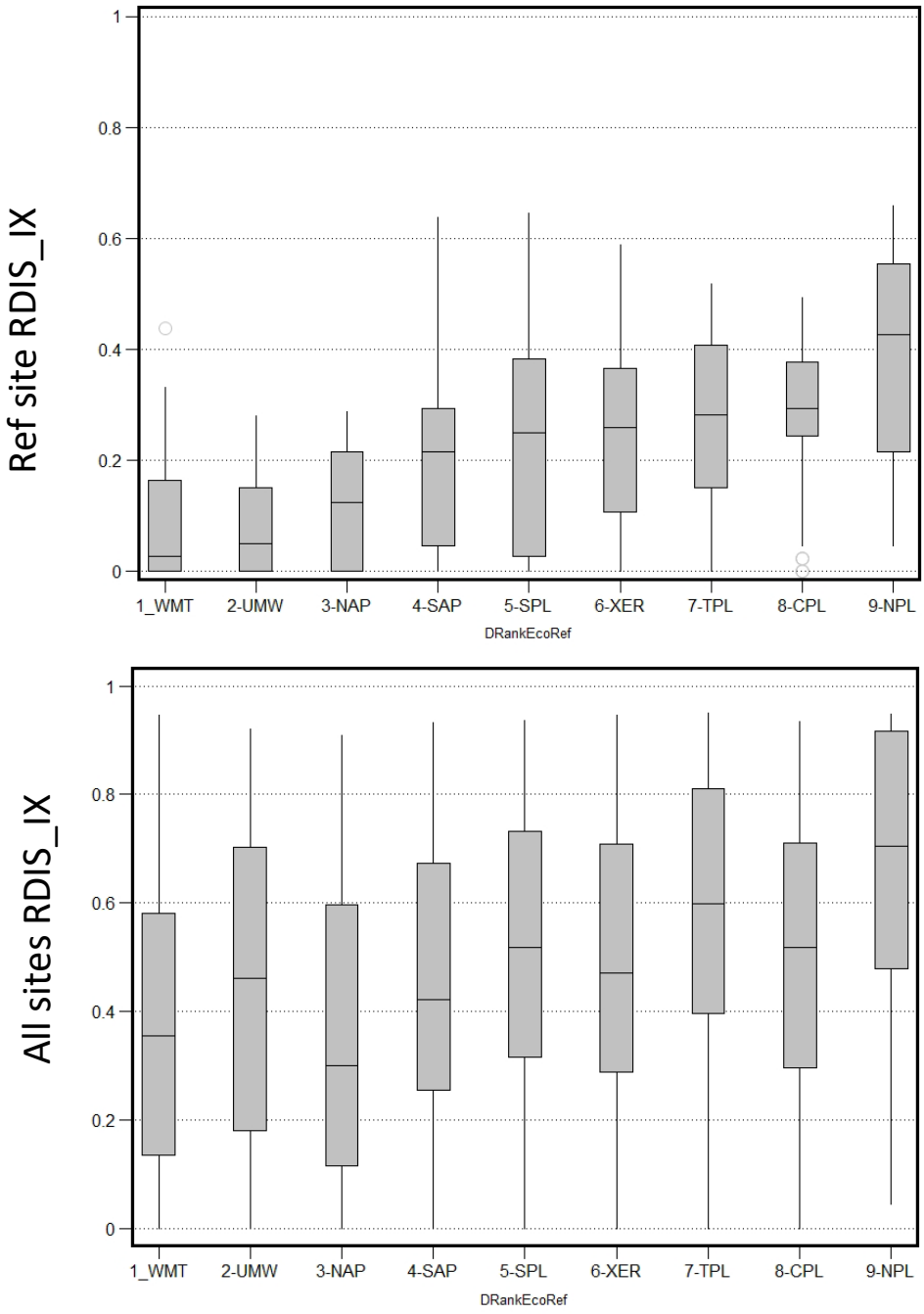


Figure 5.2. Near-shore anthropogenic disturbance (*RDis_IX)* in NLA0712 regions, ordered by their median Reference site RDis.
Upper plot: Least disturbed reference sites. Lower plot: all sites. Unweighted sample statistics are shown; box midline and lower and upper ends show median and 25th and 75th percentile values, respectively; whiskers show maximum and minimum observations within 1.5 times the interquartile range above ⁄ below box ends; circles show outliers.

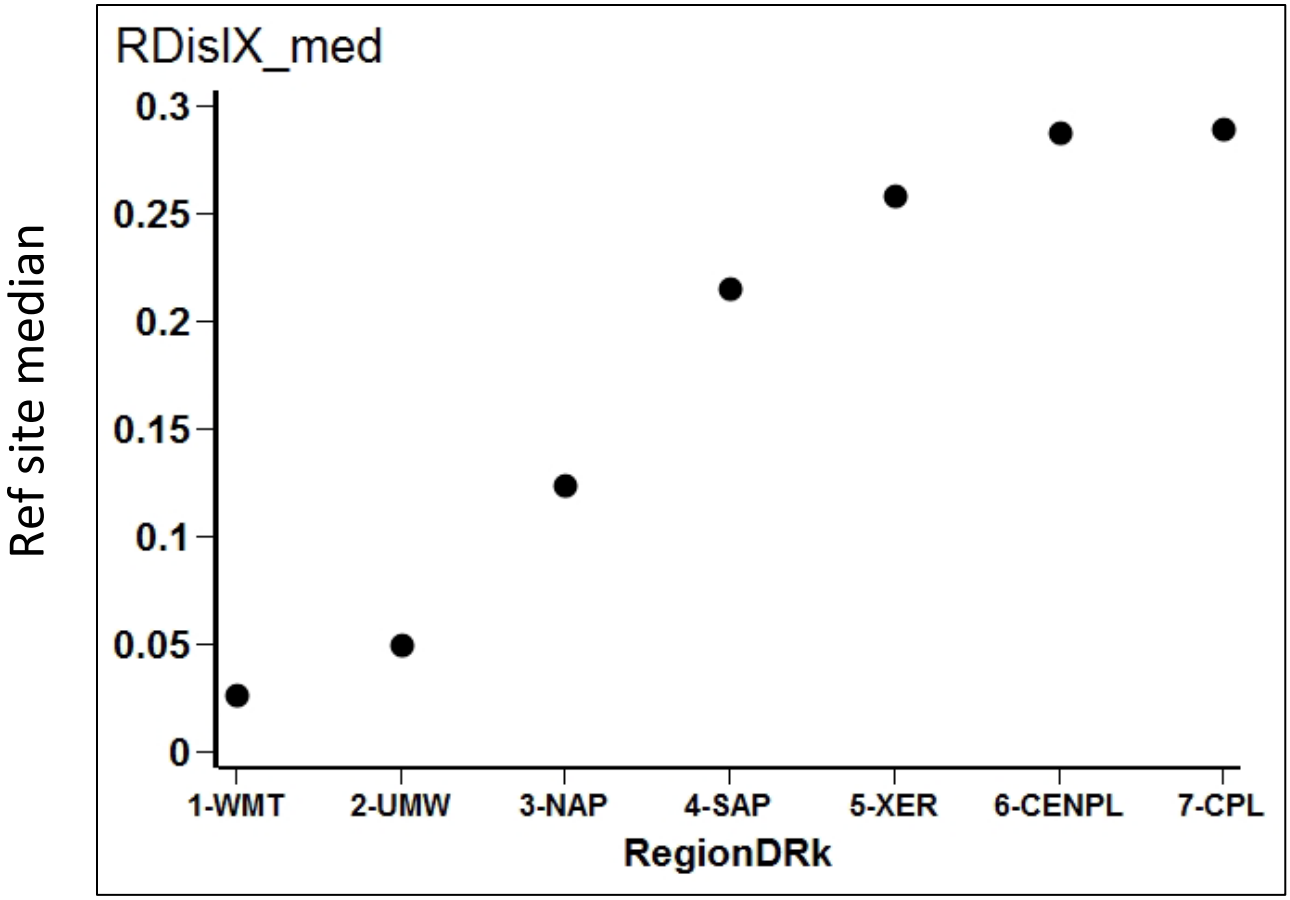
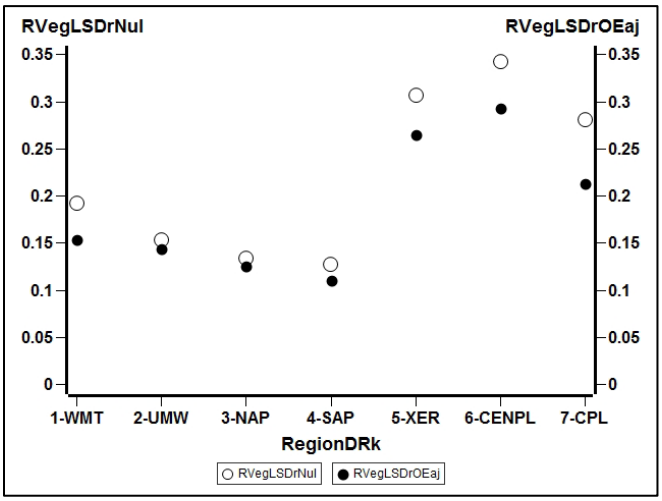
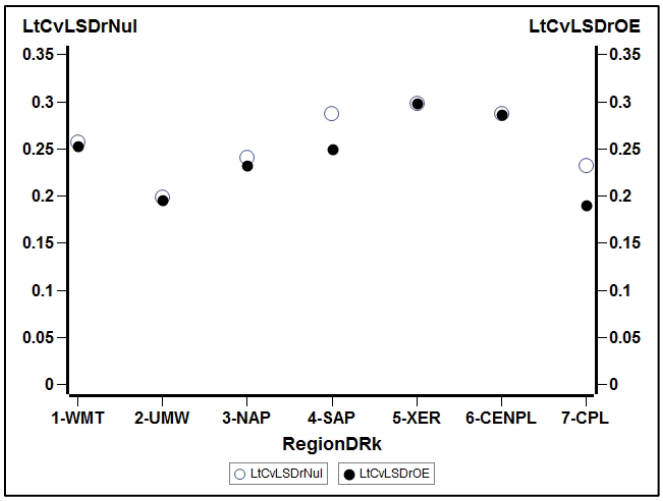


Figure 5.3. Near-shore anthropogenic disturbance in NLA0712 least disturbed reference sites (median RDis_IX), ordered by aggregated region according to the same median level of near-shore disturbance.
The NLA ECO9 regions NPL, SPL, and TPL are combined into the Central Plains (CENPL) region.

Log(RVegQ):



Log(LitCvrQ):



Log(LitRipCvrQ):

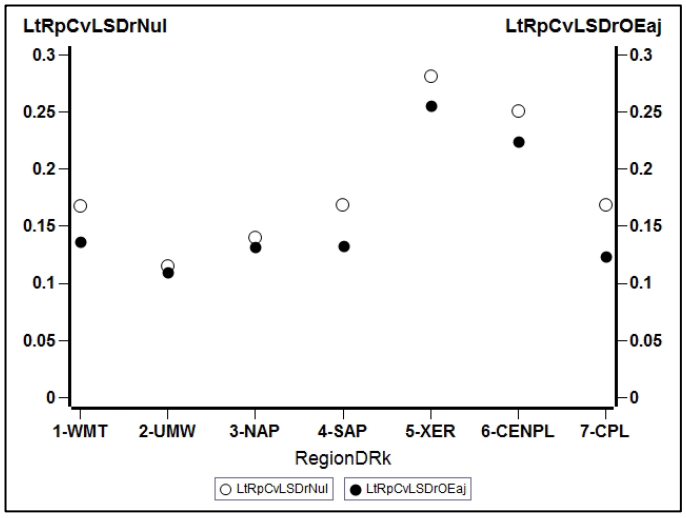


Figure 5.4. LogSD's for Null-Model and regression-based O/E model for Near-shore RVegQ, LitCvrQ, and LitRipCvrQ in the set of least disturbed lakes and reservoirs (
Table 5-1) sampled in the combined NLA 2007 and 2012 surveys.
X-axis shows the 7 modeling regions ordered by increasing median RDis_IX in 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.

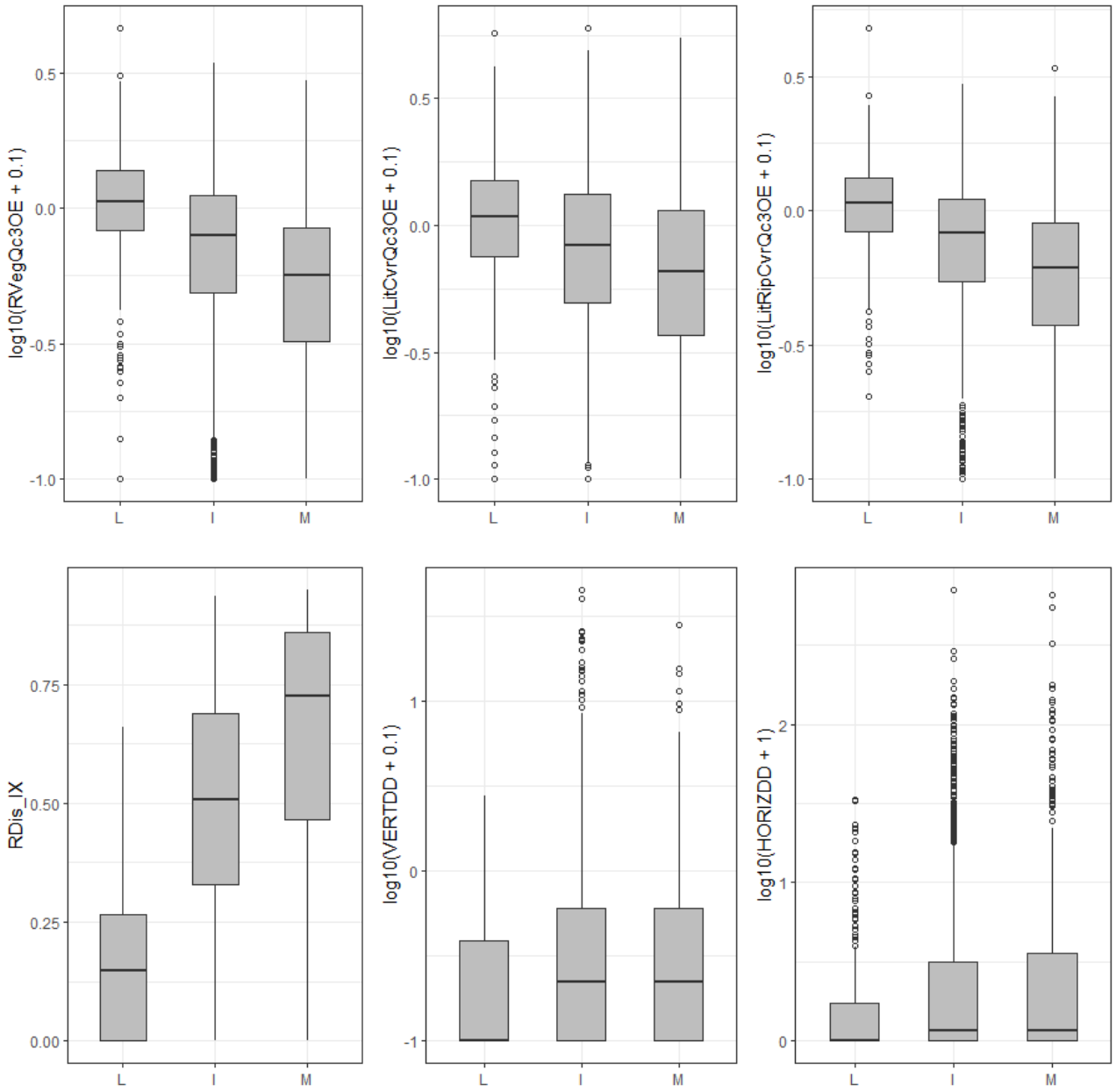


Figure 5.5. Contrasts in key NLA physical habitat index values among least disturbed reference (L), intermediate (I), and most disturbed (M) lakes in the contiguous 48 states of the U.S. based on combined NLA 2007 and 2012 data. Unweighted sample statistics are shown; box midline and lower and upper 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 least disturbed reference (L) and most disturbed (M) sites.

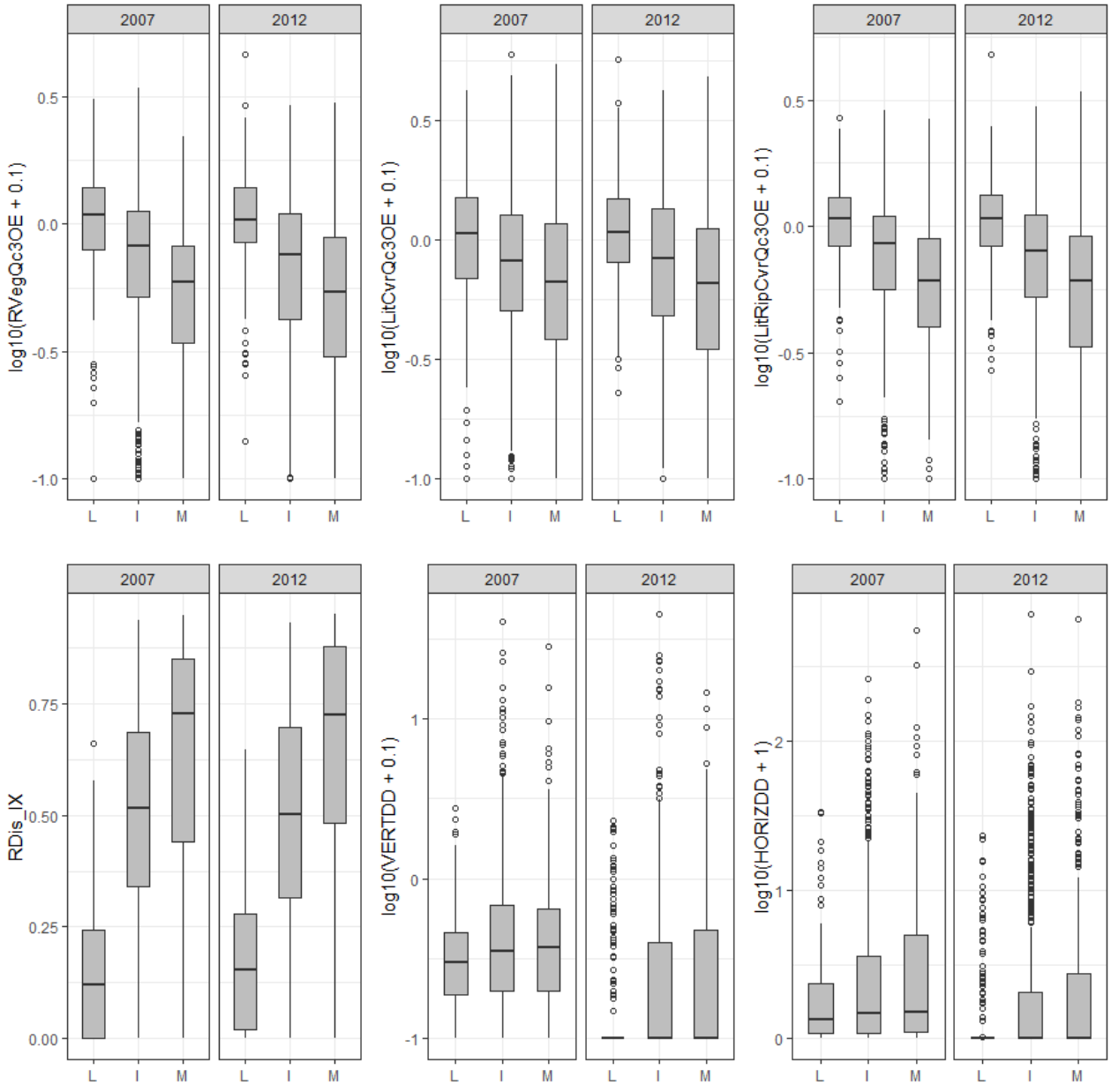


Figure 5.6. Contrasts in key NLA physical habitat index values among least disturbed reference (L), intermediate (I), and most disturbed (M) lakes in the contiguous 48 states of the U.S. shown separately for the NLA 2007 and 2012 surveys.

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 (L) and most disturbed (M) sites.

Chapter 6: Water Chemistry

6.1 Background information

The NLA public report presents the percentage of lakes in different condition class categories for water quality stressor data collected at the deepest part of each study lake. Field sampling included a depth profile and a 0-2 m depth integrated water sample. Variables analyzed and presented in the NLA 2022 report include: total nitrogen (TN), total phosphorus (TP), chlorophyll *a* (CHLA), acidity, dissolved oxygen, and atrazine. Turbidity data were also reviewed but are not presented in the public report. Acidity, dissolved oxygen, trophic state class, and atrazine benchmarks were based on established criteria and applied consistently across the nation. Good, fair and poor condition classes were established for TP, TN, and CHLA using the percentile of reference sites approach used in prior NLAs (Herlihy and Sifneos, 2013). Separate benchmarks were established for each of the nine ecoregions. The benchmarks used in the 2022 analyses are consistent with those developed for the 2017 survey. In NLA 2017, the benchmark values were revised; therefore, direct comparisons should not be made between 2007 and 2012 condition class results and those reported in 2017 and 2022. Human health water quality indicators (i.e., cyanotoxins and enterococci) are discussed in Chapter 8.

6.2 Chemical condition benchmarks

6.2.1 *Acidity*

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 (good condition class) for acidification. Sites with ANC ≤ 50 µeq/L and DOC values ≥ 6 mg/L were classified as naturally acidic due to organic acids (also good condition class). 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 or poor condition class. 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 (fair condition class).

6.2.2 *Dissolved Oxygen*

Depth profiles of dissolved oxygen were collected at the deepest location 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. Mean surface water dissolved oxygen was classified into three classes, good (≥5 mg/L), fair (3-5 mg/L), and poor (≤3 mg/L). Dissolved oxygen benchmarks of 5 mg/L and 3 mg/L represent US EPA's dissolved oxygen water

qualtiy criteria recommendations for a warmwater daily minimum for early life stages and other life stages, respectively (USEPA 1986).

6.2.3 *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, trophic state was defined using concentrations of CHLA (Table 6-1). The same trophic state classification was used for all ecoregions.

Table 6-1. Trophic State Classification used in NLA

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

6.2.4 *Total nitrogen, total phosphorus, chlorophyll a, and turbidity*

TN, TP, CHLA, and turbidity were classified into good, fair and poor condition classes based on percentiles of the nutrient reference site distribution (Herlihy and Sifneos, 2008, 2013). Because nutrients (TN, TP) were used to select biological reference sites, the biological reference sites could not be used as is for nutrient reference lakes due to circularity. The same nutrient benchmarks used in NLA 2017 were used in NLA 2022. In 2017, to develop nutrient reference sites, we compiled all sampled sites in NLA 2007, 2012, and 2017 as was done for the biological reference condition process (see Chapter 3:). All sites were then passed through the NLA biological reference screening process for their ecoregion as described in section 3.4 with one exception. To avoid complete circularity, TP and TN thresholds were removed as screening variables in the screening process.

After this initial screening, there remained a fairly strong disturbance signal in the reference sites as evidenced by looking at relationships with GIS landscape stressor variables in particular, % Agriculture watershed and % Developed watershed. In order to remove this disturbance signal, an additional GIS stressor screen was added to the process to remove from the nutrient reference site pool those sites that failed the filtering for these two metrics. For watershed % agriculture, ecoregional criteria were used: >10% for NAP, WMT, and XER lakes; >25% for NPL, SAP, SPL, and UMW lakes; >40% for CPL lakes; and >50% for TPL lakes. For watershed % developed, a >10% criterion was used for all ecoregions except the CPL where a >15% filtering criterion was used.

For calculating the nutrient condition class benchmarks used in the NLA 2017 and 2022 public reports, we used these 2007-2017 all NLA nutrient reference sites sampled from 2007-2017 (Table 6-2). When a site was sampled multiple times, only the first visit to the most recent year of sampling was used to calculate percentiles so reference sites were not double-counted. Before calculating benchmarks, a 1.5*IQR outlier analysis was done on the reference site concentrations to remove outliers. Separate benchmarks were calculated for each of the nine NARS ecoregions (Fig. 3-1). In addition,, and just in the Southern Plains, separate benchmarks were calculated for natural and manmade lakes due to large differences in least disturbed nutrient concentrations separately. Thresholds were determined for TP, TN, CHLA, and turbidity. The cutoff between good and fair condition class was set at the 75th percentile (Q3) of reference lakes, and the cutoff between fair and poor condition class was set at the 95th percentile (P95) of reference lakes (Table 6-3).

Table 6-2. Number of unique nutrient reference sites used to calculate nutrient benchmarks (2007-2017 data).

Ecoregion	Number of Nutrient Reference Sites
CPL	33
NAP	88
NPL	16
SAP	41
SPL-manmade	24
SPL-natural	20
TPL	26
UMW	87
WMT	142
XER	32
Total	509

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 benchmark versus human-made SPL lakes. Table 6-3 reports the 75th and 95th percentile-based benchmarks used to define the good, fair and poor condition classes for TP, TN, CHLA, and turbidity for each of the ecoregions.

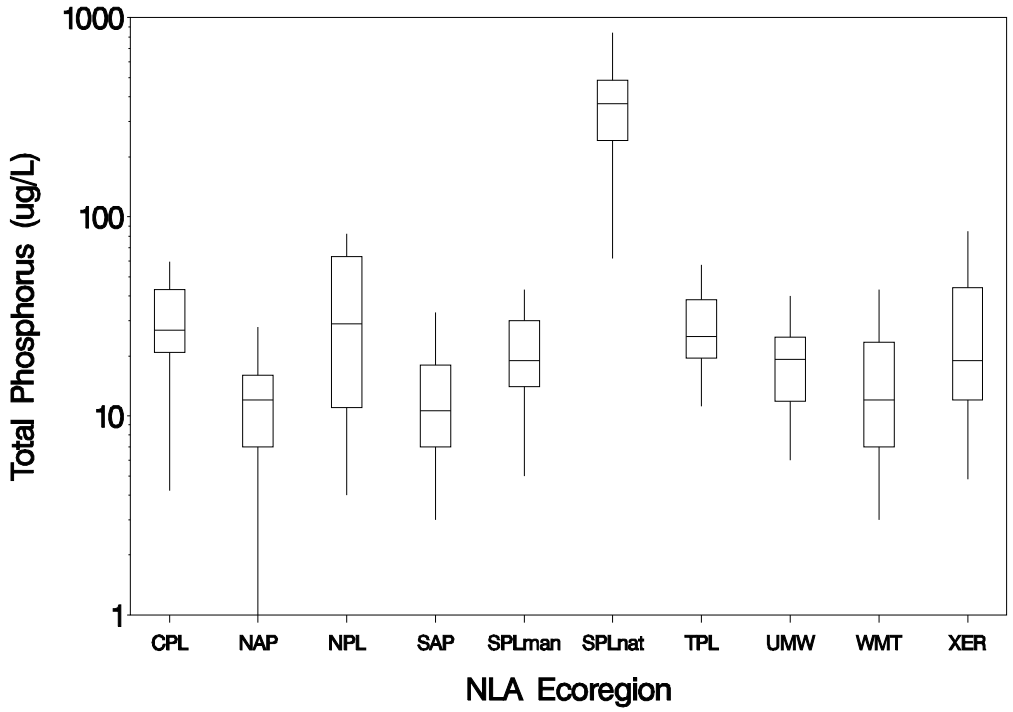


Figure 6.1. Box and whisker plot of Total Phosphorus in GIS screened, outlier removed, 2007-2017 nutrient reference sites by ecoregion. Boxes are the interquartile range, whiskers are 5th/95th percentiles.

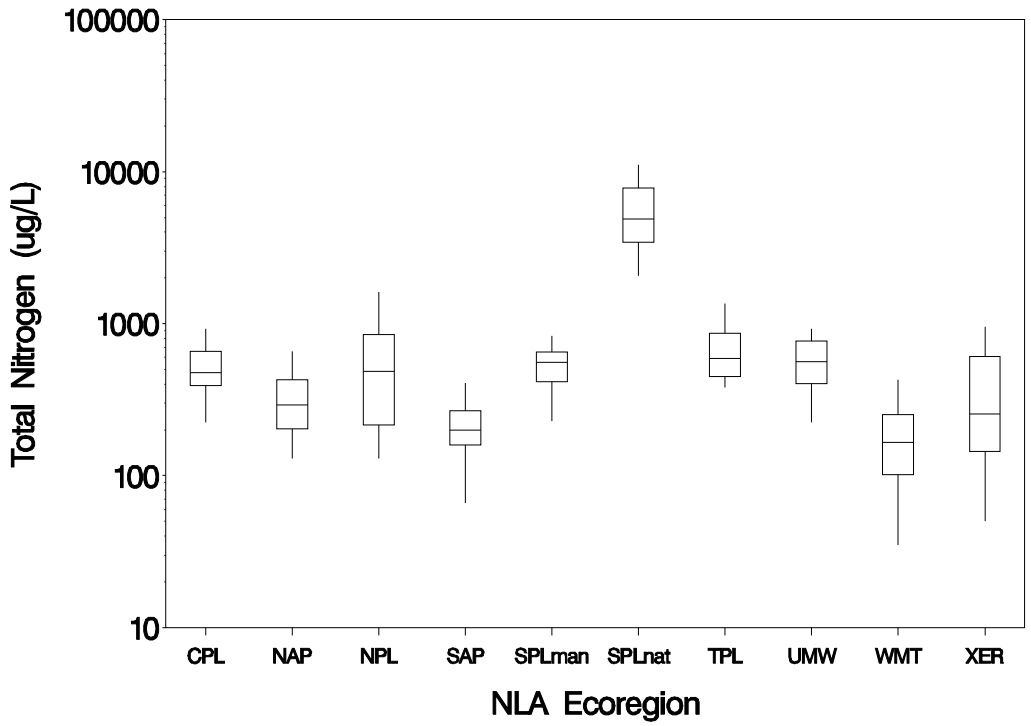


Figure 6.2. Box and whisker plot of Total Nitrogen in GIS screened, outlier removed, 2007-2017 nutrient reference sites by ecoregion. Boxes are the interquartile range, whiskers are 5th/95th percentiles.

Table 6-3. NLA 2017 good, fair, and poor benchmarks (75th/95th percentiles) for TP, TN, CHLA, and turbidity condition classes.

Ecoregion	TP (µg/L) 75th Good-fair	TP (µg/L) 95th Fair-poor	TN (µg/L) 75th Good-fair	TN (µg/L) 95th Fair-poor
CPL	43.0	59.5	659	923
NAP	16.0	27.9	428	655
NPL	63.0	82.0	849	1,620
SAP	18.0	33.0	266	409
SPL-manmade	30.0	43.0	650	830
SPL-natural	486	839	7,840	11,100
TPL	38.4	57.5	865	1,350
UMW	24.8	40.0	766	926
WMT	23.4	43.0	253	429
XER	44.0	84.8	605	954

Ecoregion	CHLA (µg/L) 75th Good-fair	CHLA (µg/L) 95th Fair-poor	Turbidity (NTU) 75th Good-fair	Turbidity (NTU) 95th Fair-poor
CPL	12.7	28.0	3.42	4.15
NAP	4.52	8.43	1.30	2.52
NPL	10.9	19.3	3.08	4.46
SAP	5.54	13.1	2.83	4.21
SPL-manmade	8.97	12.6	3.32	4.67
SPL-natural	118	219	71.3	86.4
TPL	13.9	19.8	3.64	4.23
UMW	7.43	14.6	2.18	3.32
WMT	1.86	3.86	0.910	1.60
XER	5.92	9.00	2.97	4.84

6.2.5 *Atrazine*

Atrazine water chemistry analyses were added to the NLA in 2012. Samples for atrazine were collected using a 0-2 m vertically integrated water column sampler at the open-water site. Measured concentrations were compared to nationally consistent benchmarks to estimate ecological risk. The NLA also reports on the percentage of lakes with detections and changes in detection over time. Detection is defined as a value greater than the minimum detection limit (MDL). When the MDL changed between surveys, the greatest MDL for all surveys was used to determine detection.

The NLA atrazine benchmark is the EPA's aquatic plant concentration equivalent level of concern (CE-LOC) used in the EPA's atrazine ecological exposure monitoring program. This benchmark ensures that atrazine levels will not cause significant changes in aquatic plant community structure, function and productivity (US EPA Atrazine website). In NLA 2012, the EPA used a proposed CE-LOC of 4 ppb for atrazine risk results. In NLA 2017, this value was updated to the current CE-LOC of 3.4 ppb. To report on the percentage of lakes with atrazine detections, a consistent detection value was selected. The MDL was equal to 0.046 ppb for most samples in NLA 2012 and 0.03 ppb for most samples in NLA 2017 and 2022. Therefore, detection results in the public report and data dashboard present the percentage of lakes with measured values greater than or equal to 0.046 ppb for all surveys.

6.2.6 *Within-year variability*

To examine within-year variability of water chemistry data, analysts used the revisit sites from the NLA 2007 and 2012 (2,482 sites with 192 sites with revisits) to calculate S:N estimates for the water chemistry indicators presented in Table 6-2 Overall S:N and pooled standard deviation (SD) for NLA 2007 and 2012 surface water chemistry within three concentration range classes.Table 6-2. Metrics with high S:N are more likely to show consistent responses to human caused disturbance, and S:N values ≤ 1 indicate that sampling a site twice yields as much or more metric variability as sampling two different sites (Stoddard et al., 2008).

Table 6-2 Overall S:N and pooled standard deviation (SD) for NLA 2007 and 2012 surface water chemistry within three concentration range classes. N = 192

Parameter	**S:N**	**Low**		**Medium**		**High**	
		range	SD	Range	SD	range	SD
ANC	98.3	<500 ueq/L	28.9	500-2500 ueq/L	153	>2500 ueq/L	309
Chloride	78.7	0-250 ueq/L	9.32	250-1000 ueq/L	59.1	>1000 ueq/L	373
Chlorophyll-a	3.85	0-10 ug/L	2.47	10-50 ug/L	16.9	>50 ug/L	63.6
Color	8.2	0-10 PCU	4.32	10-50 PCU	5.9	>50 PCU	40.1
Conductivity	134	0-100 uS	6.13	100-500 uS	21.3	500-2000 uS	67
DOC	97.2	0-5 mg/L	0.388	5-10 mg/L	0.687	>10 mg/L	4.35
ph	5.44	0-6	0.111	6-8	0.28	>8	0.343
Sulfate	238	0-250 ueq/L	13.3	250-1000 ueq/L	50.2	>1000 ueq/L	364
Total Nitrogen	23.2	0-250 ug/L	42.5	250-1000 ug/L	160	>1000 ug/L	818
Total Phosphorus	18.6	0-25 ug/L	5.24	25-100 ug/L	16.8	>100 ug/L	123
Turbidity	6.69	0-5 NTU	1.1	5-25 NTU	6.85	>25 NTU	33.9

Within-year sampling variability for atrazine was assessed by comparing NLA, 2012, 2017 and 2022 visit 1 and 2 condition categories and is presented in Table 6-3Table 8-1. For atrazine detection, results showed agreement in 238 (81%) of the 293 revisit sites. For atrazine risk condition, 291 (99%) of the 293 risk categores were in agreement.

Table 6-3. Atrazine detection (a) and risk condition (b) contingency tables. N = 293

a)		Atrazine Detection		
		Visit 1		
		Detected	Not-detected	Not Assessed
Visit 2	Detected	64	30	
	Not detected	24	174	
	Not Assessed			1

b)		Atrazine Risk Condition		
		Visit 1		
		At or Below Benchmark	Above Benchmark	Not Assessed
Visit 2	At or Below Benchmark	289	2	
	Above Benchmark		1	
	Not Assessed			1

6.3 Literature cited

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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 2007 National Lake Assessment (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 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.

For NLA 2022, we used the same MMIs to assess lake condition. This chapter provides corrections and clarifications to the 2017 technical report that we identified for the NLA 2022 analyses.

7.2 Methods

7.2.1 *Field methods*

Sample collection procedures for zooplankton are described in the NLA 2022 FOM (USEPA 2022a). 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 with a reducing collar (20-cm diameter) 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) having 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 vertical tow was done. At lakes between 4 and 6 m deep, two 2.5-m vertical tows were done. At lakes between 2 to 3 m deep, five 1-m vertical tows were done. At lakes less than 2 m deep, ten 0.5 m vertical tows were collected. 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

Type of disturbance	Assemblage component or metric	Hypothesized response	References
Catchment development	Biomass of small cladocerans	Increase	Gélinas and Pinel-Alloul (2008), Beaver et al. (2014)
Catchment development	Abundance of small daphnids and cladocerans	Increase	Gélinas and Pinel-Alloul (2008), Dodson et al. (2009), Van Egeren et al. (2011), Beaver et al. (2014)
Nutrients; Agricultural land use; riparian buffer presence	Species richness	Decrease	Gannon and Stemberger (1978), Dodson et al. (2005)
Nutrients, land use	Large-sized species richness (e.g., Daphnia spp., calanoid copepods)	Decrease	Stemberger and Lazorchak (1994)
Nutrients, land use	Small-sized species richness (e.g., *Ceriodaphnia*, rotifers)	Increase	Stemberger and Lazorchak (1994)
Nutrients	Proportion of calanoid copepod taxa	Decrease	Jeppesen et al. (2000), Du et al. (2015)
Nutrients	Proportion of cyclopoid copepod taxa	Increase	Jeppesen et al. (2000), Du et al. (2015)
Nutrients	Ratio of calanoid copepods to (cyclopoid copepods + cladocerans)	Decrease	Gannon and Stemberger (1978), Kane et al. (2009)
Nutrients	Mean size	Decrease	Gannon and Stemberger (1978)
Nutrients	Total biomass	Increase	Gannon and Stemberger (1978)
Nutrients	Proportion of cladoceran biomass	Decrease	Jeppesen et al. (2000), Du et al. (2015)
Nutrients	Relative abundance of calanoid copepods	Decrease	Brooks (1969), Gannon and Stemberger (1978)
Nutrients	Relative abundance of cyclopoid copepods and small-bodied cladocerans	Increase	Brooks (1969), Attayde and Bozelli (1998)
Nutrients	Omnivorous taxa richness, abundance, or biomass	Increase	Stemberger and Lazorchak (1994), Stemberger et al. (2001)
Nutrients (total P)	Biomass of rotifers and cyclopoid copepods	Increase	Du et al. (2015)
Nutrients (total P)	Biomass of cladocerans and cyclopoid copepods	Decrease	Du et al. (2015)
Nutrients, chlorophyll a, Secchi transparency, temperature, dissolved oxygen	Rotifer assemblage composition	Change	Duggan et al. (2001), (2002)
Decrease in acid neutralization capacity/calcium concentrations	Abundance of large-bodied zooplankton	Decrease	Tessier and Horwitz (1990)
Invasive species	Native species richness, abundance, or biomass	Decrease	Kane et al. (2009)

7.2.1 *Laboratory methods*

Laboratory methods for zooplankton samples are described in the NLA 2022 laboratory operations manual (USEPA 2022b). 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, only cladocerans and copepods (including copepedids) were enumerated. In the ZOFN samples, only "small" taxa were enumerated (cladocerans < 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). In 2012, only the presence of these taxa in the subsample was noted (i.e., they were not enumerated). In 2022, the laboratory recorded the number of organisms encountered in the Large/Rare search.

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:

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

The biomass (µg 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 (µg dry mass/individual) based on published length-weight relationships (Dumont et al. 1975, McCauley 1984, Lawrence et al. 1987). Biomass was then calculated as:

$$Biomass\ =\ Density\ \left(\frac{indiv}{L}\right)\ \times Biomass\ Factor\ \left(\frac{\mu g}{indiv}\right)$$

In 2012, one 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. In 2022, one laboratory processed all zooplankton samples and provided quantitative biomass data. This laboratory was different than the laboratory that processed the NLA 2012 and 2017 samples. Biomass was estimated at each site for taxa that had existing length-weight relationships, but estimates were not provided for all taxa. For sites where a taxon with a missing biomass factor comprised less than 5% of the total number of individuals counted, it was left as missing. For the remaining cases, we used available biomass factors for that taxon from other sites (including NLA 2022, NLA 2017, and NLA 2012) from the state and surrounding states to calculate a mean value to use.

NOTE: In 2021, we discovered an error in the calculation of the tow volumes for the coarse-net samples, which affected the values for density, biomass, any metrics based on either of these, and any MMIs that included metrics based on density or biomass for both 2012 and 2017. The 2012 and 2017 data were corrected and republished to the NARS website.

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 variables 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. The number of errors discovered in the NLA 2022 field and laboratory data was very low.

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 primarily 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.
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*), *Cercopagis pengoi*, *Daphnia lumholtzi*, *Sinocalanus doerri, Pseudodiaptomus forbesi*, *and Arctodiaptomus dorsalis* were considered to be introduced to North America. *Eutymora affinis* was considered to be introduced to inland lakes of the US. *Skistodiapomus pallidus* was considered to be introduced to lakes in states outside of the Mississippi-Missouri-Ohio River basins. EPA also reviewed the laboratory results for non-target taxa. Non-target taxa are excluded from enumeration and are listed in Appendix D.

For NLA 2022, we updated the master taxa list from NLA 2017 to add new taxa and associated autecological information that were identified in the coarse and fine net samples collected in 2022. The NLA 2022 taxa list for zooplankton contains 634 unique names (excluding the taxa

listed in Appendix D) for the variable TARGET_TAXON, which are used for metric calculations. This is an increase of 54 taxa from those included in the taxa list for NLA 2017.

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.

Even with a fixed count subsampling approach, taxonomic richness and metrics can be influenced by the number of individuals enumerated in a subsample (Stoddard et al. 2008). 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 Plains (CPL) and Upper Midwest (UMW) as separate bio-regions. These are the same regions as are used for the NLA benthic macroinvertebrate MMI.

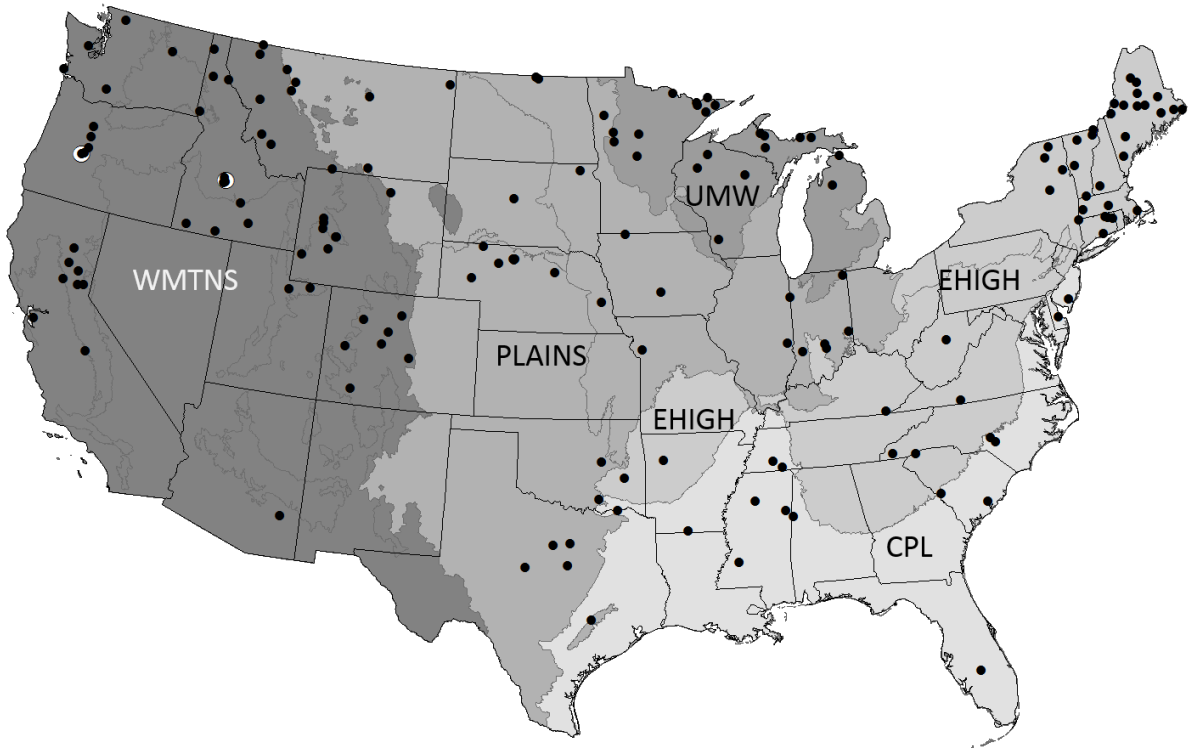

Figure 7.1 Five aggregated bio-regions used to develop zooplankton MMIs for the 2012 National Lake Assessment (CPL=Coastal Plains; EHIGH=Eastern Highlands, PLAINS= Plains, UMW=Upper Midwest, and WMTNS=Western Mountains). Solid dots indicate least disturbed sites used for developing the zooplankton MMI. 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 NLA 2012, we used the same list of sites for the zooplankton MMI 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 and excluded them from the list of least-disturbed sites.

For NLA 2017, we combined least disturbed sites sampled in 2017 with those from the NLA 2012 assessment. We retained only one visit per site by excluding revisits (VISIT_NO=2) and using the 2017 visit for sites from 2012 that were resampled in 2017. In addition, we identified three situations where we felt that the zooplankton samples from least disturbed sites were

not representative of the existing assemblage, and excluded these sites from developing condition class benchmarks:

1. Sites where at least one of the net samples were hugely dominated by unidentified copepod individuals (which included nauplii and immature copepodites).
2. Sites where no rotifers were collected.
3. Sites where less than 100 individuals were collected in either the coarse or fine net sample.

The first two of these situations were used in the NLA 2012 assessment. The third situation was added for the NLA 2017 assessment. Out of 343 least disturbed lakes, we identified 21 sites (15 from 2012 and only six from 2022) where the coarse sample had less than 100 individuals counted. At two of these sites (both from 2012), the fine net sample also had less than 100 individuals counted.

For NLA 2022, we identified least-disturbed and most disturbed sites using the same process as described above and in Section 3.3. However, we did not apply the additional screening criteria specific to zooplankton samples and did not use sites from 2022 to modify the existing condition class benchmarks.

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). For each combined net sample, we developed dominance metrics based on the percent of individuals represented in the most dominant taxon and represented in the three and five most dominant taxa.

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 $\leq$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 Appendix D: Zooplankton. 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.

NOTE: As described in Section 7.2.1, we had to recalculate metrics and MMI scores after correcting for the error in the calculation of tow volume. We repeated the metric screening process and determined that the existing suite of metrics included in each of the regional MMIs still performed adequately, so we retained them for use in the NLA 2022 assessment. The results presented in Section 7.5 are based on the recalculated data.

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., $t \geq 0$), 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 10/6. This resulted in an MMI score that ranged between 0 and 100.

7.5 Zooplankton MMI metric composition and performance

See *Appendix D: List of Candidate Metrics for Zooplankton* for metric descriptions.

7.5.1 *Coastal Plains MMI*

The component metrics for the Coastal Plains MMI are presented in Table 7-2. Information related to the performance of the Coastal Plains 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 as expected 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 and abundance 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 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. 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.

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	0.4*
Copepod	DOM1_300_COPE_PBIO (45.90, 100)	+1.16*	1.9
Richness/Diversity	FAM300_NAT_NTAX (5, 15)	+2.72	2.1
Rotifer	COLLO_PBIO (0, 5.64)	+1.84	7.2
Trophic	OMNI_PTAX (10.53, 47.06)	-3.35	4.3

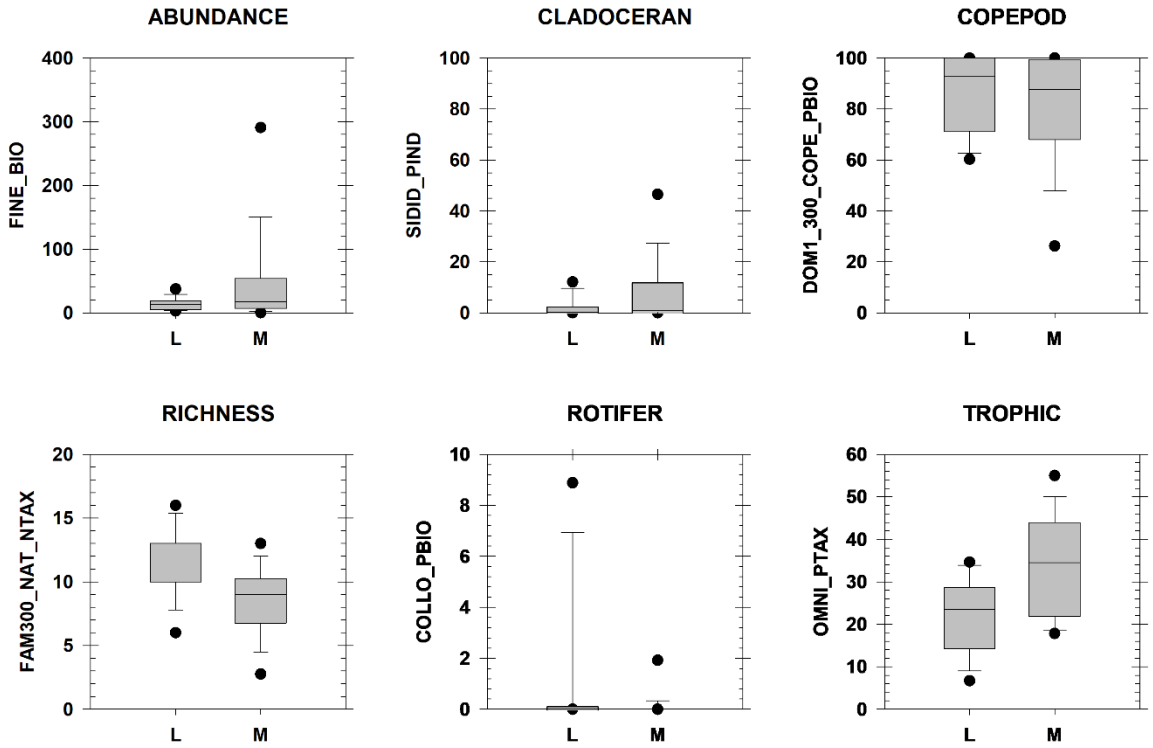

Figure 7.2. Distribution of six component metrics of the zooplankton MMI for the Coastal Plains bio-region in least disturbed (L) versus most disturbed (M) sites. Dots indicate the 5th and 95th percentiles.

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 (**Error! Reference source not found.**). An increase in cyclopoid copepods expected with increased disturbance (Table 7-1) would help to explain the observed response in both of these metrics.

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 Appendix D: Zooplanktonfor metric descriptions.

Metric Type	Metric Variable Name (floor, ceiling)	*t* value	S:N (bio-region)
Abundance/Size	ZOCN_DEN (0.216450,259.3050)	-1.89	2.2
Cladoceran	SMCLAD_PBIO (0, 57.31)	-2.91	1.3
Copepod	COPE_NAT_DEN (8.8236,398.397)	-1.70	1.5
Richness/Diversity	COARSE_NAT_PTAX (22.22,57.14)	+1.71*	0.2*
Rotifer	ROT_PBIO (0.79,86.39)	-1.94	1.2*
Trophic	OMNI300_PTAX (12.50, 44.44)	-2.48	1.8

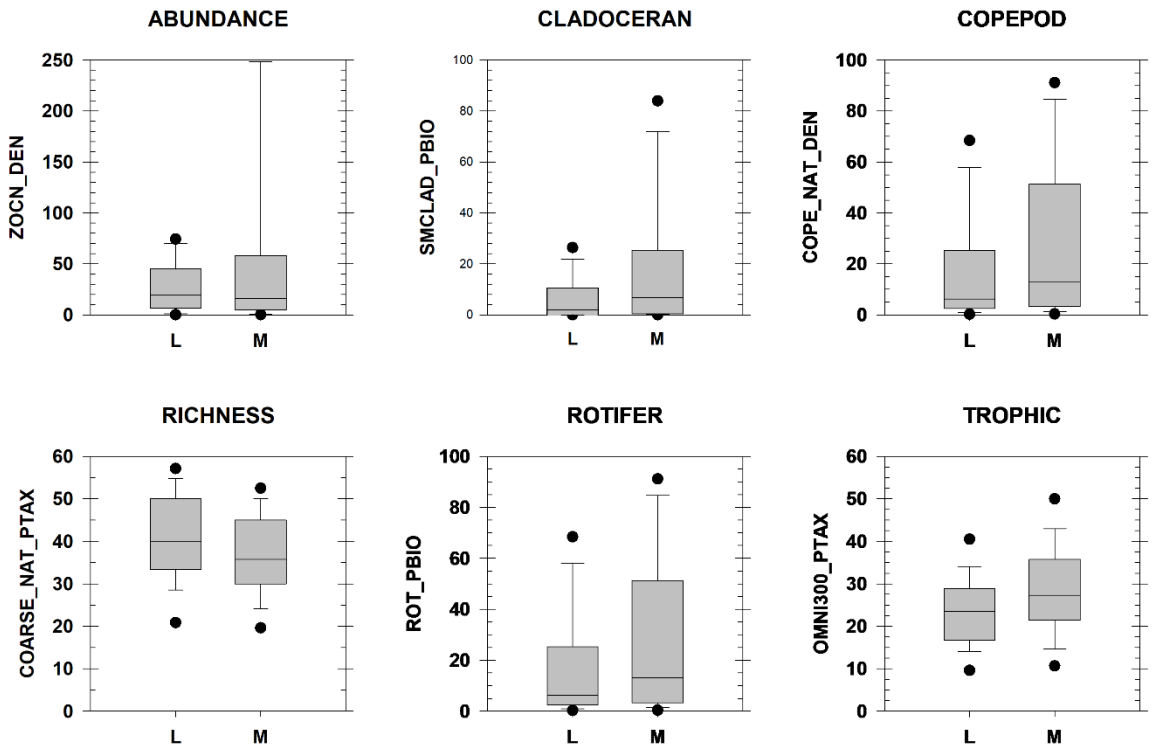

Figure 7.3 Distribution of six component metrics of the zooplankton MMI for the Eastern Highlands bio-region in least disturbed (L) versus most disturbed (M) sites. Dots indicate the 5th and 95th percentiles.

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 7-4. 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.

Metric Type	Metric Variable Name (floor, ceiling)	*t* value	S:N (bio-region)
Abundance/Size	FINE300_NAT_PBIO (0.66, 85.12)	+1.89	5.8
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, 15)	+2.20	2.6
Rotifer	ROT_NTAX (3, 17)	+2.63	1.7
Trophic	COPE_HERB_PDEN (0, 29.93)	-2.45	9.1

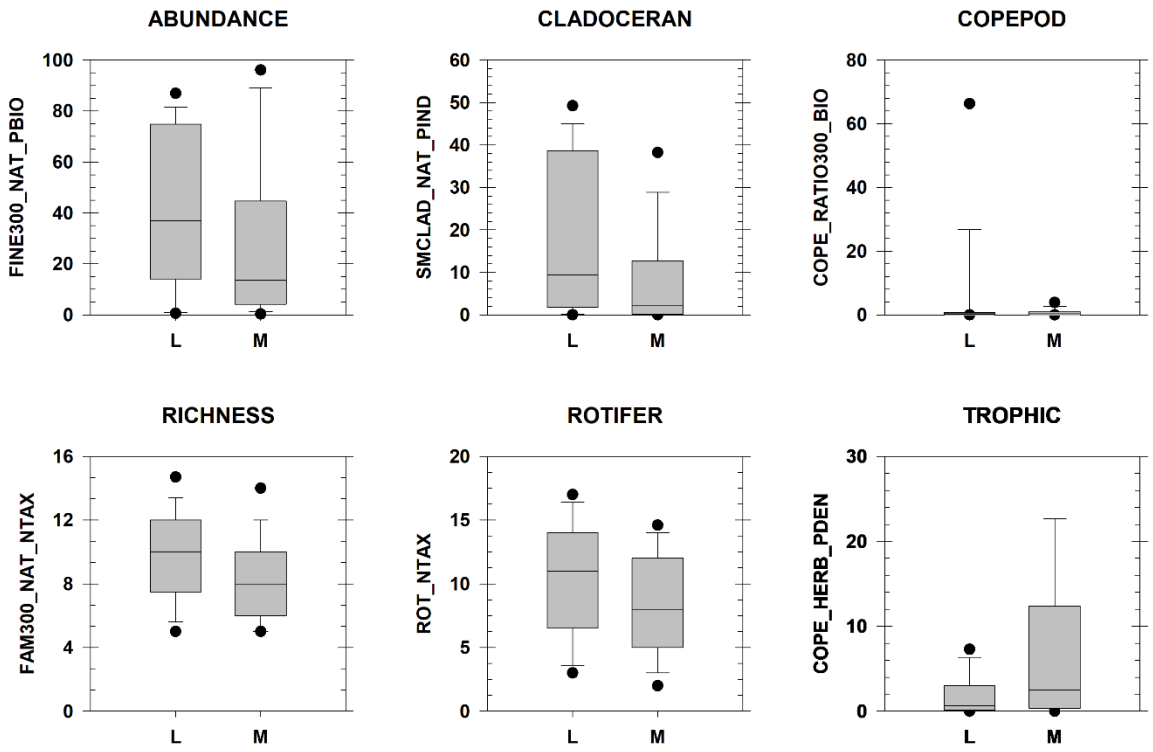

Figure 7.4. Distribution of six component metrics of the zooplankton MMI for the Plains bio-region in least disturbed (L) versus most disturbed (M) sites. Dots indicate the 5th and 95th percentiles.

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). The value for the abundance metric (2348) resulted from essentially no difference in the values between the small number of revisit samples.

Only three of the six metrics responded to disturbance as expected (Figure 7.5**Error! Reference source not found.** ; 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 taxonomic 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.

Metric Type	Metric Variable Name (floor, ceiling)	*t* value	S:N (bio-region)
Abundance/Size	TOTL_NAT_PIND (96.75, 100)	+1.47*	2348
Cladoceran	BOSM300_NAT_PTAX (0, 12.5)	+2.72	1.3
Copepod	CALAN300_NAT_BIO (0,65.037544)	-2.17	9.9
Richness/Diversity	FINE_PTAX (37.50, 77.78	+1.87	1.4
Rotifer	DOM1_ROT_PBIO (25.30, 93.60)	-2.46	3.5
Trophic	COPE_HERB300_PBIO (0.19, 59.42)	-1.99	5.1

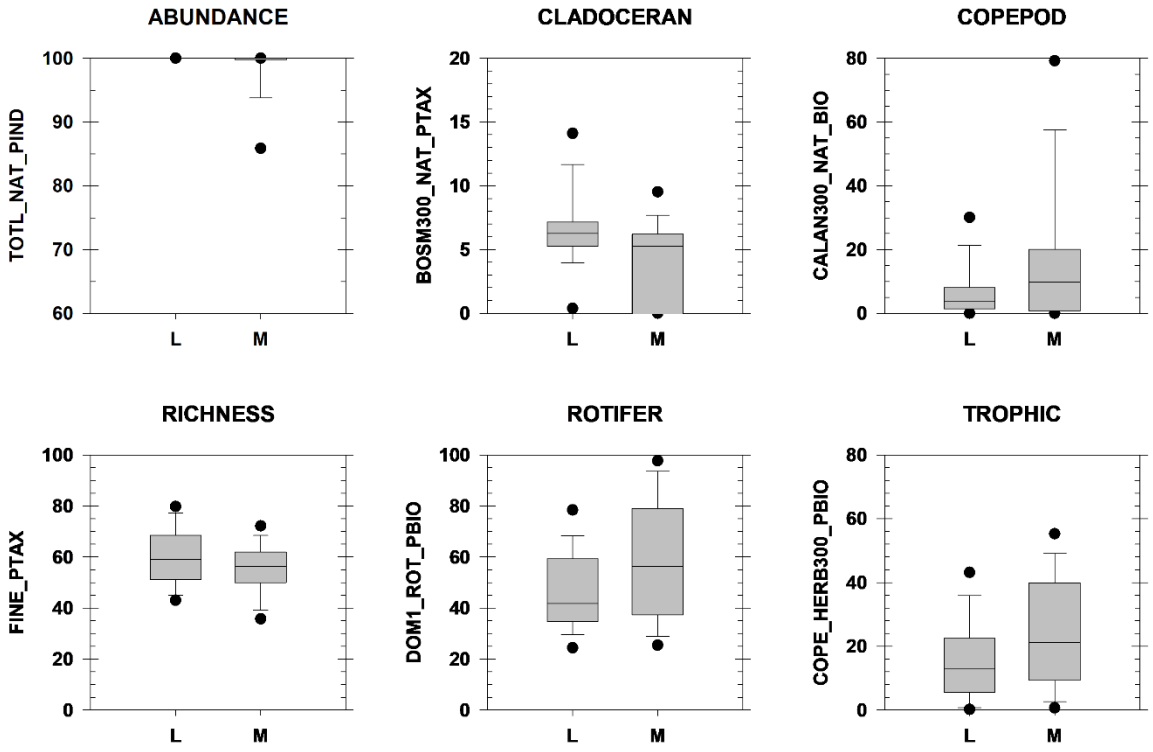

Figure 7.5. Distribution of six component metrics of the zooplankton MMI for the Upper Midwest bio-region in least disturbed (L) versus most disturbed (M) sites. Dots indicate the 5th and 95th percentiles.

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.

Metric Type	Metric Variable Name (floor, ceiling)	*t* value	S:N (bio-region)
Abundance/Size	COARSE300_NAT_PBIO (10.94, 99.26)	+1.89	5.6
Cladoceran	LGCLAD300_NAT_PTAX (0, 29.285)	+2.53	2.0
Copepod	COPE300_BIO (0.073928, 149.035677)	-2.75	2.0
Richness/Diversity	ZOFN300_NTAX (3, 15)	-1.69*	1.9
Rotifer	PLOIMA_PTAX (20, 70.835)	+0.49*	4.3
Trophic	COPE_OMNI_PTAX (0, 22.22)	-2.46	1.5

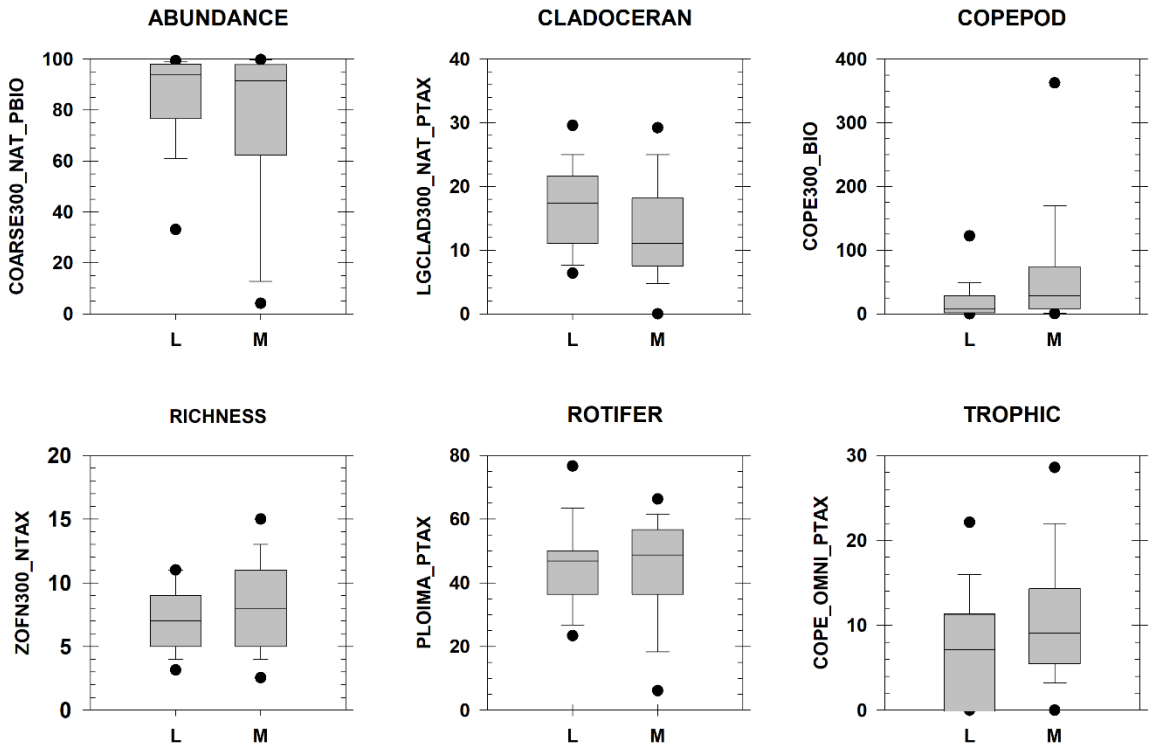

Figure 7.6. Distribution of six component metrics of the zooplankton MMI for the Western Mountains bio-region in least (L) disturbed versus most disturbed (M) sites. Dots indicate the 5th and 95th percentiles.

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). **Error! Reference source not found.** 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 Distribution of zooplankton MMI scores in least-disturbed (L) vs. most disturbed (M) sites for five bio-regions. Sample sizes are in parentheses. Dots indicate the 5th and 95th percentiles. Figure 7.8**Error! Reference source not found.** 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 7.0.

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)	0.85	0.187
Eastern Highlands (EHIGH)	-1.23	0.119
Plains (PLAINS)	1.21	0.237
Upper Midwest (UMW)	0.94	0.112
Western Mountains (WMTNS)	0.42	0.117

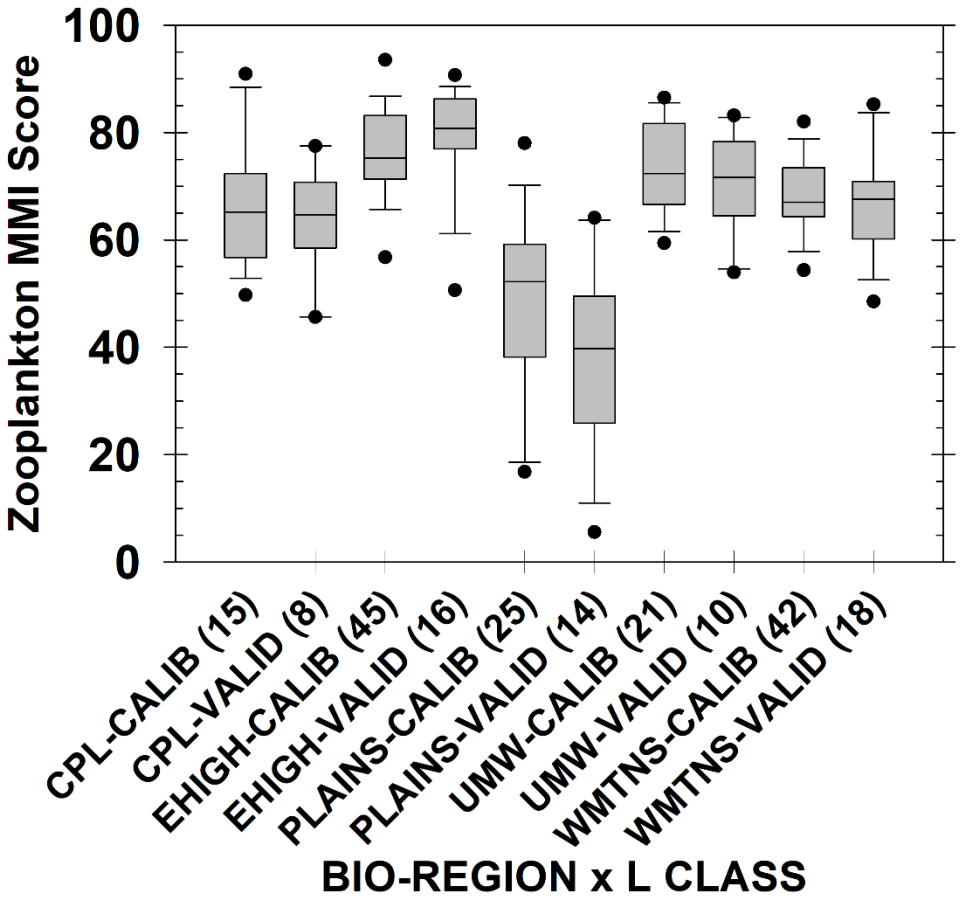

Figure 7.7. Distribution of zooplankton MMI scores in-calibration vs. validation sites for five bio-regions. Sample sizes are in parentheses. Dots indicate the 5th and 95th percentiles.

Table 7-8. RESULTS OF RESPONSIVENESS, REDUNDANCY, AND REPEATABILITY TESTS FOR NLA 2012 ZOOPLANKTON MMIs.
* For the Upper Midwest (UMW) MMI, the abundance metric scores in all least-disturbed sites were identical. Values in parentheses are correlation coefficients with the abundance metric coefficients set to missing.

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)	4.11	0.55	0.28	2.7
Eastern Highlands (EHIGH)	5.09	0.43	0.17	2.5
Plains (PLAINS)	5.49	0.57	0.20	3.6
Upper Midwest (UMW)	5.78	1.0 (0.61)*	0.50 (0.20)*	18.0
Western Mountains (WMTNS)	6.28	0.561	0.20	3.6

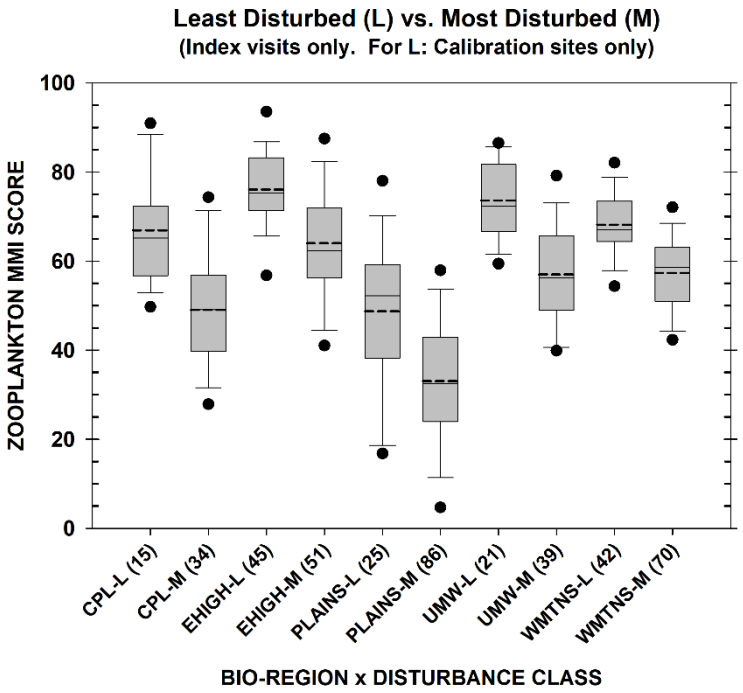

Figure 7.8 Distribution of zooplankton MMI scores in least-disturbed (L) vs. most disturbed (M) sites for five bio-regions. Sample sizes are in parentheses. Dots indicate the 5th and 95th percentiles.

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 0.42 (r=0.65) 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.

7.6.5 *Effect of natural drivers and tow length on MMI scores*

The set of lakes sampled for the NLA 2012 included both natural and human-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. human-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. human-made reservoirs for each of the five bio-regions (Figure 7.10). The distributions are similar within each bio-region except the WMTNS, where human-made lakes appear to have much lower MMI scores than natural lakes. In the Coastal Plains, human-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 human-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 settings condition benchmarks.

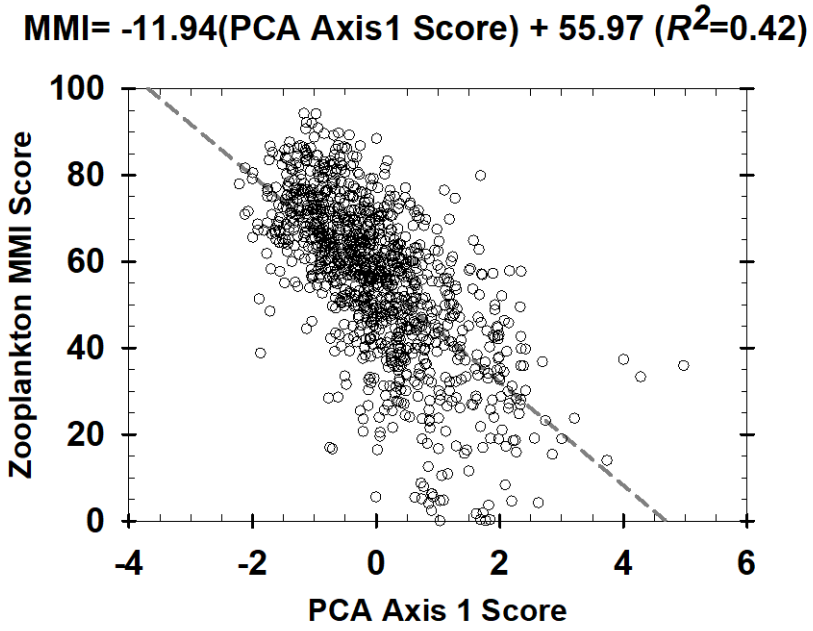

Figure 7.9. Linear regression of NLA 2012 Zooplankton MMI scores vs. first axis score from principal components analysis (PCA) based on chemical, habitat, and visual assessment stressor variables used to screen least- and most disturbed sites.

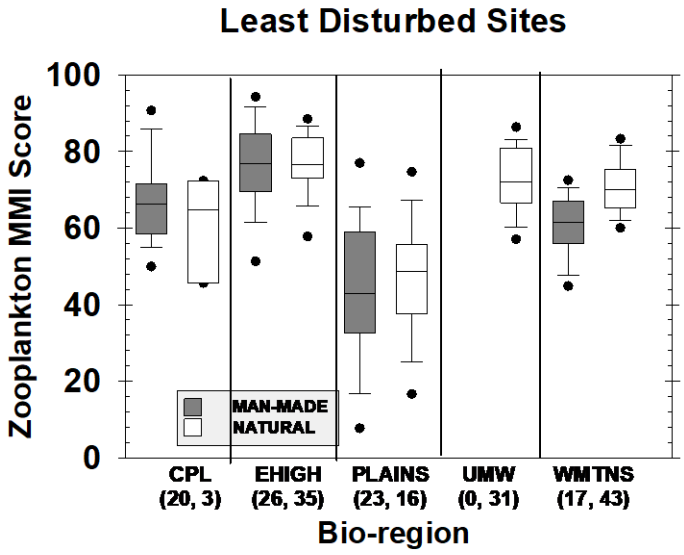

Figure 7.10. NLA 2012 Zooplankton MMI scores of human-made (shaded boxes) versus natural lakes (unshaded boxes) for least disturbed sites in five bio-regions. See Figure 7.1 for bio-region codes. Sample sizes for each type are in parentheses. Dots indicate 5th and 95th percentiles.

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.

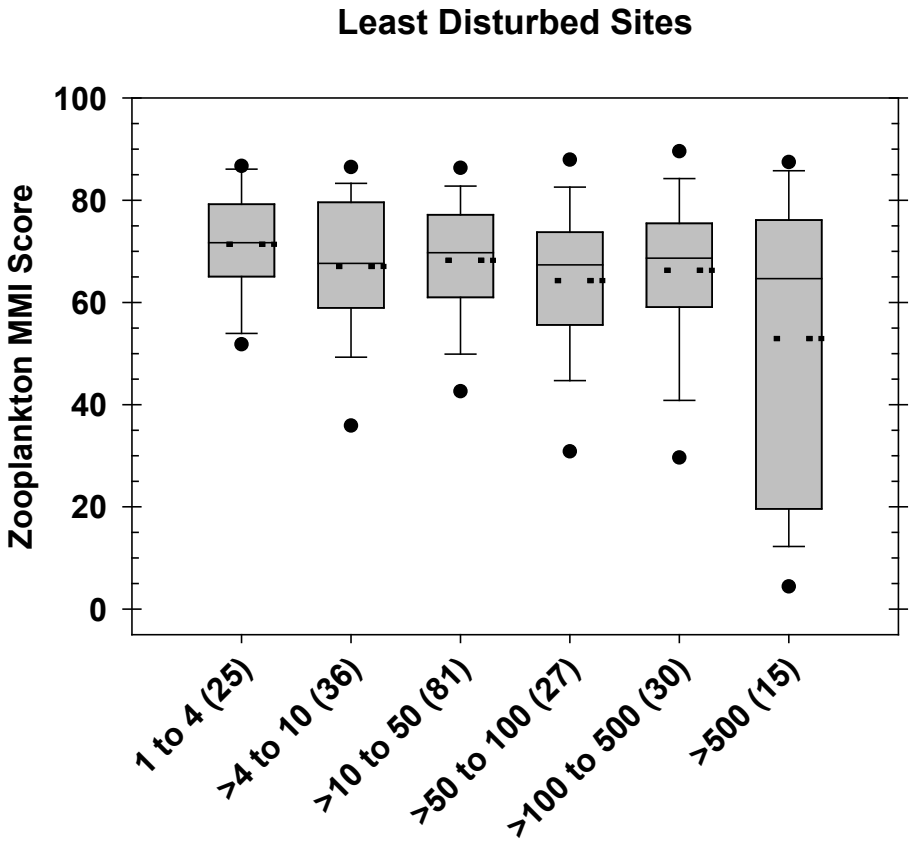

Figure 7.11. Zooplankton MMI scores versus lake size class within least disturbed lakes of the NLA 2012. Sample sizes are in parentheses. Dashed lines are mean values. Dots indicate the 5th and 95th percentiles.

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).

7.6.5.4 *Component metrics used in zooplankton MMIs for NLA 2022*

Table 7-9 summarizes the component metrics for each of the five zooplankton MMIs used in NLA 2022. There were no changes or modifications from those used in the NLA 2012.

Table 7-9. Component metrics of the zooplankton multimetric indices (MMIs) used for NLA 2022.

Metric Category	Metric Description	Direction of Response[a]	Metric Variable Name
Coastal Plains MMI			
Abundance/Biomass/Density	Biomass of fine mesh net (50 µm) taxa	INC	FINE_BIO
Cladoceran	% of total individuals that are within the cladoceran family Sididae	INC	SIDID_PIND
Copepod	% of biomass in dominant copepod taxon (300 count subsamples)	DEC	DOM1_300_COPE_BIO
Richness/Diversity	Number of native families (300 count subsamples)	DEC	FAM300_NAT_NTAX
Rotifer	% of total biomass within the rotifer order Collothecaceae	DEC	COLLO_PBIO
Trophic	% of taxa that are omnivorous	INC	OMNI_PTAX
Eastern Highlands MMI			
Abundance/Biomass/Density	Density of individuals collected in coarse mesh net (150-µm)	INC	ZOCN_DEN
Cladoceran	% Biomass represented by small cladoceran individuals	INC	SMCLAD_PBIO
Copepod	Density represented by native copepod individuals	INC	COPE_NAT_DEN
Richness/Diversity	% of taxa that are larger-sized and native	DEC	COARSE_NAT_PTAX
Rotifer	Percent total biomass from rotifers	INC	ROT_PBIO
Trophic	Percent of taxa that are omnivorous (300-count subsamples)	INC	OMNI300_PTAX
Plains MMI			
Abundance/Biomass/Density	% of biomass represented in individuals of smaller-sized native taxa (300-count subsamples)	DEC	FINE300_NAT_PBIO
Cladoceran	% of native individuals within the suborder Cladocera that are "small" (coarse and fine net samples combined)	DEC	SMCLAD_NAT_PIND

Metric Category	Metric Description	Direction of Response[a]	Metric Variable Name
Copepod	Ratio of Calanoids to (Cladocera+Cyclopoids) based on biomass (300-count subsamples).	DEC	COPE_RATIO_300_BIO
Richness/Diversity	Total native family richness (300-count subsamples)	DEC	FAM300_NAT_NTAX
Rotifer	Number of rotifer taxa	DEC	ROT_NTAX
Trophic	% of total density represented by herbivorous copepods	INC	COPE_HERB_PDEN
Upper Midwest MMI			
Abundance/Biomass/Density	% of native individuals	DEC	TOTL_NAT_PIND
Cladoceran	% of native taxa that are within the cladoceran family Bosminidae (300-count subsamples)	DEC	BOSM300_NAT_PTAX
Copepod	Biomass of individuals within native calanoid taxa (300-count subsamples)	INC	CALAN300_NAT_BIO
Richness/Diversity	% of fine mesh net (50 µm) taxa	DEC	FINE_PTAX
Rotifer	Percent of rotifer biomass in dominant rotifer taxon	INC	DOM1_ROT_PBIO
Trophic	Percent of biomass represented by herbivorous copepods (300-count subsamples)	INC	COPE_HERB300_PBIO
Western Mountains MMI			
Abundance/Biomass/Density	% biomass of individuals of native coarse mesh net (150 µm) taxa (300-count subsamples)	IDEC	COARSE300_NAT_PBIO
Cladoceran	% of distinct native taxa that are large cladocerans (300-count subsamples)	DEC	LGCLAD300_NAT_PTAX
Copepod	Total biomass of copepod individuals within the subclass Copepoda (300-count subsamples)	INC	COPE300_BIO
Richness/Diversity	Number of taxa in the fine net (50-µm) sample (300-count subsample)	INC	ZOFN300_NTAX
Rotifer	% taxa that are within the rotifer order Ploima	DEC	PLOIMA_PTAX
Trophic	% taxa that are omnivorous copepods	INC	COPE_OMNI_PTAX

[a] Direction of response to increased disturbance: INC= response increases with increased disturbance, DEC=response decreases with increased disturbance.

7.7 Thresholds for assigning ecological condition

7.7.1 *NLA 2012*

For the NLA 2012, we followed Stoddard et al. (2008) in using the set of least disturbed sites (including calibration and validation sites) to set ecological condition benchmarks 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 6). 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 benchmarks, 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 benchmark adjustment statistical analysis.

The best regression model had two different slopes and separate intercepts for each bio-region (Table 7-10). 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 the PLAINS, and 74.39 in the WMTNS. Table 7-11 shows both the raw (unadjusted sample) 5th and 25th percentiles and the regression model adjusted percentiles that we are using as the MMI benchmarks. In three bio-regions (CPL, EHIGH, and UMW), the adjustment resulted in as slight lowering (< 2 points) of the Good/Fair benchmark value. In the PLAINS and WMTNS bio-regions, the Good/Fair benchmark values were increased (4.6 to 5.6 points). Adjustment lowered the Fair/Poor benchmark values in the CPL, EHIGH, and UMW bio-regions by 2.7 to 6.7 points. The Fair/Poor benchmark value was increased by 14.5 points in the PLAINS bio-region, and 3.9 points in the WMTNS bio-region.

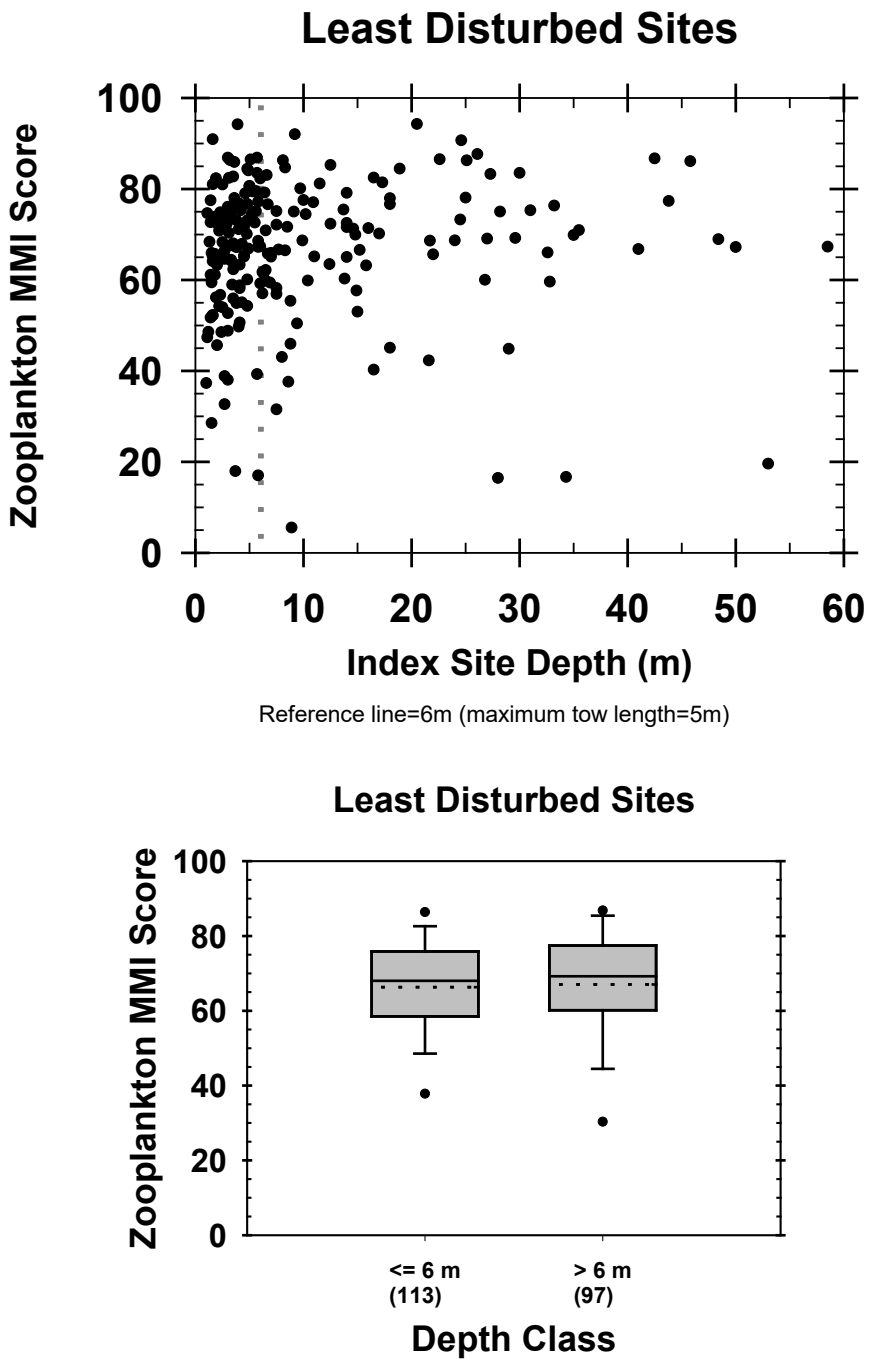

Figure 7.12.Zooplankton MMI scores versus site depth for least disturbed sites.
Upper panel shows MMI scores versus actual site depth. The reference line of 6 m separates shallower lakes where the entire water column was sampled and deeper lakes where part of the water column was not sampled. The lower 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 7-10. 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 7-11. ECOLOGICAL CONDITION BENCHMARKS FOR ZOOPLANKTON MMI SCORES (NLA 2012 ONLY) 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.

Bio-Region	*n*[a]	Good/Fair Benchmark (P_{25})		Fair/Poor Benchmark (P_5)		Range of MMI scores in Least disturbed Sites
		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.

7.7.2 *NLA 2017*

The process used to develop condition class benchmarks in the NLA 2012 was modified as follows for the NLA 2017:

1. We excluded within-year revisits (see Section 2.1.3) and used the 2017 visit for sites that were sampled in both 2012 and 2017.
2. We did not try to adjust the benchmarks for varying quality among regions by using the ”hindcasting” approach described by Herlihy et al. (2008).

Before calculating benchmarks for each of the five bio-regions, we removed outliers based on a 1.5*IQR outlier analysis of the MMI scores in least disturbed sites (Tukey 1977). 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). The revised benchmark values (Table 7-12) were used to assign condition classes for the NLA 2017 sites, and to re-assign condition classes for the NLA 2012 sites (so that the change in condition status could be estimated).

Table 7-12 Ecological condition benchmarks for NLA 2017 zooplankton MMI scores based on the distribution of least disturbed sites in five aggregated ecoregions (bio-regions).
Poor condition indicates a site is different from least disturbed condition. Fair condition indicates a site is somewhat different from least disturbed condition. Good condition indicates a site is similar to least disturbed condition.

Bio-region	Number of Least Disturbed Zooplankton Sites[a]	Good-Fair Benchmark	Fair-Poor Benchmark
Coastal Plains	23	59.42	53.77
Eastern Highlands	88	73.595	60.03
Plains	61	36.72	28.17
Upper Midwest	61	63.68	52.03
Western Mountains	102	60.78	51.32

[a] Based on a single visit per site from the NLA 2012 and the NLA 2017 and after excluding sites where less than 100 individuals were collected in either the coarse or fine net sample, anomalous samples, and statistical outliers.

7.7.3 *NLA 2022*

For the NLA 2022, we used the same benchmarks for assigning condition class as those presented in Table 7-12.

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 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 benchmark values for them based on a very small number of least disturbed lakes.

The regional zooplankton MMIs have the following 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) and would reduce the precision associated with biomass estimates due to lumping of taxa to coarser levels. Many richness metrics didnot perform well in the 2012 NLA, but stronger richness signals may be observed in future rounds of the NLA. Many density- and biomass-based metrics did perform well, thus laboratory analyses will require the determination of biomass, which increases costs.

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 most 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 described in this document.

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 are not aware of any studies that have conducted an evaluation of an exhaustive list of candidate zooplankton metrics such as we developed for the NLA; it is possible that there has not been the incentive to do so up to now. We hope that the success of the initial NLA zooplankton MMIs will increase interest in the use of zooplankton metrics and indices in lake bioassessment activities. This would lead to additional information related to responses of zooplankton assemblages to various types of human disturbance.

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; Hébert et al. (2016)), 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. Tolerance 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 NLA 2007 that would allow tolerance values to be developed and applied to the NLA zooplankton MMI, albeit at a coarser taxonomic level than species, and tolerance values derived from NLA 2012 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 NLA does 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.

The primary modifications to the NLA zooplankton MMI indicator implemented for the NLA 2017 were focused on defining the reference distribution for ecological condition benchmark calculations. Adding a minimum count criterion for excluding least disturbed sites before calculating ecological condition benchmarks is consistent with what is done for the NLA benthic macroinvertebrate MMI. We excluded more NLA 2012 sites with this screen than NLA 2017 sites. The observed decrease may have been due to clarifications made in the field NLA 2017 operations manual and during training to help reduce the occurrence of problematic samples. For sites that were not least disturbed, we did not treat sites with low counts differently, unless there was evidence that any zooplankton sample was compromised.

We combined least disturbed sites from the NLA 2012 and the NLA 2017 to increase the sample sizes to provide more robust estimates of the percentiles on which the condition class benchmarks are based. This is consistent with what has been done for several other NLA indicators that derive benchmarks based on least disturbed condition. Sample sizes were substantially increased in four of the five bio-regions. The sample size for the Coastal Plains (CPL; n=24) was only increased by two sites over what was available in the NLA 2012.

Finally, we have determined for other indicators and NARS assessments that adjusting the percentiles used as benchmarks for ecological condition class assignments using the approach described in Herlihy et al. (2008) does not yield benchmarks that are much different from the unadjusted percentiles for nearly all aggregated ecoregions (or bio-regions). The adjustment process requires additional time and effort and is more complicated to explain. Having increased sample sizes of least disturbed sites from combining multiple surveys may be a factor in the increased comparability of the unadjusted and adjusted percentiles.

Several aspects of the zooplankton MMI development process warrant further work:

1. Evaluating MMIs constructed using the best-performing metrics regardless of their metric category.
2. Investigating metrics that perform well, but whose response to disturbance appears to be contrary to our current expectations.
3. Developing better autecological information for zooplankton taxa, especially with respect to tolerance to environmental stressors.

All of these aspects are still applicable after the NLA 2017 study and could lead to refinements of the MMI process before the next round of the NLA is implemented in 2017.

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Chapter 8: Human Health Water Quality Indicators

8.1 Enterococci indicator

The EPA developed and validated a molecular testing method employing quantitative polymerase chain reaction (qPCR) as a rapid approach for the detection of enterococci in recreational water (USEPA 2015). NLA used this method to estimate the presence and quantity of these fecal indicator bacteria in the nation's lakes. The statistical benchmark value of 1280 calibrator cell equivalents (CCE)/100 mL from EPA's 2012 *Recreational Water Quality Criteria* document (USEPA 2012)was then applied to the enterococci data to assess the recreational condition of coastal waters.

8.1.1 *Field collection*

To collect enterococci samples, field crews took a water sample from the last littoral station or the launch site in an area that was approximately 1 m deep at about 0.3 m (12 inches) below the water. Following collection, crews placed the sample in a cooler and kept it on ice prior to filtration of two 50 mL volumes. Samples were filtered and frozen on dry ice within 6 hours of collection. The frozen filters were shipped to the laboratory on dry ice. A sterile phosphate buffer solution (PBS) blank was also filtered at revisit sites durring one of the two visits.

8.1.2 *Lab methods*

The sample collections and the laboratory method followed EPA's Enterococcus qPCR Method 1609.1 (USEPA 2015);). Method 1609.1 describes a quantitative polymerase chain reaction (qPCR) procedure for the detection of DNA from enterococci bacteria in ambient water matrices based on the amplification and detection of a specific region of the large subunit ribosomal RNA gene (lsrRNA, 23S rRNA) from these organisms. This method uses an arithmetic formula (the comparative cycle benchmark (CT) method; Applied Biosystems, 1997) to calculate the ratio of Enterococcus lsrRNA gene target sequence copies (TSC) recovered in total DNA extracts from the water samples relative to those recovered from similarly prepared extracts of calibrator samples containing a consistent, pre-determined quantity of Enterococcus cells. Mean estimates of the absolute quantities of TSC recovered from the calibrator sample extracts were then used to determine the quantities of TSC in the water samples and then converted to CCE values as described in the section below. To normalize results for potential differences in DNA recovery, monitor signal inhibition or fluorescence quenching of the PCR analysis caused by a sample matrix component, or detect possible technical error, CT measurements of sample processing control (SPC) and internal amplification control (IAC) target sequences were performed as described in Method 1609.1.

8.1.3 *Analysis and application of benchmarks*

8.1.3.1 *Calibration*

Estimates of absolute TSC recoveries from the calibrator samples were determined from standard curves using EPA-developed plasmid DNA standards of known TSC concentrations as described in Method 1609.1. Estimates of TSC recovered from the test samples were determined by the comparative cycle threshold (CT) method, as also described in Method 1609.1. Before applying the EPA benchmark to the qPCR data, it was necessary to convert the TSC estimates to CCE values.

The standardized approach developed for this conversion is to assume 15 TSC/CCE (USEPA 2015). This approach allows the CCE values to be directly compared to the EPA RWQC values (Haugland et al., 2014). A slightly modified approach was employed in the earlier NRSA 2008-09 study to obtain the same conversions of TSC to standardized CCE units.

8.1.3.2 *Benchmarks*

For the data analysis of the enterococci measurements determined by Method 1609.1, analysts used a benchmark as defined and outlined in EPA's recommended recreational criteria document for protecting human health in ambient waters designated for swimming (USEPA 2012). Enterococci CCE/100 mL values were compared to the EPA benchmark of 1280 CCE/100 mL.

Within-year sampling variability was assessed by comparing NLA 2022 visit 1 and 2 condition categories and is presented in Table 8-1. For conditions categories of "at or below benchmark", "above benhcmark" and "not assessed", results showed agreement in 84 (87.5%) of the 96 revisit sites sampled in 2022.

Table 8-1 Enterococci condition contingency table; N = 96.

		Enterococci Condition		
		Visit 1		
		At or Below Benchmark	Above Benchmark	Not Assessed
Visit 2	At or Below Benchmark	82	5	1
	Above Benchmark	6	2	
	Not Assessed			

8.2 Cyanobacteria toxins (Cyanotoxins)

Cyanobacteria are one-celled photosynthetic organisms that normally occur at low levels. Under eutrophic conditions, cyanobacteria can multiply rapidly. Not all cyanobacterial blooms are toxic, but some may release toxins, such as microcystins and cylindrospermopsin. For the NLA, both microcystins and cylindrospermopsin were analyzed.

Recreational exposure is typically a result of inhalation, skin contact, or accidental ingestion. When people are exposed to cyanotoxins, adverse health effects may range from a mild skin rash to serious illness or in rare circumstances, death. Acute illnesses caused by short-term exposure to cyanobacteria and cyanotoxins during recreational activities include hay fever-like symptoms, skin rashes, respiratory and gastrointestinal distress.

Microcystins refers to an entire group of toxins (all of the different congeners, rather than just one congener). Cyanobacteria can produce one or many different congeners at any one time, including Microcystin - LR (used in the kit's calibration standards), Microcystin - LA, and Microcystin - RR. The different letters on the end signify the chemical structure (each one is slightly different) which makes each congener different.

8.2.1 *Field methods*

Samples for cyanotoxin analyses were collected using a 0-2 m vertically integrated water column sampler at the open-water site. Water from the photic zone was emptied into a 4L cubitainer and then transferred to a 500 mL bottle. The bottle was kept on ice and then stored frozen until analysis. Both microcystins and cylindrospermopsin were analyzed from the 500 mL bottle.

8.2.2 *Analysis and application of benchmarks*

Microcystins were measured using an enzyme-linked immunosorbent assay (ELISA) procedure with an Abraxis Microcystins-ADDA Test Kit. For freshwater samples, the procedure's reporting range is 0.15 μ g/L to 5.0 μ g/L and the minimum detection level (MDL) is 0.10 µg/L. Microcystins concentrations were evaluated against the EPA recommended criterion and swimming advisory level of 8 µg/L (USEPA 2019).

The cylindrospermopsin sample was measured using an enzyme-linked immunosorbent assay (ELISA) procedure with an Abraxis Cylindrospermopsin Test Kit. For freshwater samples, the procedure'sreporting range is 0.02 μ g/L to 2.0 μ g/L and the MDL is 0.04 µg/L. Cylindrospermopsin concentrations were evaluated against the EPA recommended criterion and swimming advisory level of 15 µg/L (USEPA 2019).

The NLA also reports on the percentage of lakes with detections of cyanotoxins and changes in detection over time. Detection is defined as a value greater than the MDL. When the MDL changed between surveys, the greatest MDL for all surveys is used to determine detect/not detected.

Within-year sampling variability for microcystin condition was assessed by comparing NLA 2012, 2017 and 2022 visit 1 and 2 condition categories and is presented in Table 8-3Table 8-1. For microcystin detection, results showed agreement in 221 (75%) of the 293 revisit sites

sampled over three survey years. For microcystin risk condition, 289 (98%) of the 293 revisit sites over three survey years were in agreement.

Table 8-2. Microcystin detection (a) and risk condition (b) contingency tables; N = 293.

a)		Microcystin Detection		
		Visit 1		
		Detected	Not-detected	Not Assessed
Visit 2	Detected	72	39	
	Not detected	33	148	
	Not Assessed			1

b)		Microcystin Risk Condition		
		Visit 1		
		At or Below Benchmark	Above Benchmark	Not Assessed
Visit 2	At or Below Benchmark	287	2	
	Above Benchmark	2	1	
	Not Assessed			1

Within-year sampling variability for cylindrospermopsin was assessed by comparing NLA 2017 and 2022 visit 1 and 2 condition categories and is presented in Table 8-3Table 8-1. For detection, results showed agreement in 177 of the 193 revisit sites. For risk condition, 100% of the risk categores were the same.

Table 8-3. Cylindrospermopsin detection (a) and risk condition (b) contingency tables; N = 193.

a)		Cylindrospermopsin Detection		
		Visit 1		
		Detected	Not-detected	Not Assessed
Visit 2	Detected	12	11	
	Not detected	4	165	
	Not Assessed			1

b)		Cylindrospermopsin Risk Condition		
		Visit 1		
		At or Below Benchmark	Above Benchmark	Not Assessed
Visit 2	At or Below Benchmark	192		
	Above Benchmark			
	Not Assessed			1

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Chapter 9: Human Health Fish Tissue Indicators

Fish are time-integrating indicators of persistent pollutants, and the bioaccumulation of contaminants in fish tissue has important human health implications. Contaminants in fish pose various health risks to human consumers (e.g., cancer risks, and noncancer risks such as reproductive effects or impacts to neurological development). The NLA 2022 human health fish tissue indicator consists of the collection of whole fish samples for homogenized fillet analyses. These samples provide information on the national distribution of selected persistent, bioaccumulative, and toxic (PBT) chemical residues (specifically, mercury, polychlorinated biphenyls, or PCBs, and per- and polyfluoroalkyl substances, or PFAS) in fish species that people might catch and eat. Results of analyses of mercury, PCB, and PFAS fillet tissue concentrations are presented for this indicator.

9.1 Field fish collection

The human health fish tissue indicator field and analysis procedures described below were based on the EPA's *National Study of Chemical Residues in Lake Fish Tissue* (USEPA 2009) and the EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories*, Volumes 1-2 (third edition) (USEPA 2000a).

The NLA crews attempted to collect whole fish samples for the fillet tissue indicator from a subsample of approximately two-thirds of the lakes included in the survey design. In total, 413 whole fish composite samples were collected from the 636 designated lakes in the lower 48 states. Each lake had a surface area >1 hectare and contained at least 1,000 square meters of open, unvegetated water and a permanent population of predator fish species. The fish samples collected for fillet tissue analysis consisted of a composite of predator fish specimens[1] from each site. Additional criteria for each fish composite sample included fish that were:

- All of the same fish species that are commonly caught and consumed by humans,
- Harvestable size per legal requirements or of consumable size if there were no harvest limits,
- At least 190 mm in length and of similar size so that the smallest individual in the composite was no less than 75% of the total length of the largest individual in the composite, and
- Sufficiently abundant within the lake.

Crews were provided with a recommended list of primary and secondary fish species (Table 9-1), but they could choose an appropriate substitute (based on the criteria listed above) if none of the recommended fish species were available. Fish collection data were screened to exclude individual fish specimens with lengths less than 190 mm or composite samples where field crews collected non-target or unacceptable substitute species.

To prepare fillet composite samples for chemical analysis, fish composite samples from each site were scaled and filleted in the laboratory. In filleting individual fish, muscle tissue was removed from both sides of each fish leaving the skin on and the belly flap attached to the fillet. Fillets from the individual specimens that comprised a composite sample were homogenized together before being analyzed for contaminants.

[1] Use of composite sampling for screening studies is a cost-effective way to estimate average contaminant concentrations while also ensuring that there is sufficient fish tissue to analyze for all contaminants of concern and to archive surplus tissue, when possible.

Table 9-1. Primary and secondary NLA target species for human health fish collection

PRIMARY PREDATOR HUMAN HEALTH FISH TARGET SPECIES		
FAMILY	SCIENTIFIC NAME	COMMON NAME
Centrarchidae	*Micropterus salmoides*	Largemouth Bass
	Micropterus dolomieu	Smallmouth Bass
	Pomoxis nigromaculatus	Black Crappie
	Pomoxis annularis	White Crappie
Percidae	*Sander vitreus*	Walleye
	Perca flavescens	Yellow Perch
Moronidae	*Morone chrysops*	White Bass
Esocidae	*Esox lucius*	Northern Pike
Salmonidae	*Salvelinus namaycush*	Lake Trout
	Salmo trutta	Brown Trout
	Oncorhynchus mykiss	Rainbow Trout
	Salvelinus fontinalis	Brook Trout
SECONDARY PREDATOR HUMAN HEALTH FISH SPECIES		
FAMILY	SCIENTIFIC NAME	COMMON NAME
Centrarchidae	*Lepomis macrochirus*	Bluegill
	Ambloplites rupestris	Rock Bass
	Micropterus punctulatus	Spotted Bass
Percidae	*Sander canadensis*	Sauger
Moronidae	*Morone saxatilis*	Striped Bass
	Morone americana	White Perch
Esocidae	*Esox niger*	Chain Pickerel
Salmonidae	*Oncorhynchus clarkii*	Cutthroat Trout
	Coregonus clupeaformis	Lake Whitefish
	Prosopium williamsoni	Mountain Whitefish

9.2 Mercury analysis and fish tissue screening levels to protect human health

All fish tissue samples were analyzed for total mercury. The samples were prepared using EPA Method 1631B, Appendix A (USEPA 2001a) and analyzed using EPA Method 1631E (USEPA 2002), which utilizes approximately 1 g of fillet tissue for analysis. In screening-level studies of fish contamination, the EPA guidance recommends monitoring for total mercury rather than methylmercury (an organic form of mercury) because most mercury in adult fish is in the toxic form of methylmercury which will be captured during an analysis for total mercury. Applying the assumption that all mercury is present in fish tissue as methylmercury is a conservative approach protective of human health.

The fish tissue criterion used to interpret mercury concentrations in fillet tissue for human health protection is 0.3 milligrams (mg) of methylmercury per kilogram (kg) of tissue (wet weight), or 300 parts per billion (ppb), which is EPA's fish tissue-based CWA Section 304(a) water quality criterion recommendation for methylmercury (EPA 2001b).[2] For more information on the screening levels for human health protection, see Section 9.5.

[2] Because the EPA relies on the recommended CWA Section 304(a) national water quality criterion for methylmercury to interpret the mercury results, the EPA is only reporting mercury results for general population and is not including an additional analysis and interpretation of mercury results for high-frequency fish consumers or reduced-frequency fish consumers.

Application of this criterion to the mercury fillet tissue composite data from this study identifies the proportion of lakes in the sampled population containing fish with mercury fillet tissue concentrations that are above the criterion. Mercury concentration data from analysis of homogenized fish fillet composite samples are available to download from the NLA Fish Tissue Study webpage. Summary statistics, including the number of detections, are reported in Table 9-2, and the proportion of lakes with sample exceedances above the mercury criterion is reported in Table 9-3.

9.3 PCB analysis and fish tissue screening levels to protect human health

All fish tissue samples were analyzed for PCBs. EPA Method 1668C (USEPA 2010) was used to analyze approximately 10 g of homogenized fillet tissue from each fish composite sample to provide results for the full suite of 209 PCB congeners. The total PCB concentration for each sample was determined by summing the results for any of the 209 congeners that were detected, using zero for any congeners that were not detected in the sample.

In the main report, *National Lakes Assessment: The Fourth Collaborative Survey of Lakes in the United States*, the EPA included total PCB results for general fish consumers (those who may eat one 8-ounce meal of locally caught fish per week), for high-frequency fish consumers (those who may eat four or five 8-ounce meals of locally caught fish per week), and for reduced-frequency fish consumers (those who may eat one 8-ounce meal of locally caught fish per month). The EPA used fish tissue screening levels, expressed as wet-weight concentrations of total PCBs, to protect human health by characterizing cancer human health risks for these three levels of fish consumers. For more information on the fish tissue screening levels for human health protection, see Section 9.5.

Application of these screening levels to the PCB fillet tissue data identifies the proportion of lakes in the sampled population containing fish with total PCB fillet concentrations that are above each total PCB fish tissue screening level. PCB concentration data from the analysis of homogenized fish fillet composite samples are available to download from the NLA Fish Tissue Study webpage. Summary statistics, including the number of detections, are reported in Table 9-2, and the proportion of lakes with sample exceedances above each screening level for three levels of fish consumers is reported in Table 9-3.

9.4 PFAS analysis and results

All fish tissue samples were analyzed for 40 per- and polyfluoroalkyl substances (PFAS), using EPA Method 1633 (USEPA 2024a). This method, which utilizes approximately 2 g of fillet tissue for analysis, uses high performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) and applies isotope dilution to determine the concentration of each of the 40 PFAS.

In the main report, *National Lakes Assessment: The Fourth Collaborative Survey of Lakes in the United States*, EPA reports results on frequencies of detection of the most commonly detected PFAS (i.e., those PFAS that were detected in at least 20 percent of the fillet tissue samples). In addition, EPA reports the estimates of the number of lakes in the sampled population containing fish with detectable levels of PFAS. PFAS concentration data from fish fillet tissue composite samples are available to download from the NLA Fish Tissue Study webpage. Summary statistics for PFAS, including the number of detections for each of the 40 tested PFAS are provided in Table 9-2.

Table 9-2. NLA 2022 fish tissue fillet composite sample summary data

Chemical	Number of Detections	Detection Frequency (%)	MDL (ppb)[a]	Measured Minimum Concentration (ppb)[b]	Weighted Median Concentration (ppb)	Measured Maximum Concentration (ppb)
Mercury	413	100	0.8	4.5	308.956	1660
Total PCBs	413	100	0.000134 – 0.000797	0.013	0.958	131.482
Perfluoroalkyl carboxylic acids						
PFBA	3	0.73	0.372	0.472	<MDL	0.687
PFPeA	1	0.24	0.077	0.141	<MDL	0.141
PFHxA	0	0	0.179	0	<MDL	0
PFHpA	5	1.21	0.081	0.084	<MDL	0.263
PFOA	4	0. 97	0.152	0.175	<MDL	1.550
PFNA	95	23.00	0.127	0.123	<MDL	5.750
PFDA	330	79.90	0.134	0.131	0.486	134
PFUnA	349	84.50	0.171	0.170	0.737	28.500
PFDoA	293	70.94	0.087	0.087	0.212	35.700
PFTrDA	205	49.64	0.234	0.230	0.258	9.99
PFTeDA	164	39.71	0.168	0.159	<MDL	13.0
Perfluoroalkyl sulfonic acids						
PFBS	0	0	0.090	0	<MDL	0
PFPeS	0	0	0.061	0	<MDL	0
PFHxS	18	4.36	0.050	0.055	<MDL	0.416
PFHpS	14	3.39	0.042	0.042	<MDL	0.251
PFOS	357	86.44	0.218	0.209	3.168	526
PFNS	7	1.69	0.066	0.074	<MDL	0.291
PFDS	92	22.28	0.062	0.059	<MDL	5.160
PFDoS	1	0.24	0.113	0.121	<MDL	0.121
Fluorotelomer sulfonic acids						
4:2 FTS	0	0	0.462	0	<MDL	0
6:2 FTS	0	0	7.870	0	<MDL	0
8:2 FTS	0	0	1.190	0	<MDL	0
Perfluorooctane sulfonamides						
PFOSA	10	2.42	0.143	0.148	<MDL	0.670
N-MeFOSA	0	0	0.372	0	<MDL	0.031
N-EtFOSA	0	0	0.227	0	<MDL	0.013
Perfluorooctane sulfonamidoacetic acids						
N-MeFOSAA	15	3.63	0.089	0.090	<MDL	0.551
N-EtFOSAA	9	2.18	0.087	0.091	<MDL	2.010
Perfluorooctane sulfonamide ethanols						
N-MeFOSE	0	0	6.330	0	<MDL	0.080
N-EtFOSE	18	4.36	2.380	2.410	<MDL	5.630
Per- and Polyfluoroether carboxylic acids						
HFPO-DA	0	0	0.288	0	<MDL	0

Chemical	Number of Detections	Detection Frequency (%)	MDL (ppb)[a]	Measured Minimum Concentration (ppb)[b]	Weighted Median Concentration (ppb)	Measured Maximum Concentration (ppb)
ADONA	0	0	0.384	0	<MDL	0
PFMPA	0	0	0.059	0	<MDL	0
PFMBA	2	0.48	0.073	0.132	<MDL	0.157
NFDHA	0	0	1.600	0	<MDL	0
Ether sulfonic acids						
9Cl-PF3ONS	0	0	0.359	0	<MDL	0
11Cl-PF3OudS	0	0	0.290	0	<MDL	0
PFEESA	0	0	0.033	0	<MDL	0
Fluorotelomer carboxylic acids						
3:3 FTCA	1	0.24	0.304	1.250	<MDL	1.250
5:3 FTCA	1	0.24	3.150	972.000	<MDL	972.000
7:3 FTCA	7	1.69	1.300	1.280	<MDL	299.000

[a] MDL = Method Detection Limit in ppb, wet weight, based on the nominal sample mass analyzed. Because some samples were analyzed using a slightly larger mass, some of the minimum values in this table may be slightly below the nominal MDL values shown. PCB MDLs presented as a range because there are 209 PCB congeners with associated MDLs.

[b] The minimum and maximum concentrations are the measured minimum and maximum values from 413 sites in 2022. A value of zero was assigned to any PCB congener or PFAS compound that was not detected in the sample.

9.5 Calculation of fish tissue screening levels for human health protection

For methylmercury, the EPA used the Agency's recommended CWA Section 304(a) fish tissue-based ambient water quality criterion for methylmercury (EPA 2001b) as the screening level for human health protection to evaluate mercury fish fillet tissue results. (Note: EPA applies the conservative assumption that all mercury in fish is methylmercury and therefore measures total mercury in fillet tissue to be most protective of human health.)

For PCBs, because there is no EPA recommended CWA Section 304(a) fish-tissue based ambient water quality criterion for protection of human health for PCBs, the EPA followed the approach in its Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories (USEPA 2000a) to calculate fish tissue screening levels for human health protection. Three screening levels were calculated corresponding to three different rates of fish consumption: high-frequency fish consumers (those who may eat four to five 8-ounce meals of locally caught fish per week), general fish consumers (those who eat one 8-ounce meal of locally caught fish per week) and reduced-frequency fish consumers (those who eat an 8-ounce meal of locally caught fish just once per month).

Each screening level (SL) calculation is based on chemical-specific toxicity information, expressed as a reference dose value (RfD, mg/kg day) for noncancer health effects, or a cancer slope factor (CSF, mg/kg day^{-1}) for cancer health effects, a cancer risk level (CRL, unitless) for cancer health effects, and assumptions about the body weight of people who eat fish (BW, kg), and the amount of fish they eat (FCR, kg/day).

Each screening level is expressed as a fish fillet tissue contaminant level (ng/g or ppb) that, if exceeded, may adversely impact human health among people who eat a specified amount of fish. See Table 9-3 for the chemical- and fish consumption-specific screening levels and the estimated portion of lakes that contained fish with fillet tissue that exceeded screening levels corresponding to each contaminant and fish consumption rate.

Because PCBs can have both cancer and noncancer health effects, the EPA calculated fish tissue screening levels for each type of health effect and used the lower of the two screening levels. For each rate of fish consumption (general fish consumers, high-frequency fish consumers and reduced-frequency fish consumers), the EPA developed two fish tissue screening levels for PCB human health impacts for the purpose of directly comparing to fish fillet tissue results – one based on noncancer effects, and one based on cancer effects. The screening levels represent the concentration of total PCBs in fish tissue that should not be exceeded based on three levels of fish consumption rates ranging from 0.142 kg of fish/day (for high-frequency fish consumers who may consume four or five 8-ounce serving of locally caught fish per week) to 0.0324 kg of fish/day (for general fish consumers who may consume one 8- ounce serving of locally caught fish per week)to 0.00745 kg of fish/day (for reduced-frequency fish consumers those who may eat one 8-ounce serving of freshwater fish per month). The PCB screening levels were also based on a human adult body weight default value of 80 kg[3] and a RfD of 0.00002 mg/kg day or a cancer slope factor of 2 $(mg/kg/d)^{-1}$ (USEPA 1994) and a cancer risk level of 10^{-5}. For the screening level for general fish consumers, EPA used a fish consumption rate of 32 grams per day (or one 8-ounce meal of locally caught river fish per week), consistent with the U.S. Department of Agriculture and Department of Health and Human Services' Dietary Guidelines for Americans, 2020-2025 (USDA and HHS 2020). For the screening level for high-frequency fish consumers (such as subsistence or recreational fishers or individuals from underserved populations), EPA used a fish consumption rate of 142 grams per day (or four to five 8-ounce meals of locally caught river fish per week) which is described in the EPA 2000 Human Health Methodology (USEPA 2000b). Because the total PCBs screening levels associated with cancer effects were lower than the screening levels associated with noncancer effects, the EPA applied only the screening levels associated with cancer effects. This conservative approach is also likely to be protective against noncancer effects, which may occur at higher levels of total PCB contamination.

Table 9-3. NLA 2022 fish fillet tissue sampled population exceedances for mercury and total polychlorinated biphenyls (PCBs)

Chemical	Percent Detection, %	Percent of Lakes in the Sampled Population with Fish that Exceeded the Mercury Criterion Level, % (ppb. Note: 1 ppb = 1 ng/g)		
Mercury	100%	51% (300 ppb)		
Chemical	Percent Detection, %	Percent of Lakes in the Sampled Population with Fish that Exceeded the Total PCBs Screening Levels for Different Levels of Fish Consumers, % (Calculated Screening Level, ppb. Note: 1 ppb = 1 ng/g)		
		High-Frequency Consumer	General Consumer	Reduced-Frequency Consumer
		Four to five 8-oz meals/week	One 8-oz meal/week	One 8-oz meal/month
Total PCBs	100%	23% (2.8 ppb)	6% (12 ppb)	2% (54 ppb)

[3] The EPA's toxicity assessment for PCBs summarizes health effects to the general population of adults over a lifetime of exposure, so a national default estimated body weight for adults was used to derive screening levels for PCBs (see Exposure Factors Handbook, Chapter 8, Table 8-1).

9.6 Literature cited

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Chapter 10: From Analysis to Results

10.1 Background information

In the NLA 2022 public report, lake condition estimates based on chemical, physical and biological information are expressed as percentage 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 (981). Extent estimates for biological indicators and other measures are used to calculate relative and attributable risk.

10.2 Population estimates

The survey design for the NLA, discussed in Chapter 2 of this document, produces a spatially balanced sample using the NHDPlus HR for 1-5 ha lakes as the sampling frame. Each lake has a known probability of being sampled (Stevens and Olsen 1999, Stevens and Olsen 2000, Stevens and Olsen 2004). A sample weight is assigned to each individual site as the inverse of the probability of that lake being sampled. Sample weights can be adjusted for different survey populations (e.g., sampled population or target population; see Chapter 2 and Appendix B) and are expressed as number of lakes. In 2017, EPA determined it was appropriate to adjust the site weights used to calculate the population estimates to represent the percentage of lakes relative to the target population and continued to present the results this way in 2022. Results presented in NLA 2007 and 2012 were relative to the sampled population.

The probability of a site being sampled was related to lake size class and was stratified by state. Site weights for the survey were adjusted to account for additional lakes (i.e., oversample lakes) that were evaluated when the primary lakes were not sampled (e.g., due to denial of access, being non-target). These 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 percentage of the entire resource) in a particular condition class for the entire contiguous 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. The NLA 2022 national results are the focus in the public report. Regional results are also presented for some indicators. All regional scale and subpopulation results are presented in the interactive dashboard.

10.2.1 *Subpopulations*

10.2.1.1 *Ecoregions*

The EPA has defined ecoregions at various scale, ranging from the coarse ecoregions at the continental scale (Level I) to finer ecoregions that divide the land into smaller units (Level II or IV). The nine ecoregions used in NLA are aggregations of the Level III ecoregions delineated by

EPA for the continental U.S. These nine ecoregions include the Northern Appalachians (NAP), Southern Appalachians (SAP), Coastal Plains (CPL), Upper Midwest (UMW), Temperate Plains (TPL), Southern Plains (SPL), Northern Plains (NPL), Western Mountains (WMT), and Xeric (XER). Additional information on the NLA ecoregions is available on the NARS website.

10.2.1.2 *Lake origin: natural vs. human-made*

The NLA condition estimates can also be explored and analyzed by lake origin. Unfortunately, there is not a clear dichotomy between natural and human-made lakes. Many naturally existing lakes are altered hydrologically to widely varying degrees by flow control structures, lake level augmentation, and other human activities. For NLA analyses, we defined human-made lakes as only those that are totally artificial, either impounded streams/rivers (reservoirs) or excavated basins, an adaption of the definition developed by Whittier et al. (2002) during the EMAP lake surveys. Excavated lakes are formed by flooding of quarries, borrow pits or any other type of human dug hole and usually lack flowing outlets. Impoundments were originally lotic waterbodies now turned into lentic waterbodies intentionally by humans. In our definition for NLA purposes, human-made lakes are those where no lake existed prior to European settlement. These include millponds, created residential, agricultural, or recreational ponds and lakes, as well as reservoirs created for flood control, water supply, or hydroelectric production. Every other type of lake is considered natural, even if the flow or shape is highly altered by humans.

It was not always easy to assign lake origin to NLA sample lakes. The following information was used after sampling to determine the classification for each lake:

- Lake name (reservoir in name);
- Google Earth views (or ArcGIS Explorer Desktop);
- Online topographic maps (ArcGIS Explorer Desktop or DeLorme Topo USA);
- Field collected data (i.e., assessment form including determination of Seepage/Drainage/Reservoir and dams; verification form with general comments; maximum lake depth);
- Initial site evaluation/reconnaissance determination;
- GNIS waterbody type;
- Internet lake history searches; and
- Ecoregion location.

The process used to determine lake origin in NLA has evolved based in part on lessons learned and in part due to advances in technology (e.g., availability of online images and maps and free apps such as Google Earth and ArcGIS, Desktop Explorer). As a first step, we look for agreement of the initial reconnaissance with the field crew classification, followed by a quick map or Google Earth review. When there are discrepancies in this information, a more in depth analysis was conducted. No one source of information on lake origin by itself, was definitive. Sources sometimes give conflicting answers; therefore, we used a weight of evidence approach to make the classification in difficult cases. General guidelines included the following.

1. Ecoregion location review. The Southern Appalachians have almost no natural lakes, any lake there classified as natural should be checked. Natural lakes are common in glaciated ecoregions and less common elsewhere. In NLA in the past, the ratio of human-made to natural lakes is about 1:1.
2. Google Earth review. Google Earth views are good to get the lay of the land and look for obvious dams and human activities/roads around the lake. A lake with no roads or human activity around them are unlikely to be human-made. We examined digital topographic maps for dams, or other evidence of impoundment such as a substantial elevation drop from lake surface to the outlet stream, a straight shoreline, or a road crossing at the outlet.
3. Comparison of the mapped elevation change at the outlet to the maximum lake depth. If maximum lake depth is greater than any possible dam/elevation change, it's not a human-made impoundment by our definition.
4. Historical information search. Most named lakes have a surprising amount of information about them on the internet (note this doesn't work well for very small lakes, or lakes with no name).

Some common types of lakes were especially problematic when assigning lake origin.

Oxbow/riverine flood plain lakes. Classic oxbow lakes are inherently natural. However, many old oxbow lakes or lakes in riverine floodplain are highly altered by human activities (e.g., road/railroad berms, bridges, dikes) and look very artificial. Unless we could tell that these lakes were actually created (dug out) by humans, we classified them all as natural.

Wetland complex lakes. A number of lakes are part of wetland complexes. Many of them are in areas heavily managed by humans (e.g., state and federal wildlife/wetland management areas). These lakes are often very shallow and augmented to hold more water, and the flows are highly regulated for purposes of wetland management. Whether these lakes met the NLA definition of a lake (< 1 m deep and 10,000 m2 of open water) in the past or not is almost impossible to determine. It's likely that in the past, some of these were what we now call wetlands and were not NLA target lakes. We have, however, classified all of these types of lakes as natural in that there was very likely some type of a wetland/waterbody there in the past, pre-human development.

Augmented natural lakes. Many natural lakes are flow altered by human activity either by outlet flow control or raising the height of the lake with some kind of dam. The dams on these lakes are often very apparent when looking through the various sources of lake information, but we consider these to be natural lakes if a lake basin existed their pre-human settlement. It can be difficult to determine if a lake basin existed in the past to separate them from what we define as human-made impoundments. Dam height (or elevation contours) versus lake depth was one approach to differentiate the two as well as doing internet history searches.

Irrigation/water district management. Water is often stored and moved around for irrigation or drinking water, especially in the Xeric West. A number of lakes are a part of water management districts where water is pumped into and out of them depending on water needs. If the lake existed in the past, even though the flow now is extremely altered by humans, we called them natural.

Quarry lakes, borrow pits, and reclaimed strip mine lakes. There are a large number of quarry or borrow pit lakes and ponds that are created by humans when they dug holes and then the holes filled with water. Since they are small and often unnamed it can be very hard to distinguish these from small unnamed natural ponds. Looking at the general landscape, lake shape, depths and crew notes are the only way to make an educated guess. For lakes within reclaimed strip mines, topographic maps may provide more information than imagery). If a major road (especially an interstate highway) is adjacent, road fill was often dug out from adjacent areas creating the borrow pit. Larger borrow pits and big quarries are sometimes turned into parks and have historical information.

10.3 Lake extent estimates

The condition of each NLA probability site (i.e., good, fair, poor; above or below benchmark; detected or not-detected) is determined by the appropriate indicator values and benchmarks 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 percentage of lakes nationally or in other sub populations (e.g., ecoregions, natural vs. manmade lakes, etc) in each condition class for the target 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 percentage of lakes or number of lakes.

10.4 Stressor extent, relative risk, and attributable risk

A major goal of the National Aquatic Resource Surveys is to assess the relative importance of stressors that impact aquatic biota on a national basis. The EPA assesses the influence of stressors in three ways: stressor extent, relative risk, and population attributable risk. In NLA, each targeted and sampled lake was classified as being in either *Good*, *Fair*, or *Poor* condition, separately for each stressor variable and for each biological response variable. From this data, we estimated the stressor extent (prevalence) of lakes in *Poor* condition for a specified stressor variable. We also estimated the relative risk of each stressor for a biological response. Relative risk is the ratio of the probability of a poor biological condition when the stressor is poor to the probability of a poor biological condition when the stressor is not poor (Van Sickle et al. (2006)). Finally, we estimated the population attributable risk (AR) of each stressor for a biological response. AR combines RR and stressor extent into a single measure of the overall impact of a

stressor on a biological response, over the entire population of lakes (Van Sickle and Paulsen (2008)).

10.4.1 *Stressor extent*

For each particular stressor, the stressor extent (SE) may be reported as the number of lakes, the proportion of lakes, or the percent of lakes in *Good*, *Fair*, *Poor*, or *Not Assessed* condition. If the SE is reported as the proportion of lakes, then it can be interpreted as the probability that a lake chosen at random from the population will be in *Poor* condition for the stressor.
Stressor extent in *Poor* condition is estimated as

(1) SE_p, the sum of the sampling weights for sites that are assessed in *Poor* condition

$$SE_p = \sum_{i=1}^{n_p} w_{pi}$$

,

(2) SEP_p, as the ratio of the sums of the sampling weights for the probability selected sites that are assessed in *Poor* condition divided by the sum of the sampling weights of all the selected sites regardless of condition, i.e.,

$$SEP_p = \frac{\sum_{i=1}^{n_p} w_{pi}}{\sum_{i=1}^{n} w_i}$$

, or

(3) SER_p, the percent of stressor extent in *Poor* condition (i.e., stressor relative extent)

$$SER_p == 100 * SEP_p = 100 * \frac{\sum_{i=1}^{n_p} w_{pi}}{\sum_{i=1}^{n} w_i}$$

where w_{pi} is the weight for the *i*th selected site in the *Poor* condition category, w_i is the weight for the *i*th selected site regardless of condition category, n_p is the number of selected sites that are in *Poor* condition, and n is the total number of sites regardless of their condition category. A stressor condition category may use other terminology to identify if a site is in poor condition but generically, we use the term *Poor*. Note that the extent for a response variable is defined similarly.

10.4.2 *Relative risk and attributable risk*

To estimate relative risk and attributable risk, we restrict the sites to those that both the stressor and response variable assessed as *Good*, *Fair*, or *Poor* (or their equivalents). That is, if a site is *Not Assessed* for either the stressor or response variable, it is dropped. Next, for these sites the condition classes are combined to be either *Poor* or *Not Poor* for the stressor and response variables. For example, *Not Poor* combines the *Good* and *Fair* condition classes. Thus, each sampled lake was designated as being in either *Poor* (P) or *Not Poor* (NP) condition for each stressor and response variable separately.

To estimate the relative risk and attributable risk for one stressor (S) and one response (B) variable, we compiled a 2x2 table (Table 10-1), based on data from all lakes that were included in the probability sample and that had both the stressor and response variable measured. A separate table must be compiled for each pair of stressor and response variables.

Table 10-1. Extent estimates for response and stressor categories

# **Response (B)**	# **Stressor (S)**	
	# Not Poor (NP)	# Poor (P)
# Not Poor (NP)	# $a = \sum_{i=1}^{n_{nn}} w_{nni}$	# $b = \sum_{i=1}^{n_{np}} w_{npi}$
# Poor (P)	# $c = \sum_{i=1}^{n_{pn}} w_{pni}$	# $d = \sum_{i=1}^{n_{pp}} w_{ppi}$

Table entries (a, b, c, d) are the sums of the sampling weights of all sampled lakes that were found to have each combination of *Poor* or *Not Poor* condition for stressor and response. For example, $d = \sum_{i=1}^{n_{pp}} w_{ppi}$ where n_{pp} is the number of sites with both the stressor and response in poor condition and w_{ppi} is the weight for the *i*th site. Note that the estimates in Table 10-1 may differ from the stressor extent estimates since both the stressor and response variables must be measured at each site.

10.4.3 *Relative risk*

Relative risk (RR) is the ratio of the probability of a *Poor* biological condition when the stressor is *Poor* to the probability of a *Poor* biological condition when the stressor is *Not Poor*. That is,

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

Using the simplified notation in Table 10-1, relative risk (RR) is estimated as:

$$RR_{est} = \frac{d/(b+d)}{c/(a+c)}$$

A RR = 1.0 indicates there is no association between the stressor and response. That is, a *Poor* response condition in a lake is equally likely to occur whether or not the stressor condition is *Poor*. A RR > 1.0 indicates that a *Poor* response condition is more likely to occur when the stressor is *Poor*. For example, when the RR is 2.0, the chance that a lake is in *Poor* biological (response) condition is twice as likely when the stressor is *Poor* than when the stressor is *Not Poor*. Further details of RR and its interpretation, including estimation of a confidence interval for RR_{est}, can be found in Van Sickle et al. (2006).

10.4.4 *Attributable risk*

Population attributable risk (AR) measures what percent of the extent in *Poor* condition for a biological response variable can be attributed causally to the *Poor* condition of a specific stressor. AR is based on a scenario in which the stressor in *Poor* would be entirely eliminated from the population of lakes, e.g., by means of restoration activities. That is, all lakes in *Poor* condition for the stressor are restored to the *Not Poor* condition. AR is defined as the proportional decrease in the extent of *Poor* biological response condition that would occur if the stressor were eliminated from the population of lakes. Mathematically, AR is defined as (Van Sickle and Paulsen (2008))

$$AR = \frac{Pr(B = P) - Pr(B = P|S = NP)}{Pr(B = P)}$$

We estimated AR as

$$AR_{est} = \frac{BEP_p - c/(a+c)}{BEP_p}$$

where

$$BEP_p = \frac{(c+d)}{(a+b+c+d)}$$

and is the estimated proportion of the biological response that is in *Poor* condition. We calculated a confidence interval for AR_{est} following Van Sickle and Paulsen (2008).
An AR can take a value between 0 and 1. A value of 0 indicates either "No association" between stressor and response, or else a stressor has a zero extent, i.e., is not present in the population. A strict interpretation of AR in terms of stressor elimination, as described above, requires one to assume that the stressor-response relation is strongly causal and that stressor effects are reversible. Van Sickle and Paulsen (2008) discuss the reality of these assumptions, along with other issues such as interpreting them when multiple, correlated stressors are present, and using them to express the joint effects of multiple stressors.

However, AR can also be interpreted more informally, as a measure that combines RR and SE into a single index of the overall, population-level impact of a stressor on a response. Van Sickle and Paulsen (2008) show that the population attributable risk can be written as

$$AR = \frac{SEP_p(RR-1)}{1 + SEP_p(RR-1)}$$

This shows that the numerator of AR is the product of the SE of *Poor* stressor condition and the "excess" RR, i.e., RR-1, of that stressor. The denominator standardizes this product to yield AR values between 0 and 1. Thus, a high AR for a stressor indicates that the stressor is widely prevalent (has a high SE of *Poor* condition), and the stressor also has a large effect (high RR) in those lakes where it does have *Poor* condition.

10.4.5 *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).

10.5 Change analysis

10.5.1 *Background information*

One of the objectives of the National Lakes Assessment (NLA) is to track changes over time. The NLA conducted in 2022 was the fourth statistically valid survey of the nation's lakes and reservoirs. In NLA 2007, lakes 4 hectares and larger were sampled. As discussed earlier in this document, the NLA 2012 expanded the target population to include lakes within a smaller size class category (1-4 hectares) and this remained the same for all subsequent surveys. Because of this change in design, the change analysis was conducted on both the larger lakes (≥ 4 hectares) and all lakes (≥1 hectare) study populations.

10.5.2 *Data preparation*

Analyses focused on the change in condition from the prior survey (2017) and the longest duration for each study population. For the larger lakes study population, this included change between 2007-2022 and 2017-2022. The larger lakes analyses included all sites from NLA 2007 (1130 sites), 801 NLA 2017 sites, and 775 NLA 2022 sites (NLA 2017 and 2022 excluded 1-4 hectare lakes). For the all lakes study population, change analyses included 2012-2022 and 2017-2022 and used data collected from all lakes sampled in 2012, 2017 and 2022.

10.5.3 *Methods*

Change analysis was conducted using the spsurvey package in R (Dumelle et al. 2023). Within the GRTS (Generalized Random Tessellation Stratified) survey design, change analysis can be conducted on continuous or categorical response variables (e.g., good, fair, and poor). The analysis measures the difference between response variables of two survey time periods. For NLA 2022, the categorical response variables were used to compare changes between NLA 2007 and 2012, 2012 and 2017, and 2017 and 2022. 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 good, fair, and poor.

Change between the two years is identified as statistically significant in the interactive data dashboard and web-report when the resulting error bars around the change estimate do not cross zero. Statistical significance is provided as a way to highlight results that may warrant additional exploration and analyses.

For some indicators and subpopulations, the change in the percentage of lakes that is “not assessed” can be relatively large and may change from survey to survey. Large changes in not assessed may reflect changes in sampling or assessment success rather than actual changes in condition associated with other categories such as good, fair and poor. Therefore, when the percent of not assessed increases or decreases by more than 5 percentage points between survey cycles, EPA will not present these results in the interactive dashboard to limit potentially erroneous interpretations of condition change.

Change estimates could not be made for some indicators and some survey cycles due to differences in methodologies (e.g., zooplankton), condition categories (i.e., lake drawdown), and the timing of when indicators were added to the survey (e.g., atrazine added 2012, cylindrospermopsin added 2017 and enterococci added 2022).

10.6 Literature cited

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Chapter 11: Quality Assurance Summary

The NLA has been designed as a statistically valid report on the condition of the Nation's lakes at multiple scales, e.g., ecoregion (Level III and the aggregated nine NARS ecoregions), 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 the EPA's Office and Wetlands, Oceans and Watersheds (OWOW) and Office of Environmental Information (OEI) and a Project QA Officer. All survey participants signed an agreement to follow the QAPP standards. Compliance with the QAPP was assessed through standardized field crew training and field crew assistance visits. The QAPP addresses all aspects of the survey, including: project planning and management; data quality objectives; sampling design and site selection; indicators; field crew assistance visits; standardized/centralized data management; and data analysis. Detailed information on site selection, field protocols and the laboratory sample processing are found in the following documents:

- NLA 2022 SEG (EPA 841-B-21-008) – outlines the process to determine if a lake meets the criteria for inclusion in the target population and is accessible for sampling, and the appropriate replacement process if a lake is not sampleable;
- NLA 2022 FOM (EPA 841-B-21-011) – describes all field activites and protocols; and
- NLA 2022 LOM (EPA 841-B-21-010) – documentation of all laboratory methods.

Field Training and Sample Collection – EPA staff and contractors provided hybrid training that included the review of online videos and quizzes and in person training sessions throughout the study area. All field crew leads were required to complete all components of the NLA training and field crew members were encouraged complete as much training as possible. All field crews received an onsite assistance visit from a trained EPA staff member or contractor within the first few weeks of fieldwork. Adjustments and corrections were made on the spot for any problems identified during the assistance visit. To assure consistency, EPA supplied standard sample/data collection equipment, sample bottles, filtration supplies, and shipping supplies for all sampling events.

Revisits of Selected Field Sites - To evaluate within-year sampling variability, the NLA design called for crews to revisit 10 percent of the sites selected in the design. These sites were sampled twice in the NLA index period during a single year (visit 1 and visit 2). Useful metrics and indicators tend to have high repeatability, that is among site variability will be greater than

sampling variability based on repeat sampling at a subset of sites. To quantify repeatability, NARS uses one of two metrics 1) Signal:Noise (S:N), or the ratio of variance associated with sampling site (signal) to the variance associated with repeated visits to the same site (noise) (Kaufmann et al., 1999) or 2) condition category contingency tables. When calculating S:N, all sites are included in the signal, whereas only revisit sites contribute to the noise component.

Metrics with high S:N are more likely to show consistent responses to human caused disturbance, and S:N values ≤ 1 indicate that sampling a site twice yields as much or more metric variability as sampling two different sites (Stoddard et al., 2008). The S:N values were used by analysts in the process of selecting metrics and evaluating indicators.

Contingency tables are also used to visualize agreement between condition categories for the first and second visits. These are presented for the NLA risk indicators that track detection and risk (atrazine, cyanotoxins and enterococci).

Chemical Analyses – NLA 2022 used two labs for the water chemistry samples, the Wisconsin State Lab of Hygiene (WSLOH; Wisconsin probability and state intensification sites) and the Willamette Research Station (WRS; all remaining NLA and NES sites). For quality assurance of chemical analyses, laboratories used QC samples which are similar in composition to samples being measured. They provide estimates of precision and bias that are applicable to sample measurements. To ensure the ongoing quality of data during analyses, every batch of water samples was required to include QA samples to verify the precision and accuracy of the equipment, reagent quality, and other quality measures. These checks were completed by analyzing blanks or samples spiked with known or unknown quantities of reference materials, duplicate analyses of the same samples, blank analyses, or other appropriate evaluations. The laboratories reported QA results along with each batch of sample results. In addition, laboratories reported holding times. Holding time requirements for analyses ensure analytical results are representative of conditions at the time of sampling. To identify samples for additional investigation, EPA reviewed all laboratory QA flags, data completeness, sample ionic strength balance, completed several cross variably validity checks and noted any quality failures.

For the atrazine samples, the NLA 2022 used two labs: WSLOH (Wisconsin sites) and EnviroScience Inc. (ESCI), a sub-contracted laboratory with Great Lakes Environmental Cetner, Inc. (all remaining sites). For the cyanotoxins samples, NLA 2022 used three labs including WSLOH (Wisconsin sites), the EPA Region 4 Lab (R4 handpicked NES sites) and GreenWater Laboratories (GWL), a sub-contracted laboratory with Avanti Corporation (all remaining NLA sites). Proficiency test (PT) samples (5 concentrations per set and parameter) were sent to all labs that analysed samples for atrazine and cyanotoxins (WSLOH – 1 set; EPA R4 1 set; GWL – 2 sets). The results from these tests were used to identify samples of acceptable quality for use in the NLA assessment.

Zooplankton Laboratory QA – EPA contracted with one lab for zooplankton sample processing. This lab demonstrated that it could meet the QA/QC requirements identified in the NLA 2022 QAPP and LOM. These requirements included internal quality control (QC) checks on zooplankton identification, the use of the Integrated Taxonomic Information System for correctly naming species collected, and use of a standardized data management system. Independent taxonomists were contracted to perform QC analysis of the primary lab's samples. The external QC targeted the reidentification of 10% of the samples. The samples were randomly selected. The reidentifications were made on new aliquots taken from the original sample. Scheduling issues limited the processing of all samples selected, therefore only 7% (151 samples) were processed. Samples were assessed for within sample similarities using the Bray-Curtis Dissimilarity Index (B‑C) for each taxon. The zooplankton B-C data quality objective was 0.25 and the median B-C across all samples was 0.28. Although the median is slightly greater than the DQO, all zooplankton samples were determined acceptable for further analysis since the measure accounts for sample processing differences (each lab identified unique aliquots).

Benthic Macroinvertebrate Laboratory QA – NLA 2022 used one lab for benthic macroinvertebrate sample processing. This lab demonstrated that it could meet the QA/QC requirements identified in the NLA 2022 QAPP and LOM. These requirements 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 the national lab samples. The QC samples were randomly selected. Reidentifications were made with the same specimens (vials and slides of individual were shipped to the QC lab) for 109 (10.1%) benthic samples. The mean percent taxonomic disagreement (PTD) for the overall NLA 2022 benthic dataset was 9.3%, which is better than the programmatic measurement quality objective of 15%.

Entry of Field Data and Quality Checks– NLA used a standardized data management structure, i.e., use of the same standard field forms for data collected and centralized data management. Most field data were collected electronically using an iPad with the NLA field data mobile application. Following a review for accuracy and completeness, field crews submitted the electronic forms directly from the NLA App to NARS IM, which automated upload to the NLA 2022 SQL database. No paper field forms were submitted in the 2022 survey.

Quality of field data were reviewed on a weekly, monthly and end of season basis using numerous automated data quality checks. EPA staff and contractors then compiled a summary of data quality issues which were sent to respective field crews to correct or provide additional comments. If field data could not be corrected, crews were instructed to provide a comment as to why field data could not be collected or measured. Corrected data and new comments were resubmitted from the NLA App and updated in the NARS IM NLA 2022 SQL database.

Data Management - Information management (IM) is integral to all aspects of the program from initial selection of sampling sites through dissemination and reporting of final, validated data. Quality measures implemented for the IM system are aimed at preventing corruption of data at the time of their initial incorporation into the system and maintaining the integrity of data and information after incorporation into the system.

Reconnaissance, field observation and laboratory analysis data were transferred from NLA survey participants and collected and managed by the NARS IM center. Data and information were managed using a tiered approach. First, all data transferred from a field team or laboratory were physically organized (e.g., system folders) and stored in their original state. Next, NARS IM created a synthesized and standardized version of the data to populate a database that represented the primary source for all subsequent data requests, uses and needs. All samples were tracked from collection to the laboratory.

The IM staff applied an iterative process in reviewing the database for completeness, transcription errors, formatting compatibility, consistency issues and other quality control-related topics. This first-line data review was performed primarily by NARS IM in consultation with the NLA QA team. A second-phase data quality review consisted of evaluating the quality of data based on MQOs as described in the QAPP. This QA review was performed by the NLA QA team using a variety of qualitative and quantitative analytical and visualization approaches.

Records Management – EPA organizes and maintains all records associated with the survey. Examples of the records include: all planning documents, such as the survey design, NLA QAPP, SEG, FOM, LOM and other laboratory SOPs; QA implementation documents (e.g., QAPP signature pages, crew training, assistance visit forms, lab verification information); data and assessment files, draft reports and comments received. All data will eventually be archived in the water quality portal.

Main Report - The main report provides a summary of the findings of each of the data analyses and EPA's interpretation of them. The main report was extensively reviewed in-house by the NLA team, its partners, and other EPA experts. Because previous reports using the same analytical procedures were reviewed through an Independent External Review process, it was determined that a letter review was not required for the main report. EPA used the comments from the states and EPA's Office of Research and Development to refine the main report and improve the clarity of documentation in this document.

Appendix A: Lake Physical Habitat Expected Condition Models

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:

REF_NLA12 = Variable for disturbance level at site based on screening criteria from 2012, valid values of L (least disturbed), I (intermediate disturbance), M (most disturbed), and ? (unknown due to missing information)

REF_NLA17 = Variable for disturbance level at site based on screening criteria update for NLA 2017, valid values of L (least disturbed), I (intermediate disturbance), M (most disturbed), and ? (unknown due to missing information)

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 REF_NLA12 = L or I;
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 REF_NLA12 = L or I;

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 REF_NLA17 = L or I;
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 REF_NLA12 = L or I;

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 REF_NLA17 = L or I;

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 REF_NLA12 = L or I;
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 REF_NLA17 = L;
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 REF_NLA17 = L;
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 REF_NLA12 = L;

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 REF_NLA17 = L;
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 REF_NLA17 = L;
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 REF_NLA12 = L;
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 REF_NLA17 = L;
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 REF_NLA12 = L;

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 REF_NLA17 = L;
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 REF_NLA12 = L;

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 REF_NLA17 = L;
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 REF_NLA17 = L;
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 REF_NLA17 = L;
LitRipCvrQc3x15=10**(***L_LitRipCvrQc3x15***)-0.01;
LitCvrQc3x15=10**(***L_LitCvrQc3x15***)-0.01;

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

CENPL (NPL + SPL + TPL) Expected PHab Condition Models:

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 REF_NLA17 = L, 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;

Adjustment for reference distribution of O/E values:
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 REF_NLA17 = L
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;

Adjustment for reference distribution of O/E values:
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 REF_NLA17 = L
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;

Adjustment for reference distribution of O/E values:
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 REF_NLA17 = L;
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 REF_NLA17 = L;
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 REF_NLA17 = L;
LitRipCvrQc3x15=10**(***L_LitRipCvrQc3x15***)-0.01;

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

XER Expected PHab Condition Models:

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 REF_NLA17 = L;
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 REF_NLA17 = L;
*** 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 REF_NLA17 = L;
LitRipCvrQc3x15=10**(***L_LitRipCvrQc3x15***)-0.01;

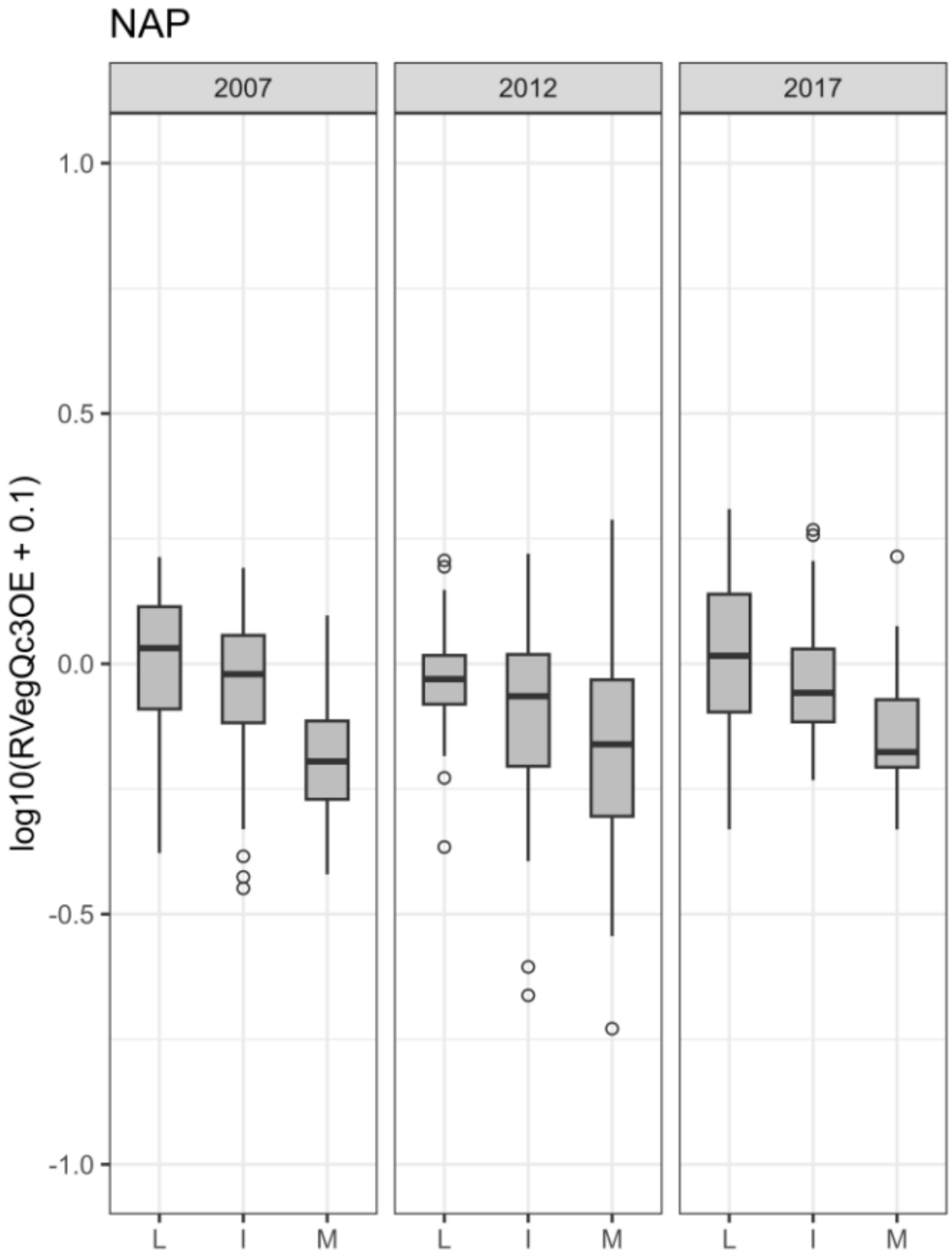
Ref O/E distribution based on mean and SD of ref sites.

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;

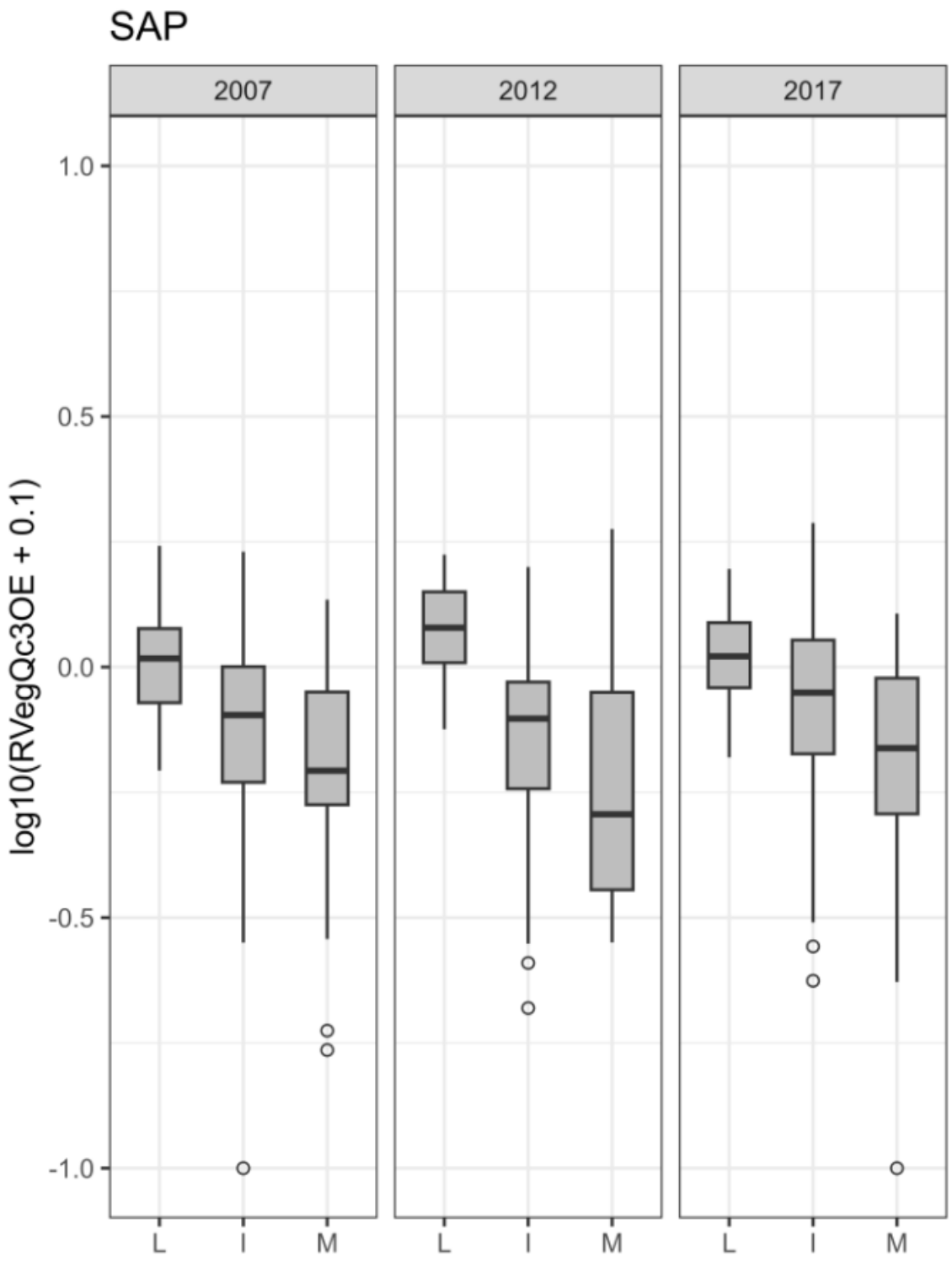
NLA 07,12,17 --- P.R.Kaufmann April 27, 2020
Log10[Observed/Expected] Lake Habitat Cover & Structural Complexity
Versus Anthropogenic Disturbance Stress (LIM-2020) and Year
For 9 Ecoregions (Sample stats, not weighted -%iles: 5/25/50/75/95
w/outliers shown as “+”

Following figures present the O/E values vs LIM for the three PHab indicators for the three surveys for each of the 9 Ecoregions.

Riparian Vegetation:



Riparian Vegetation:



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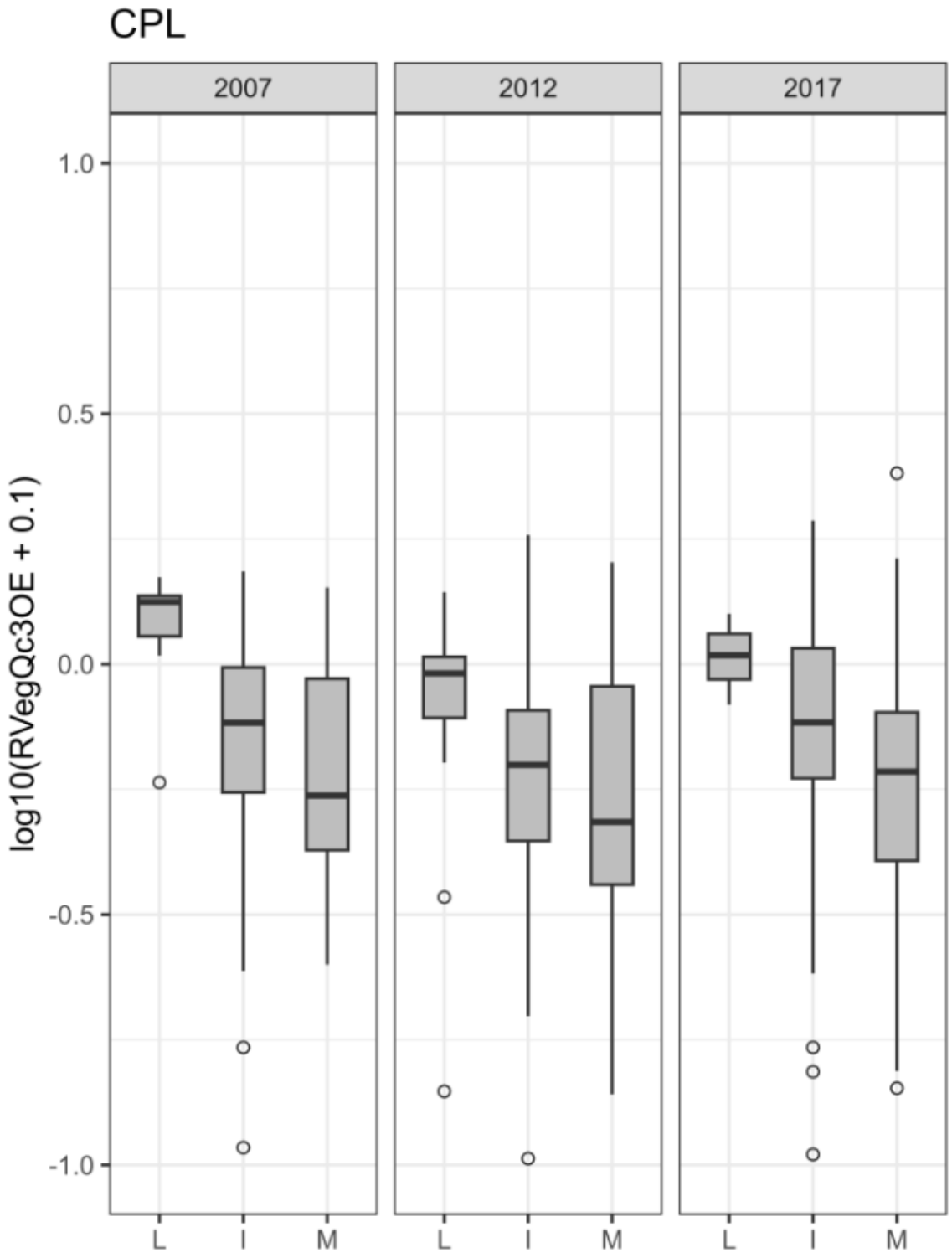

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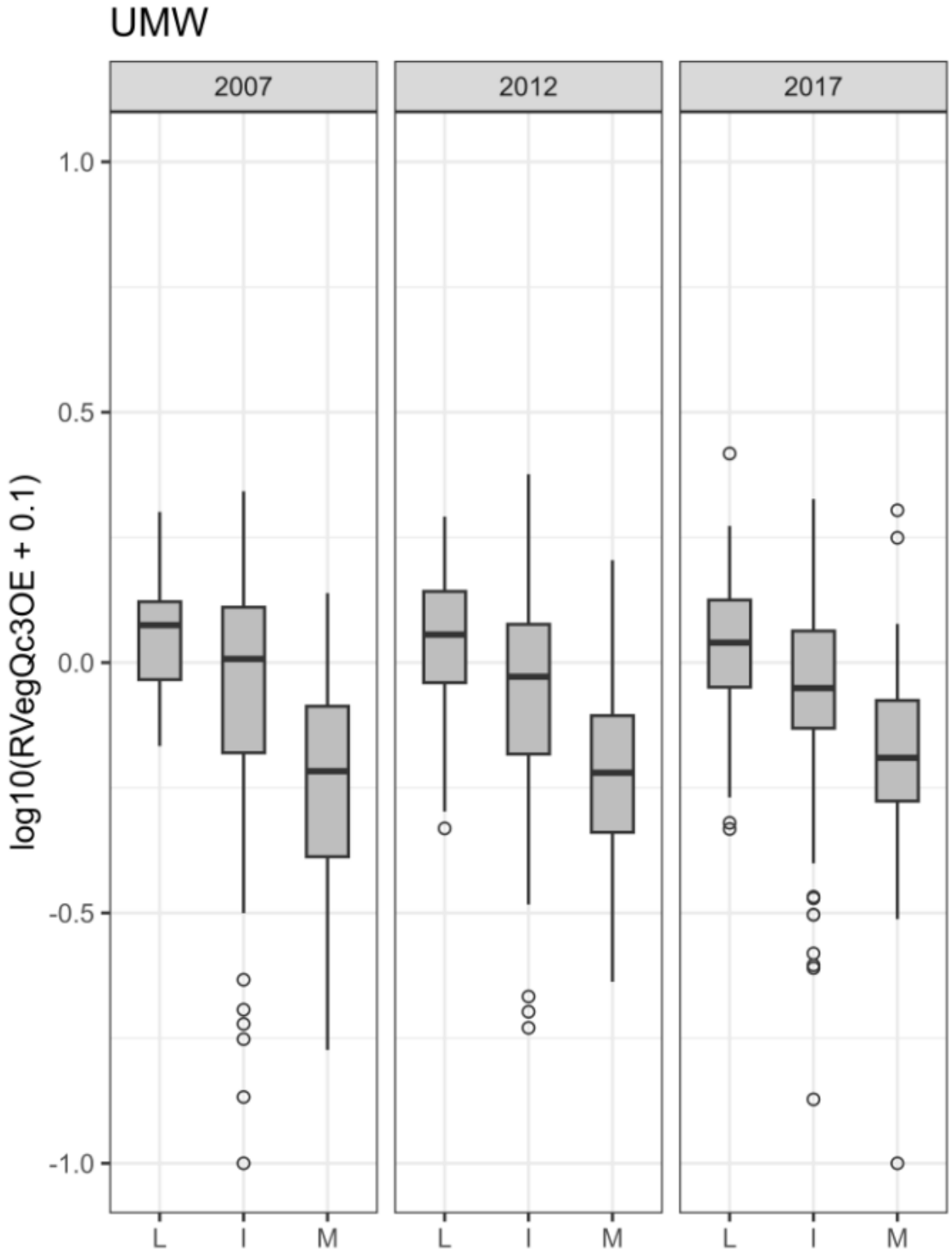

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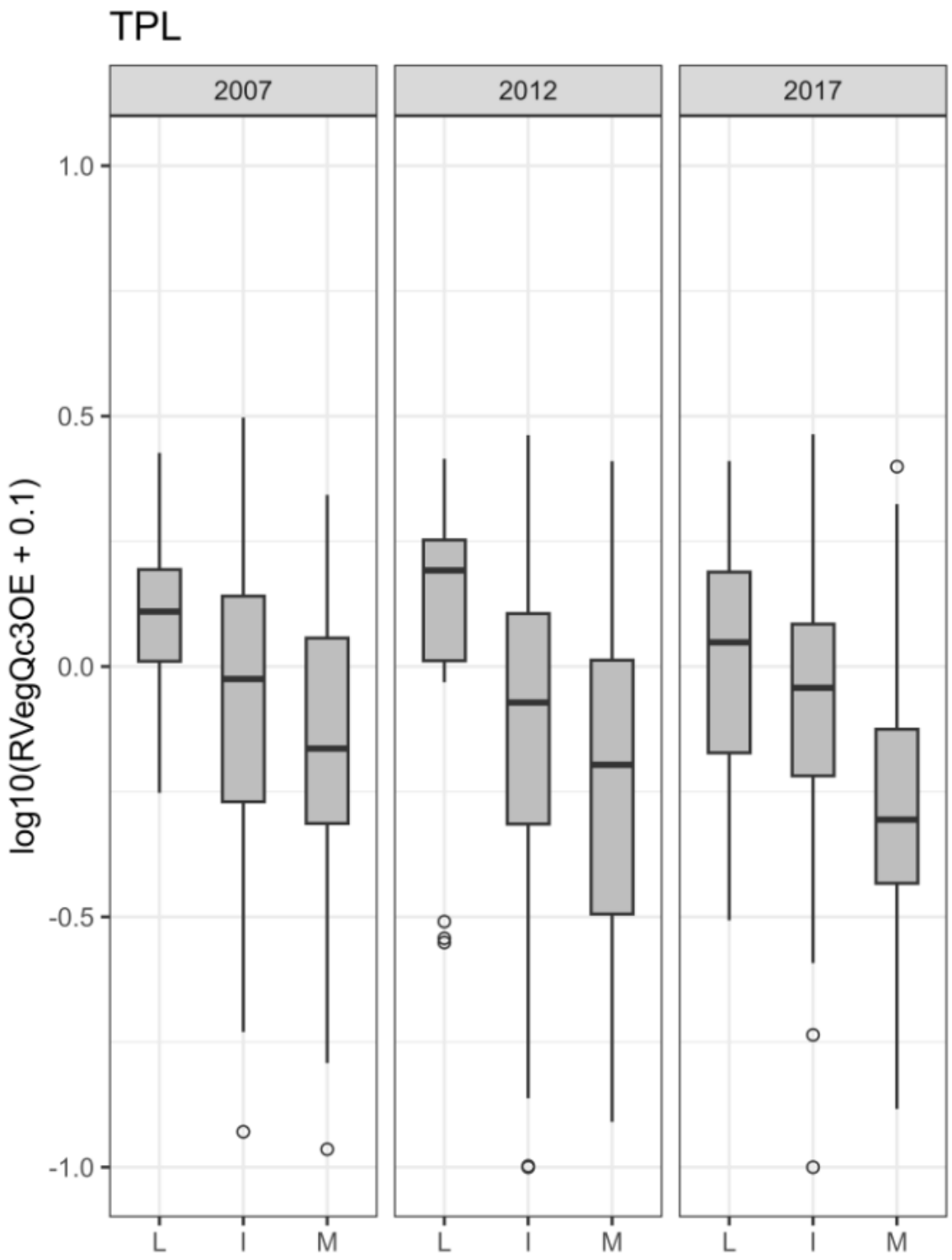

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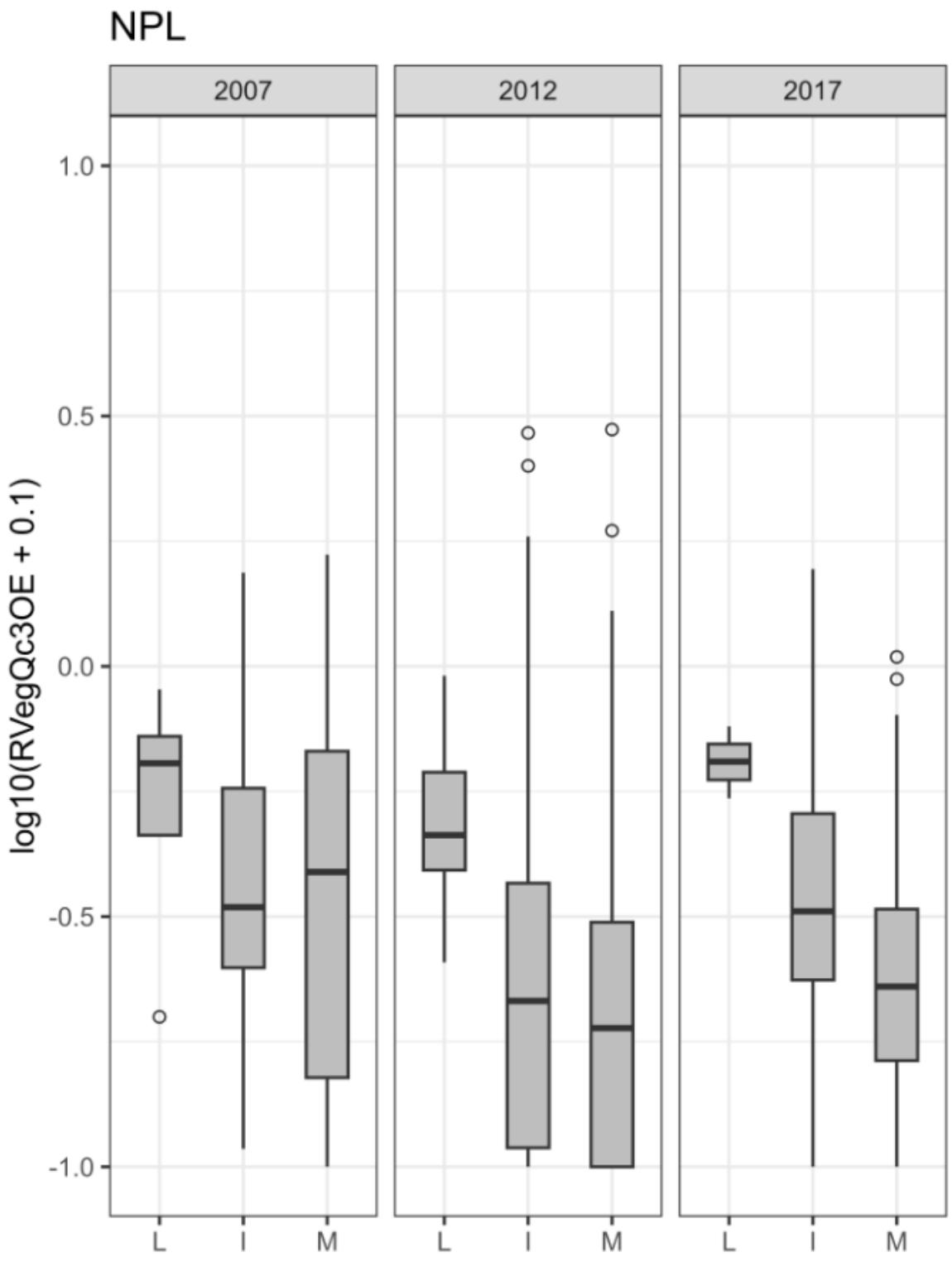

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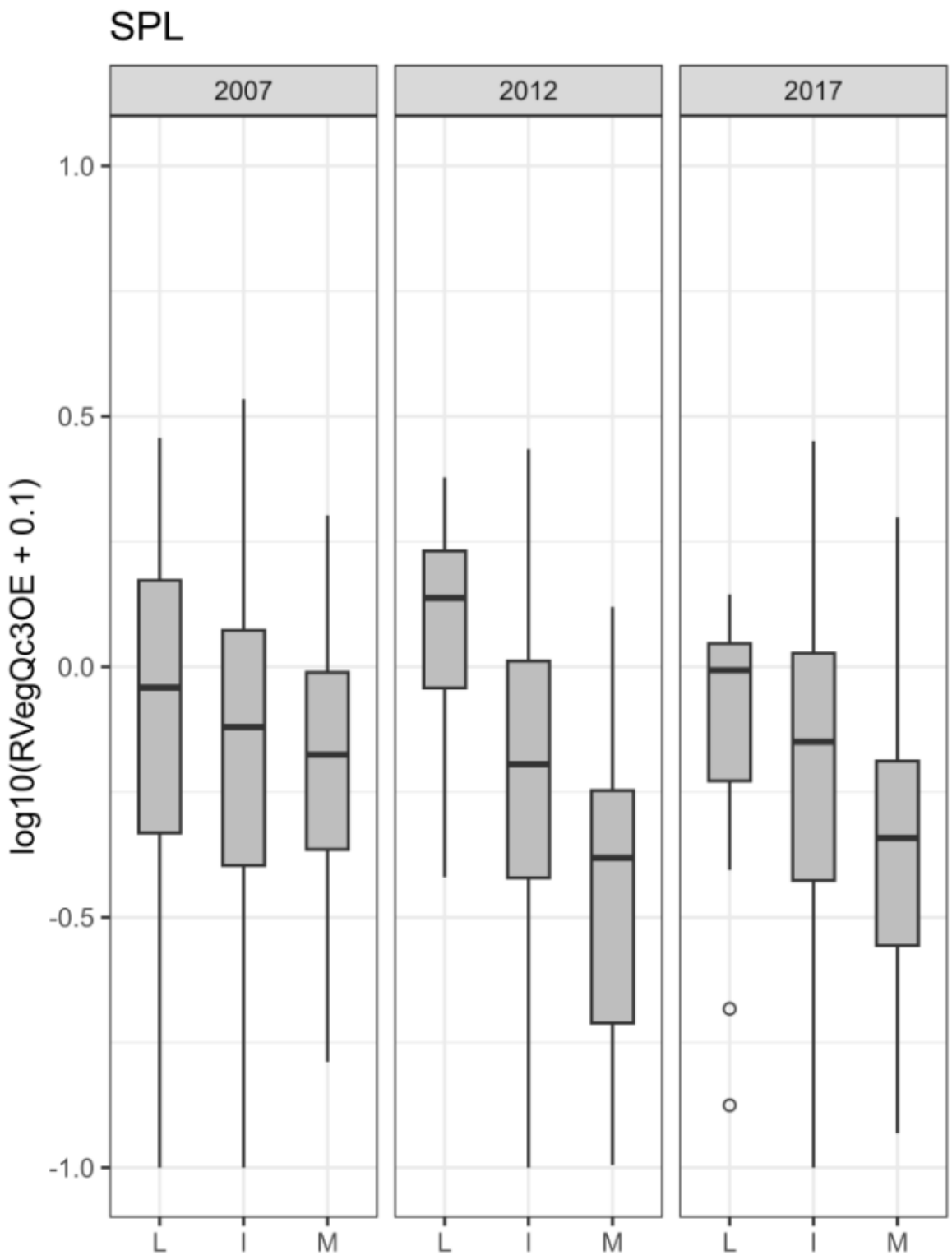

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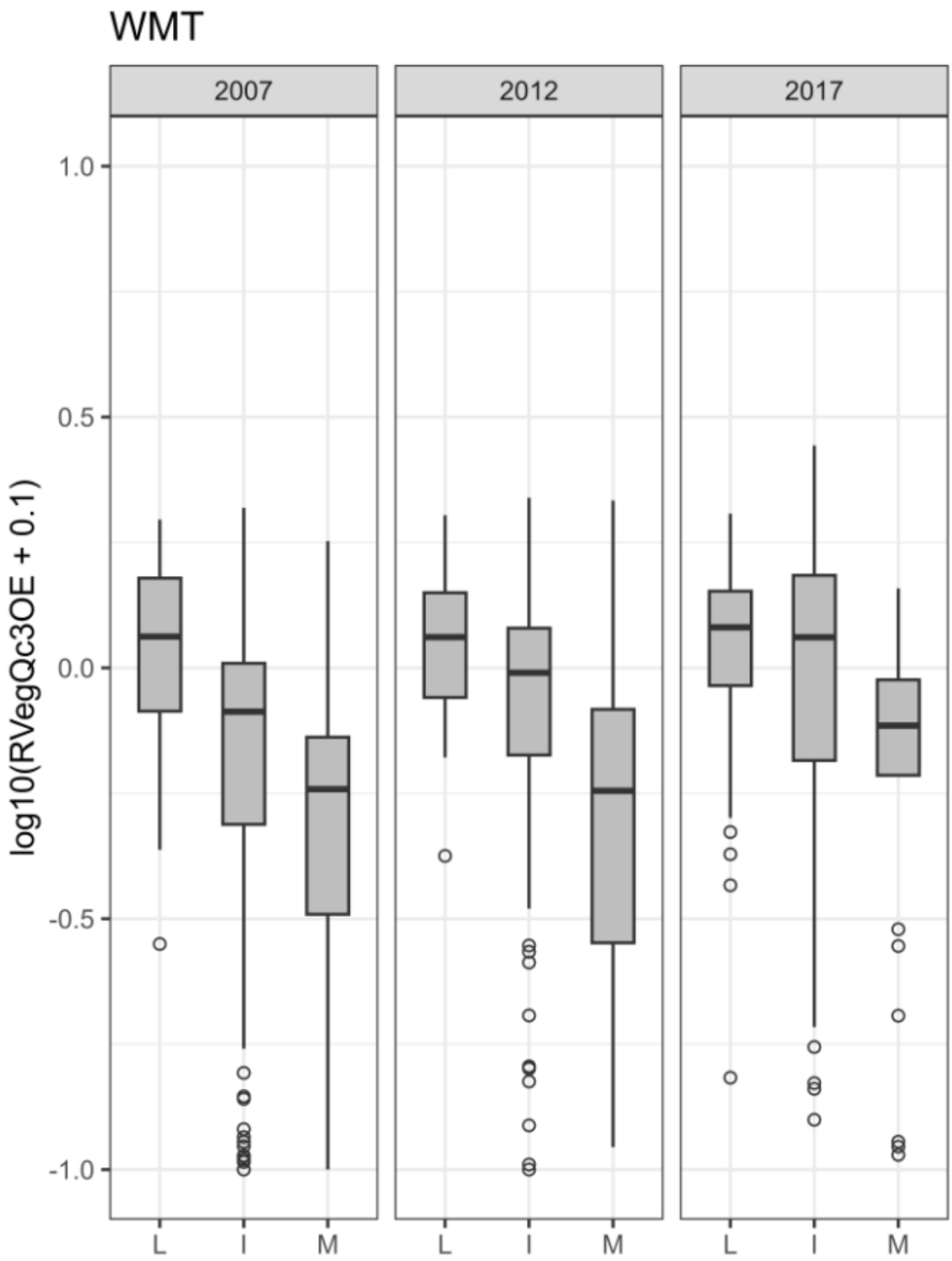
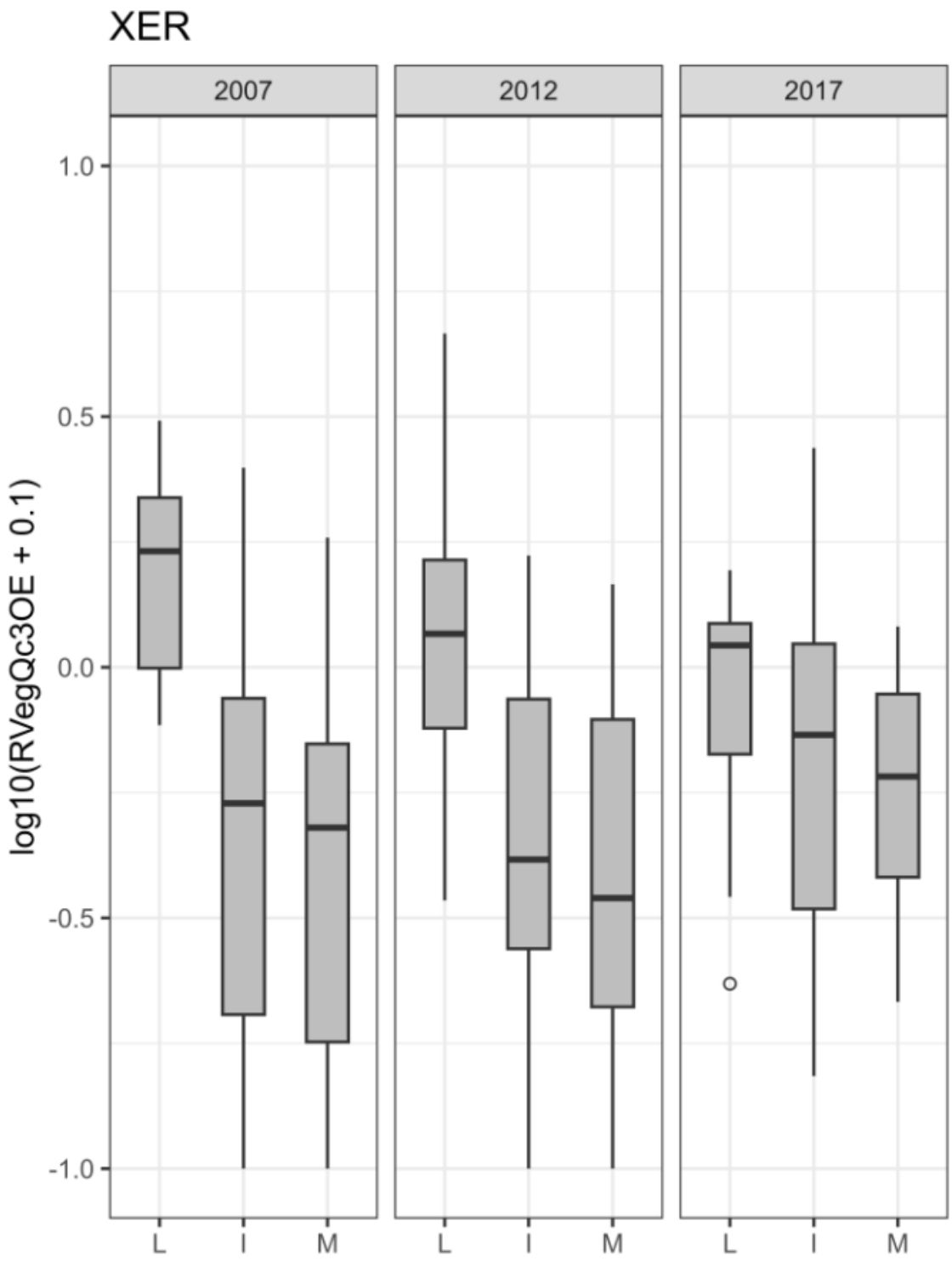
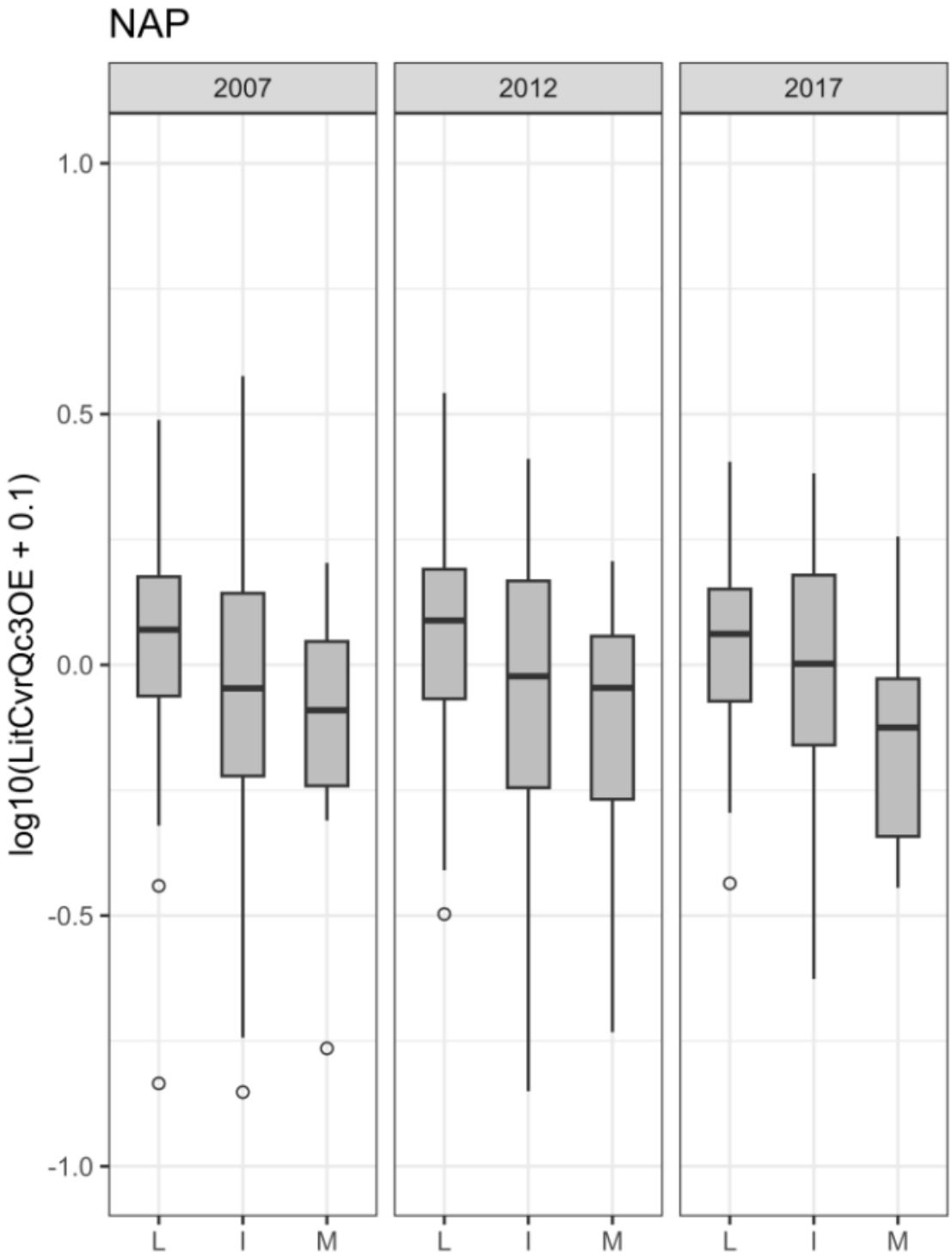


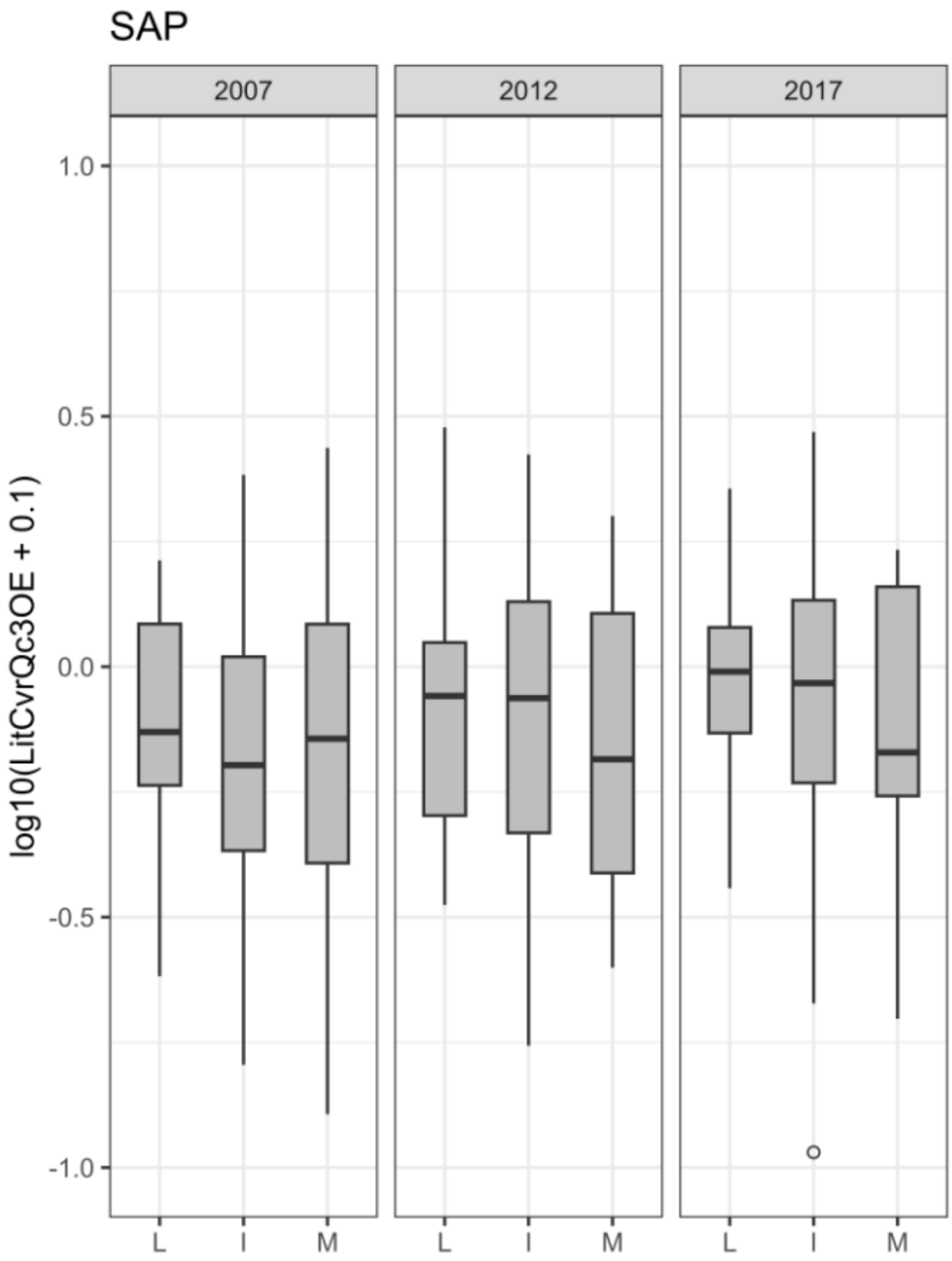
Riparian Vegetation:



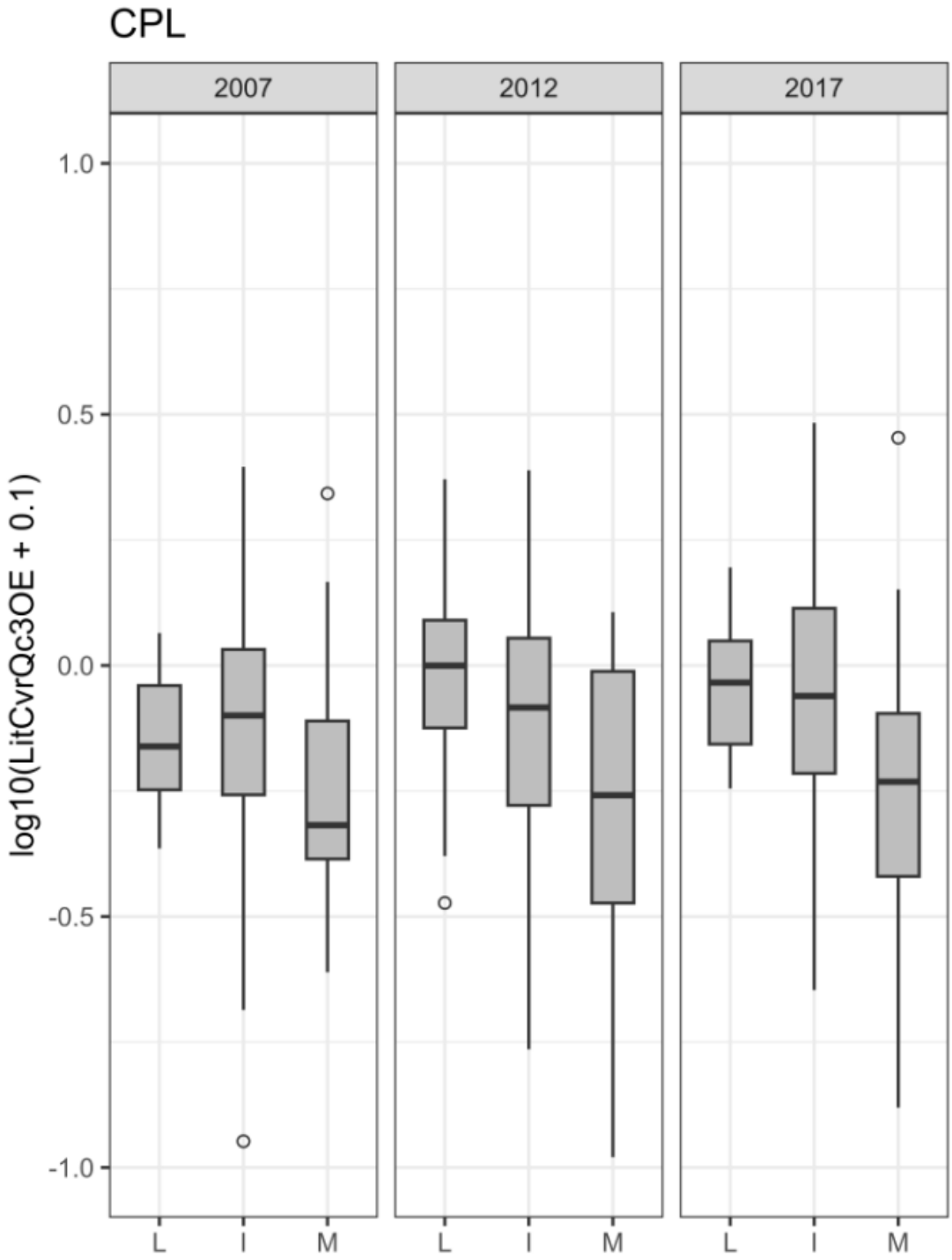
Littoral Habitat:



Littoral Habitat:



Littoral Habitat:



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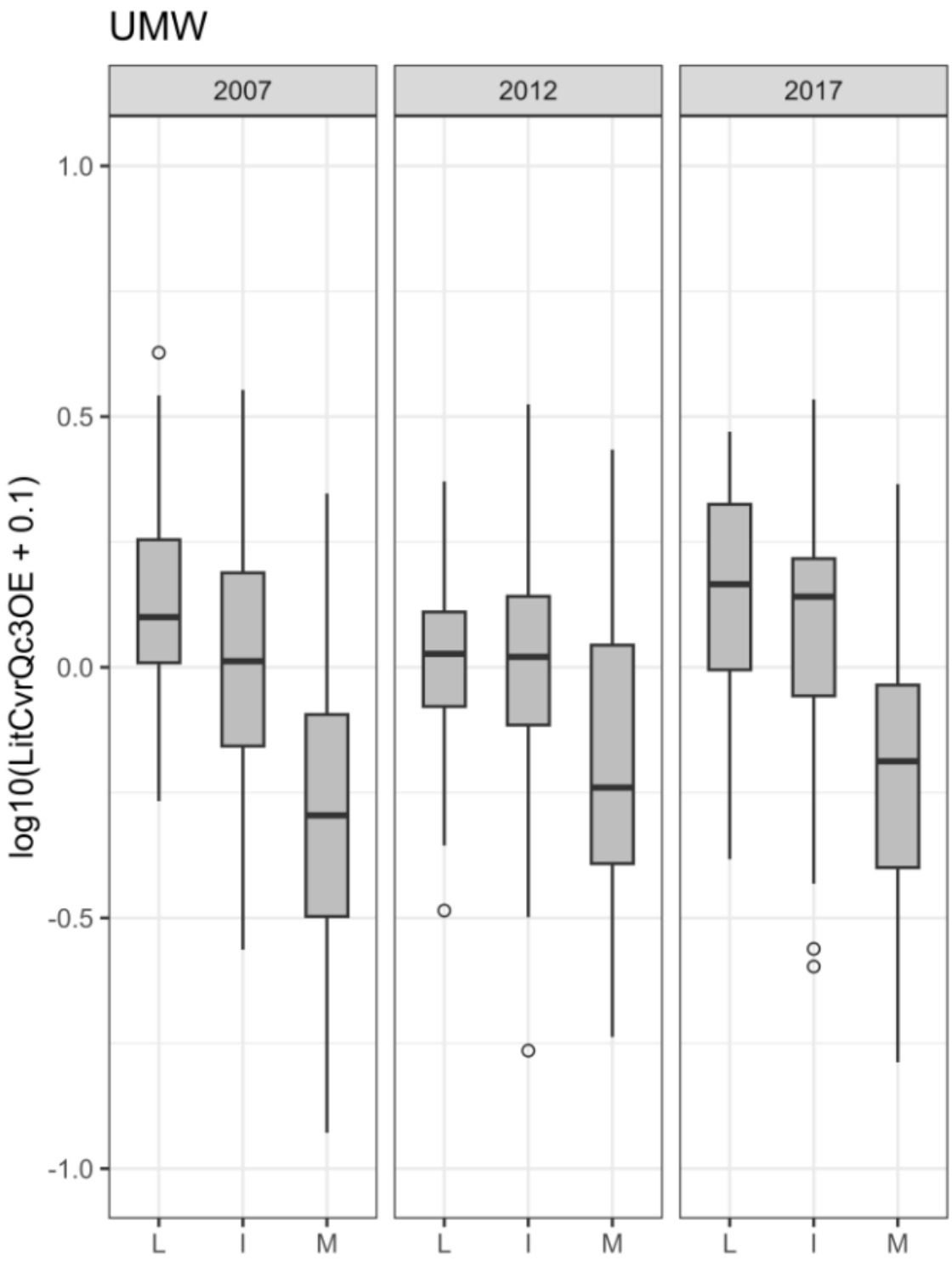
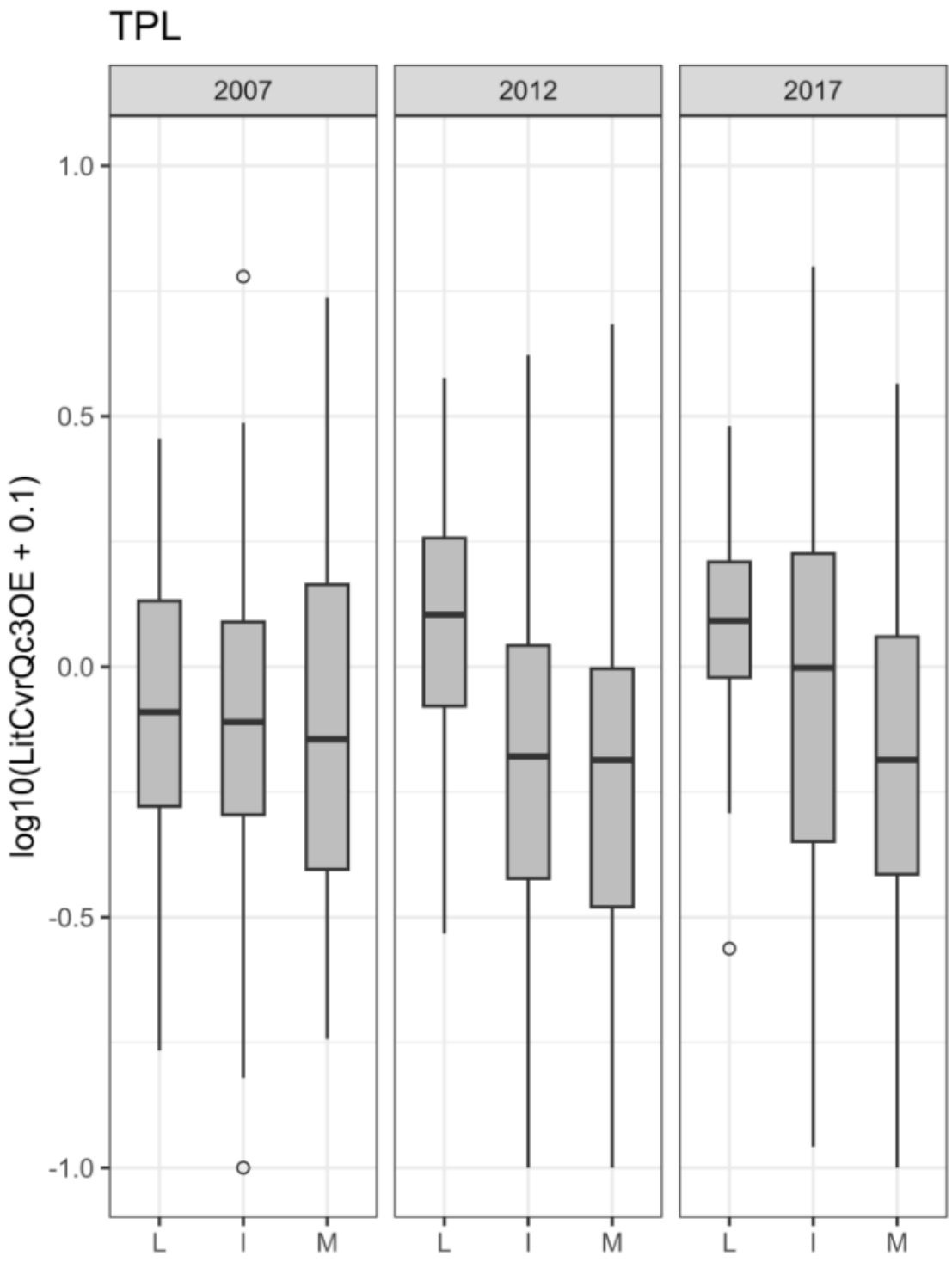
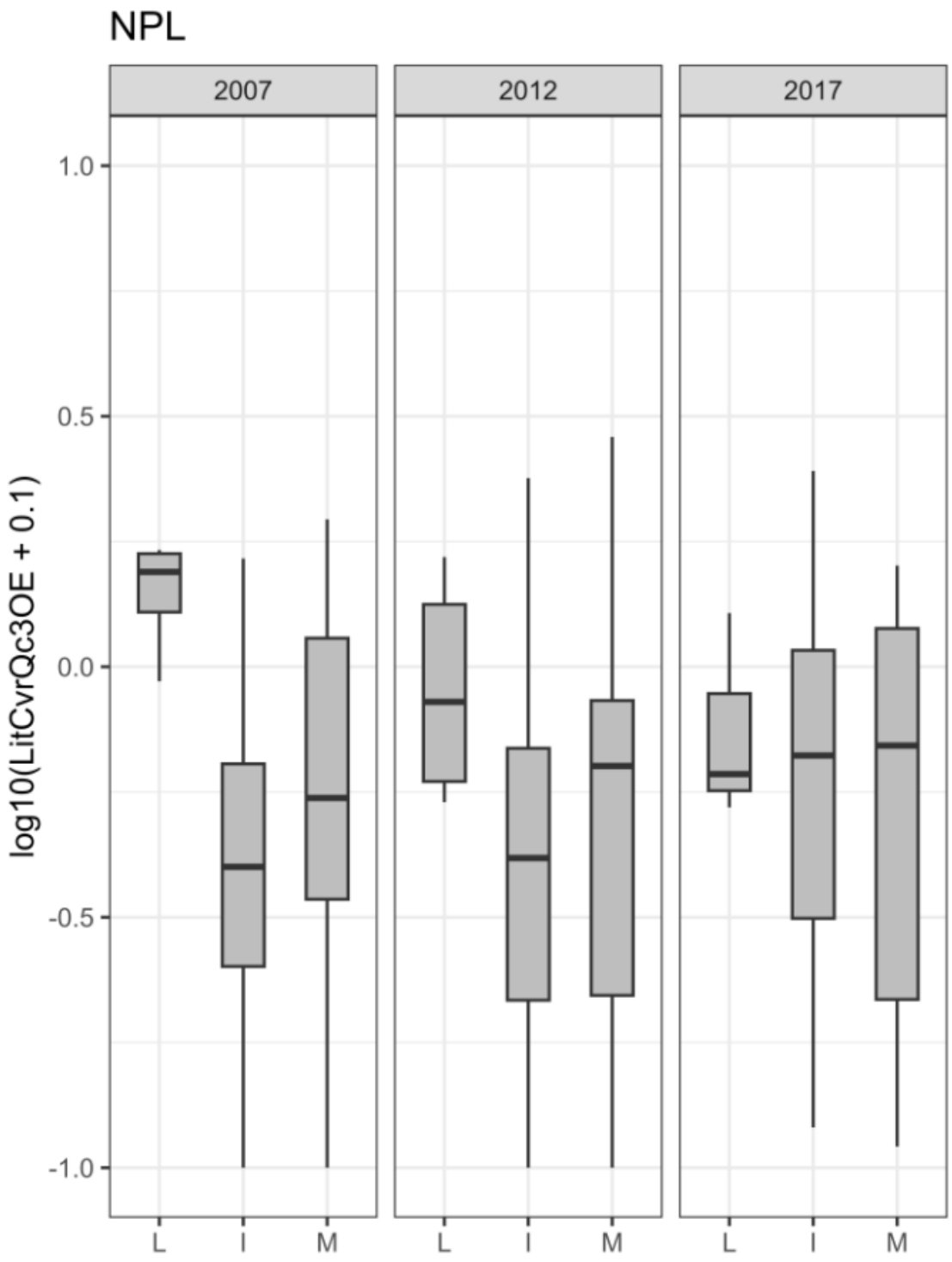


Littoral Habitat:



Littoral Habitat:



Littoral Habitat
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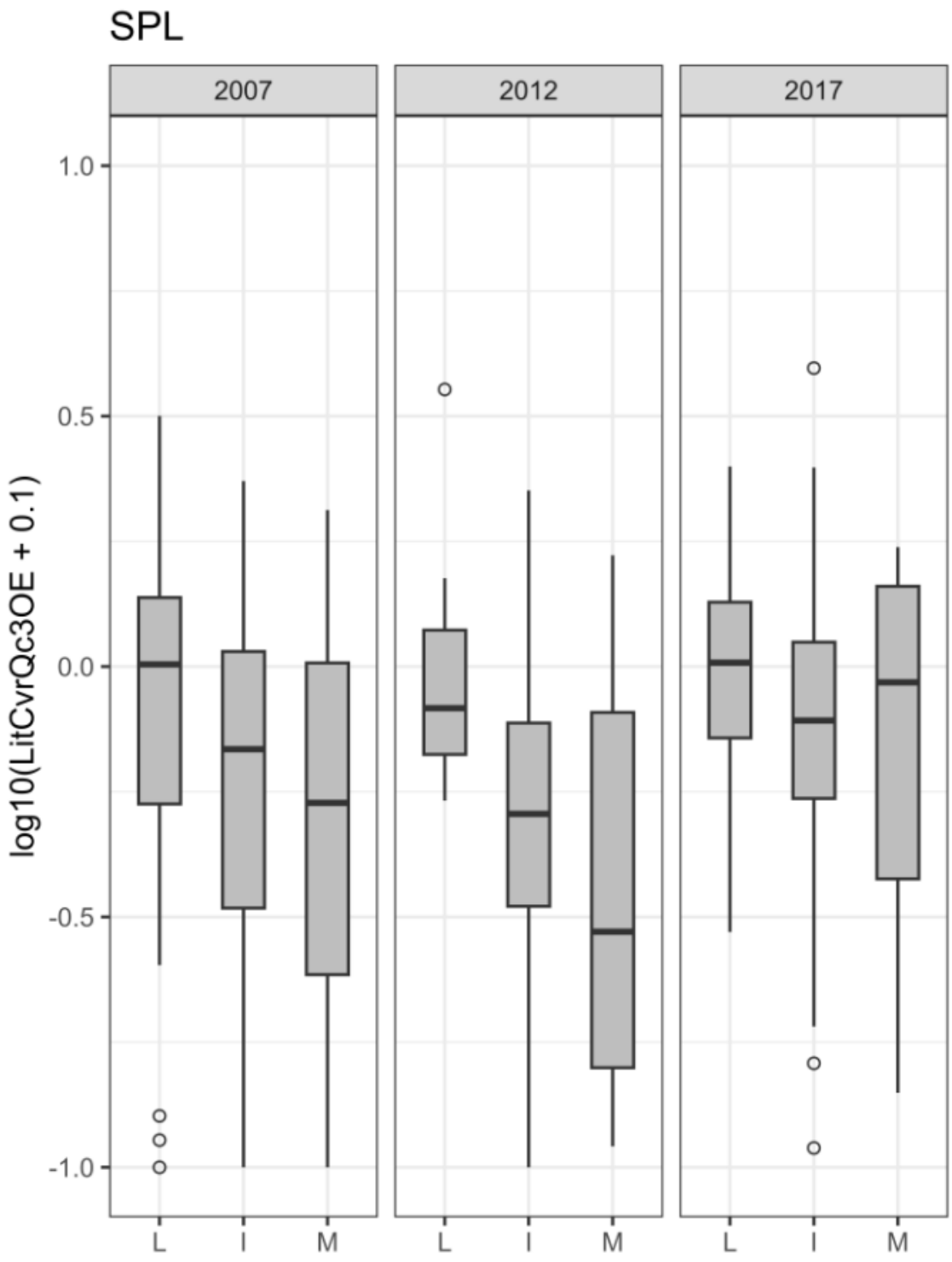

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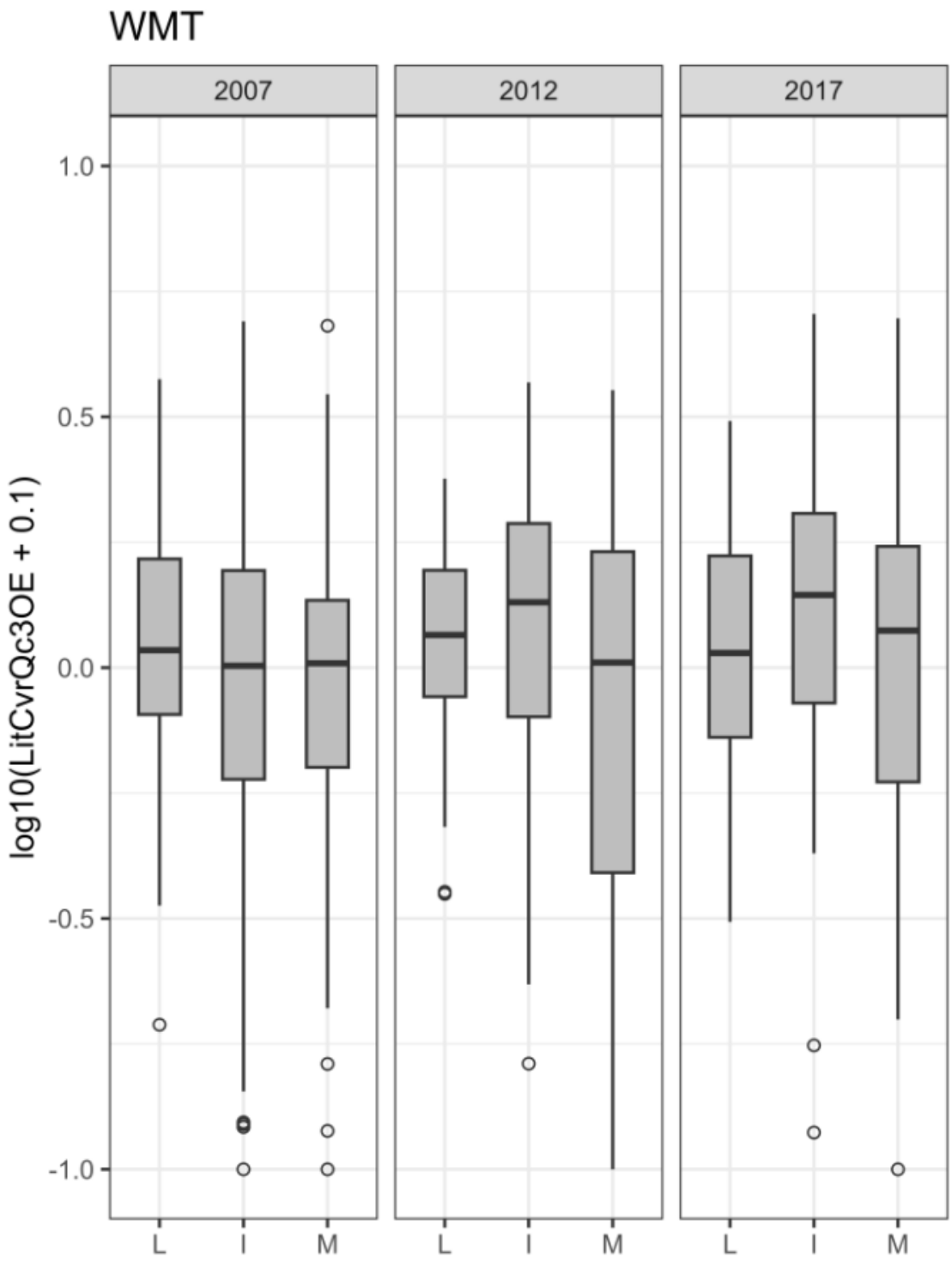
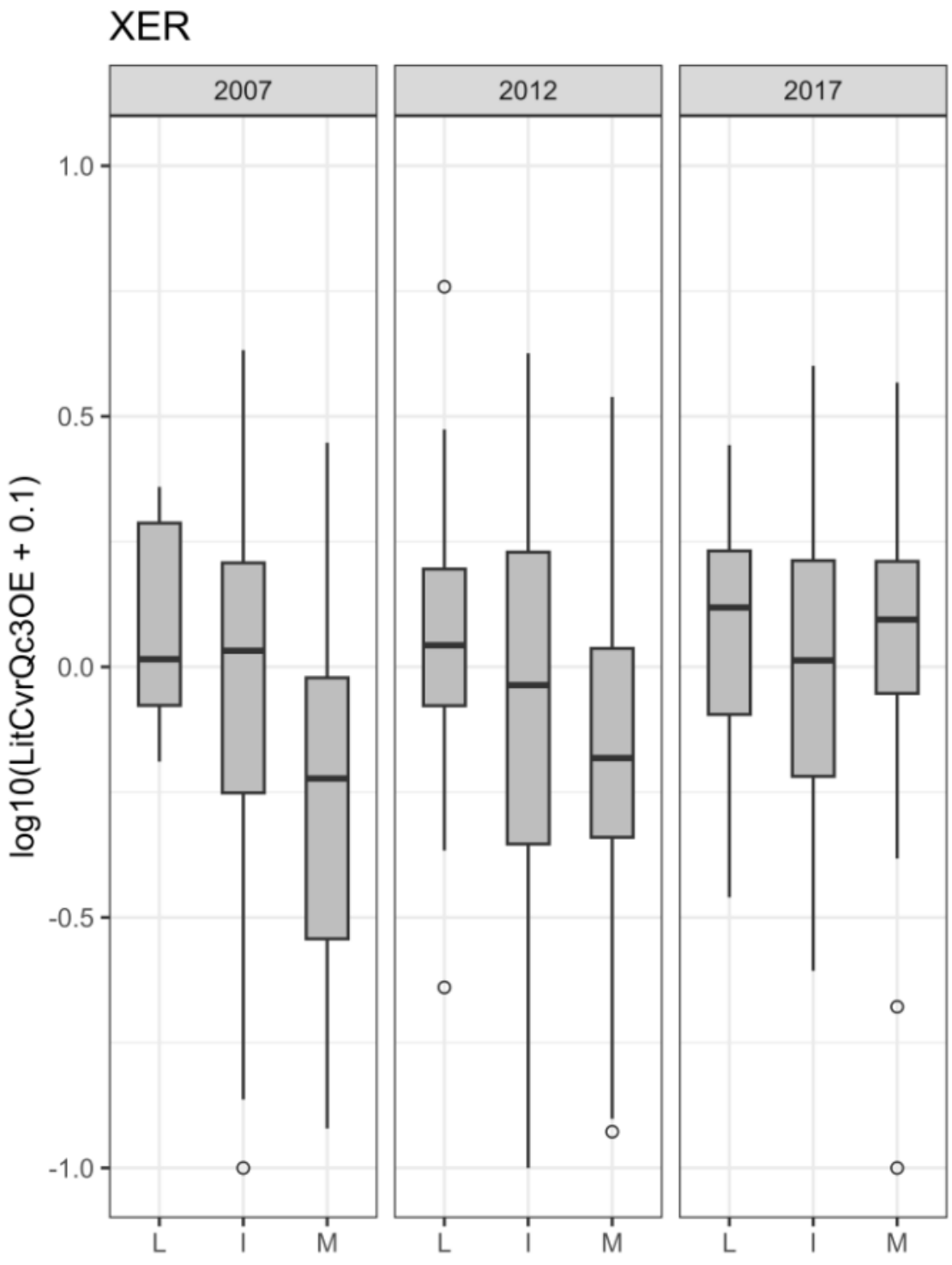
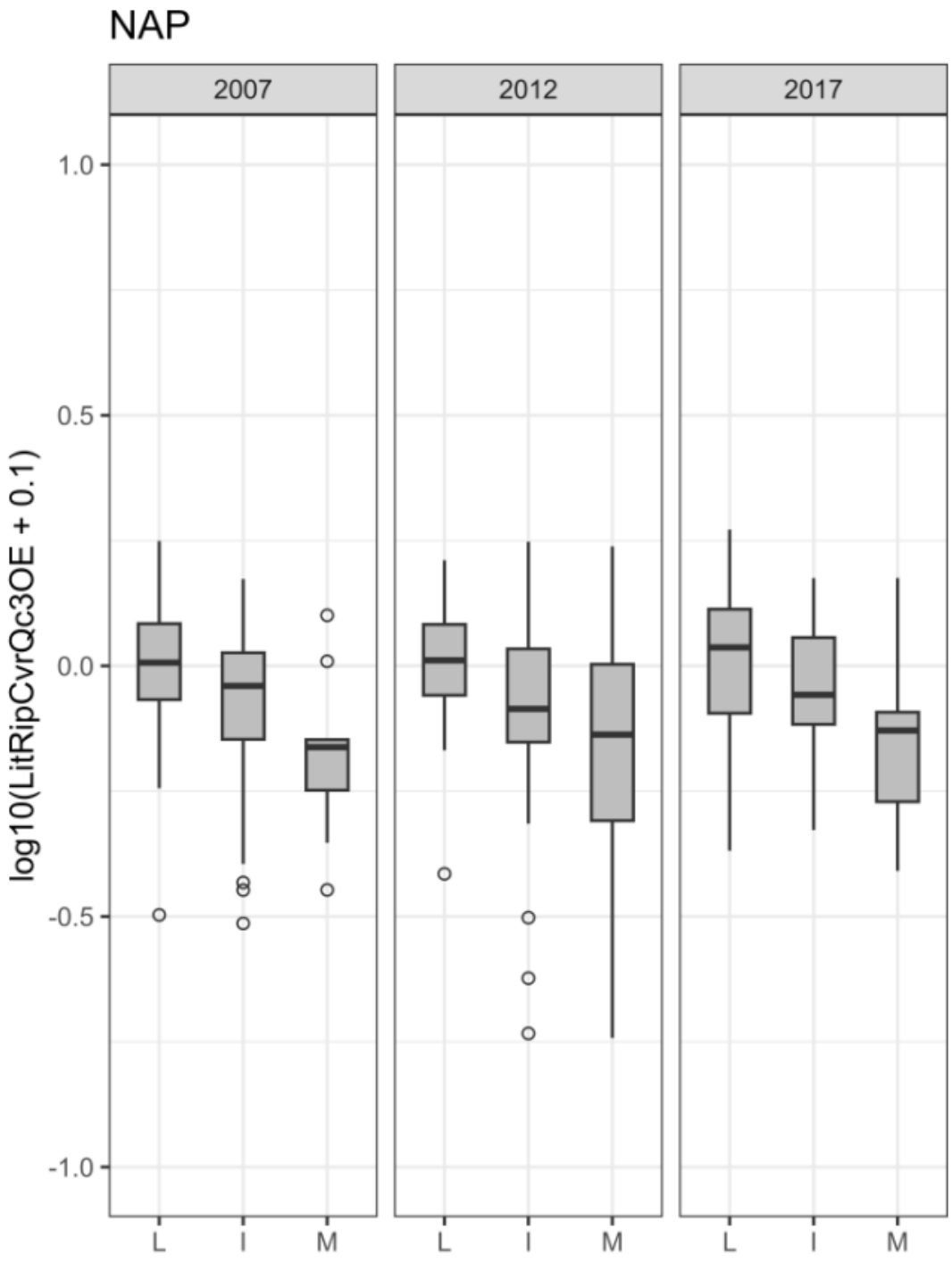


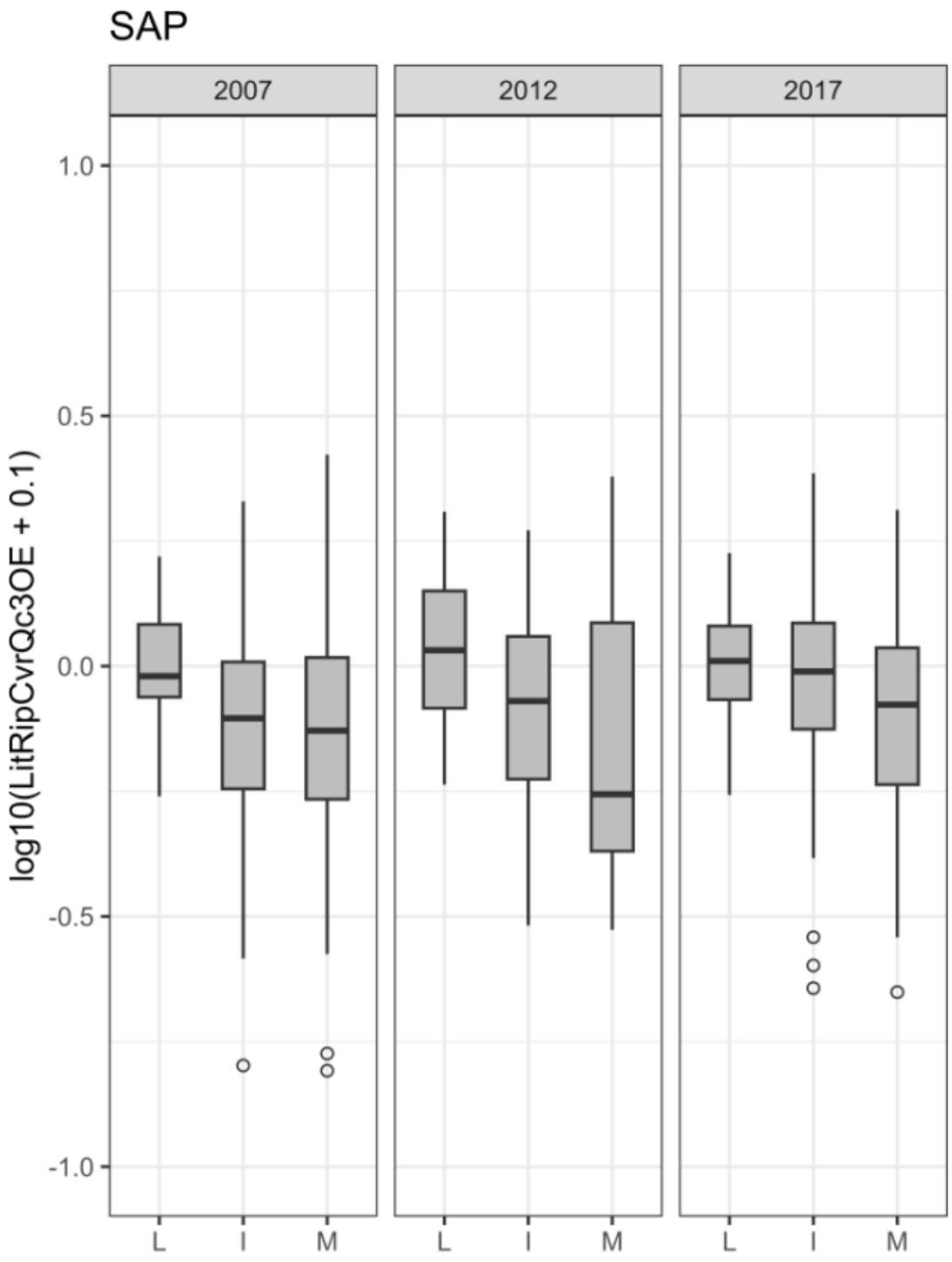
Littoral Habitat:



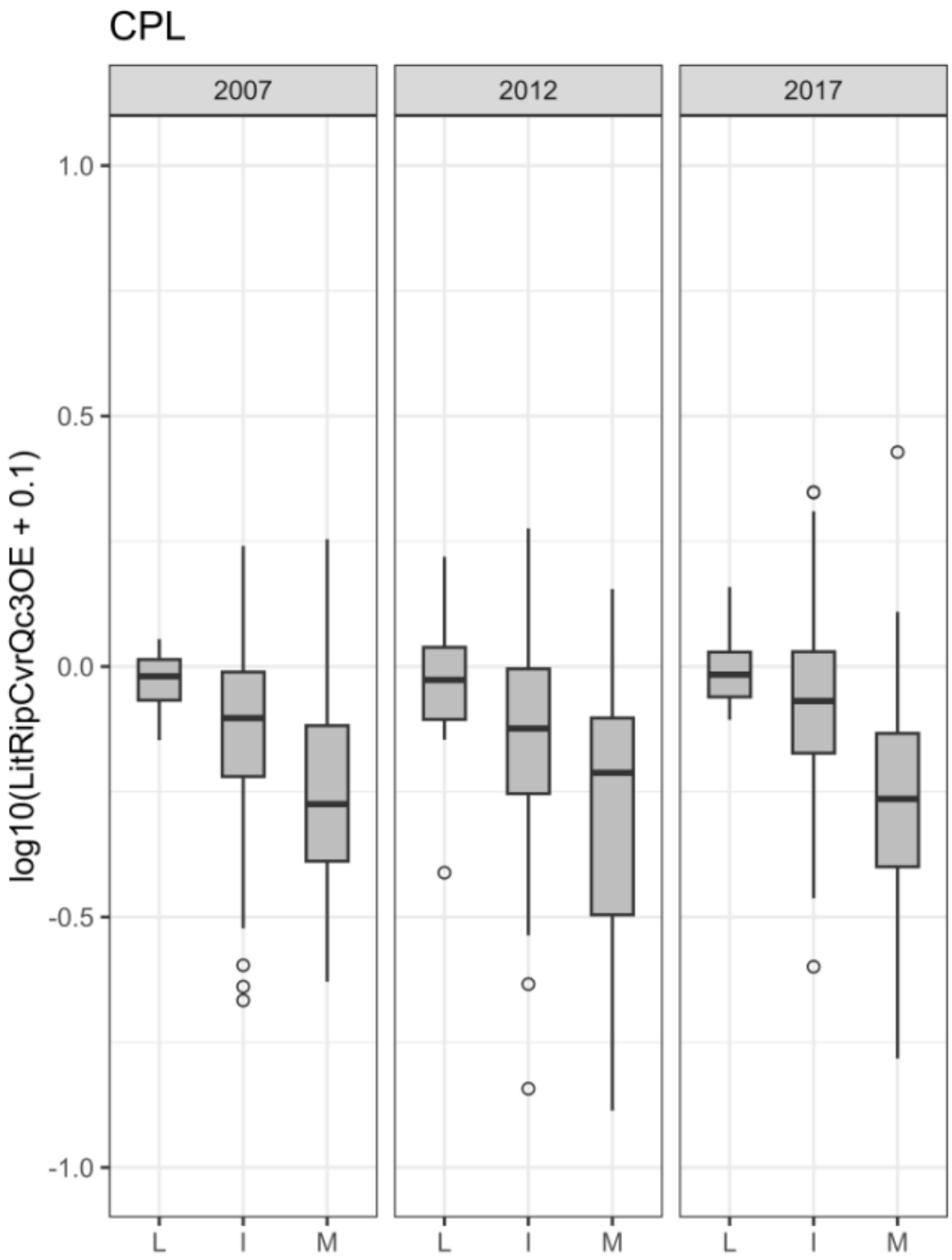
Littoral-Riparian Complexity:



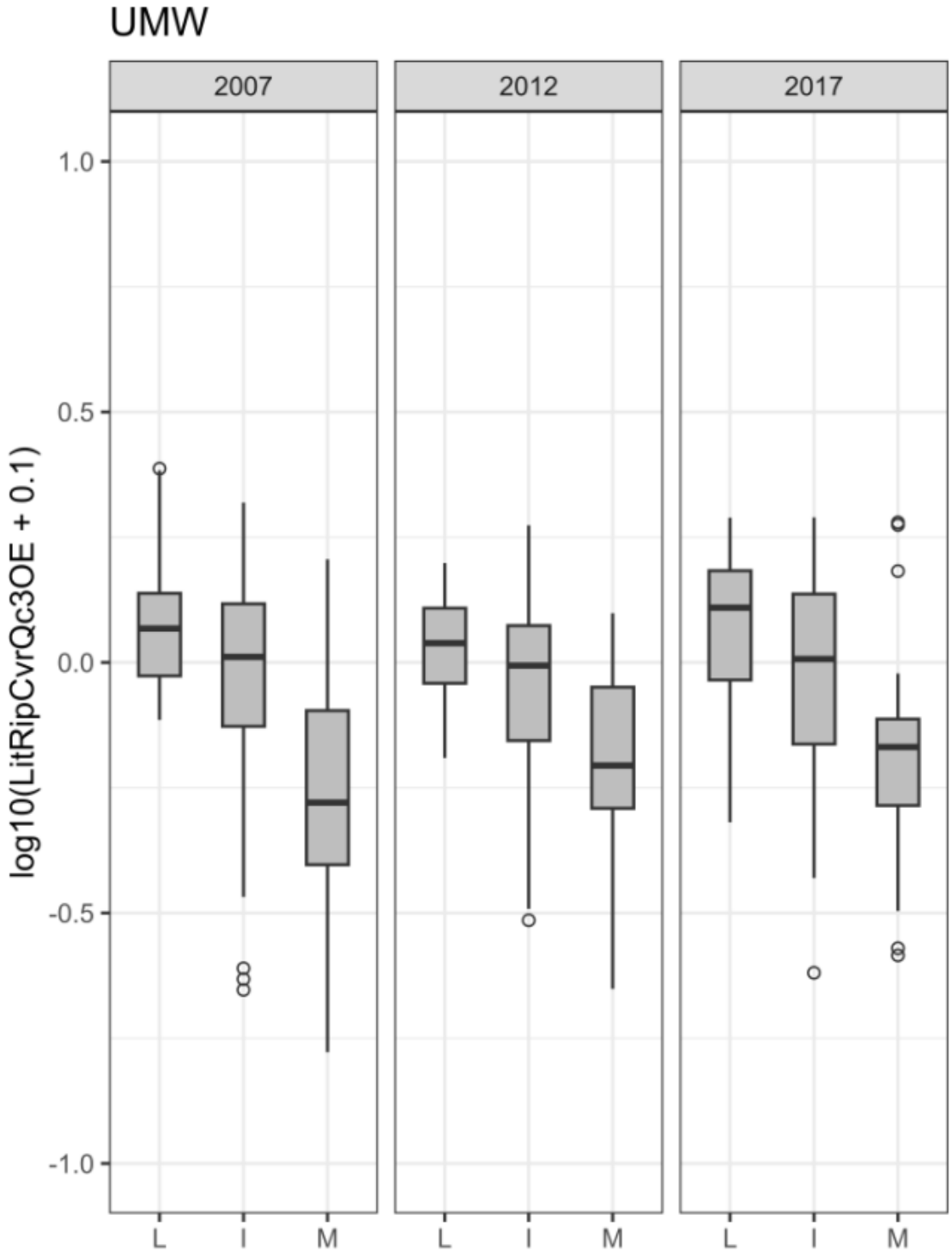
Littoral-Riparian Complexity:



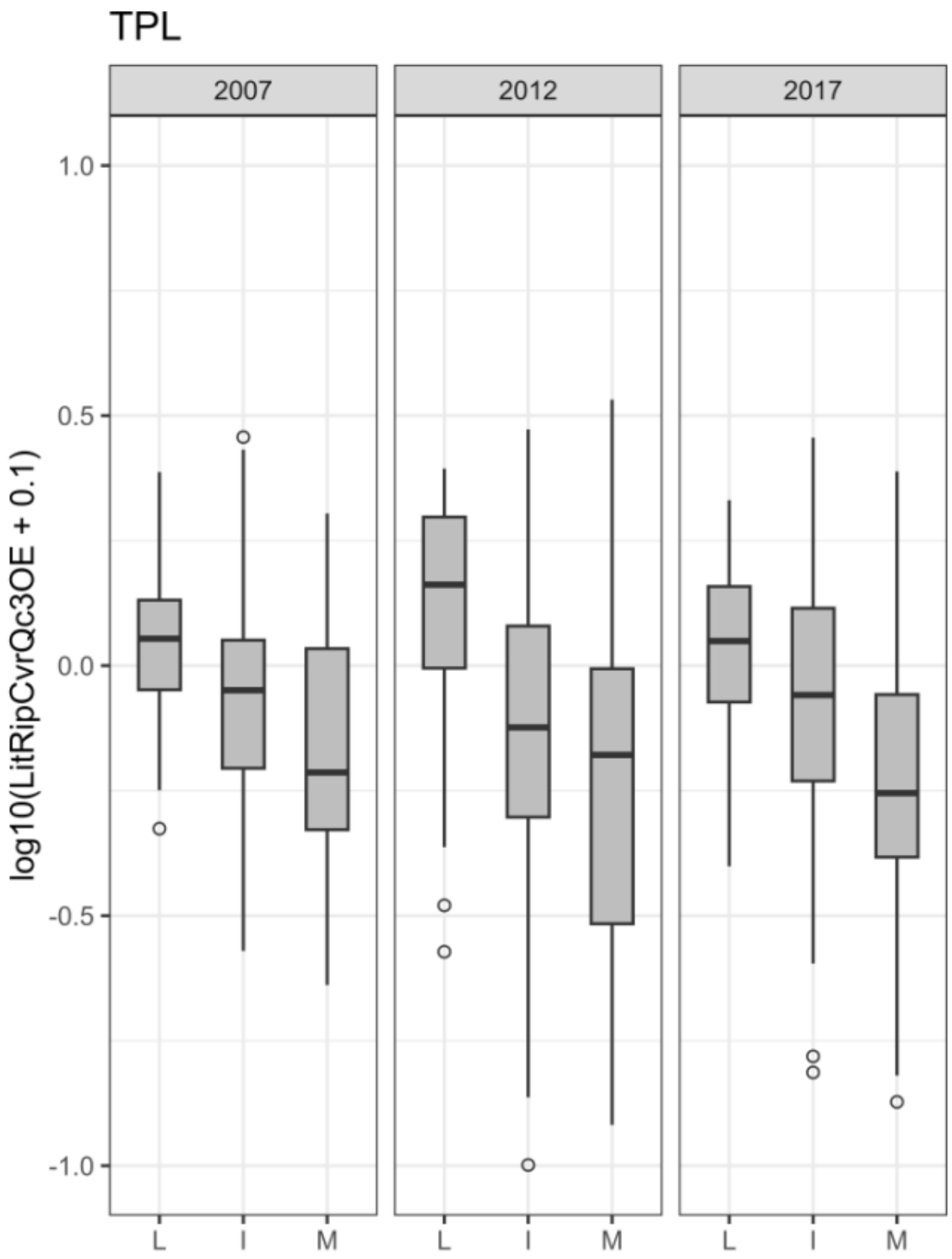
Littoral-Riparian Complexity:



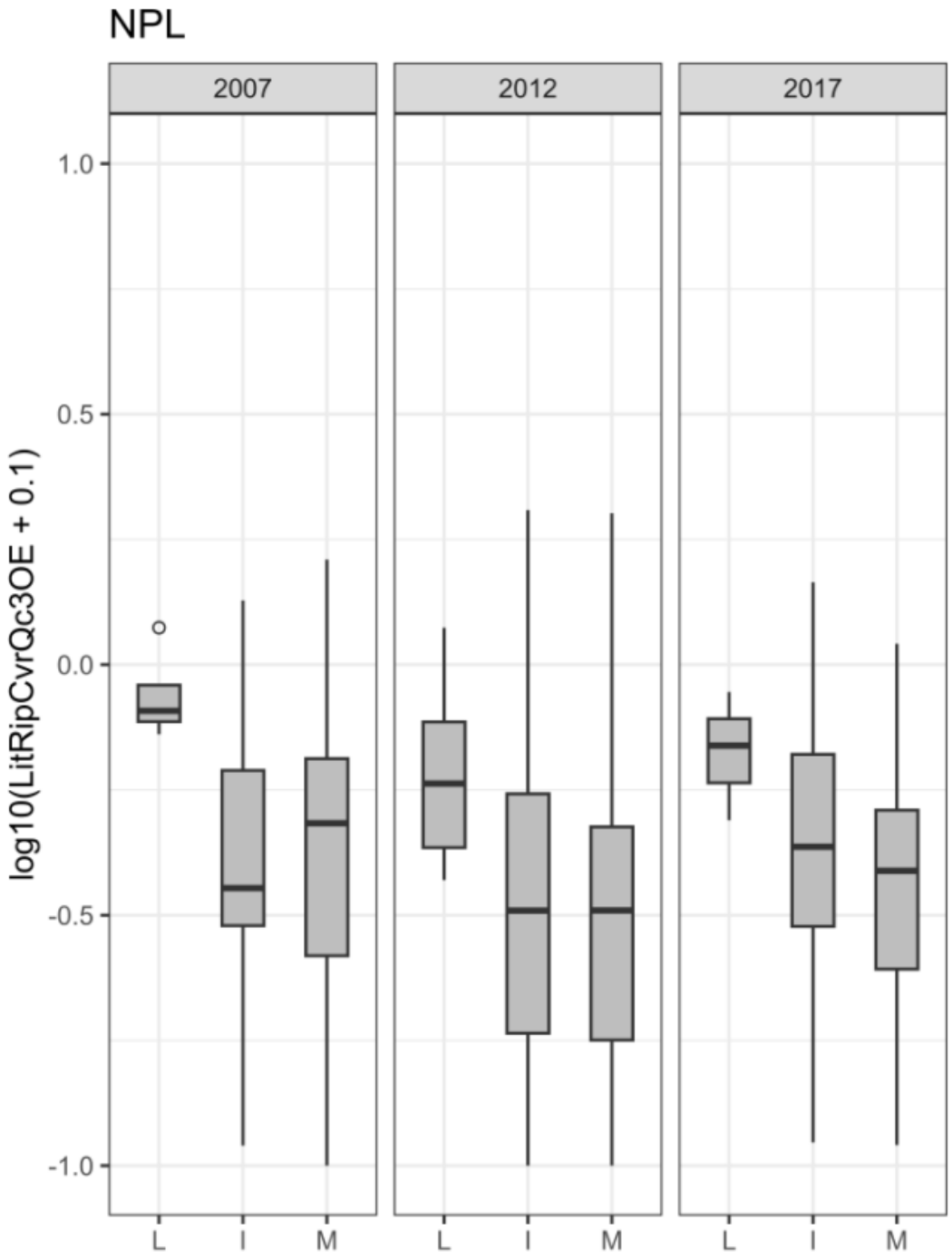
Littoral-Riparian Complexity:



Littoral-Riparian Complexity:



Littoral-Riparian Complexity:



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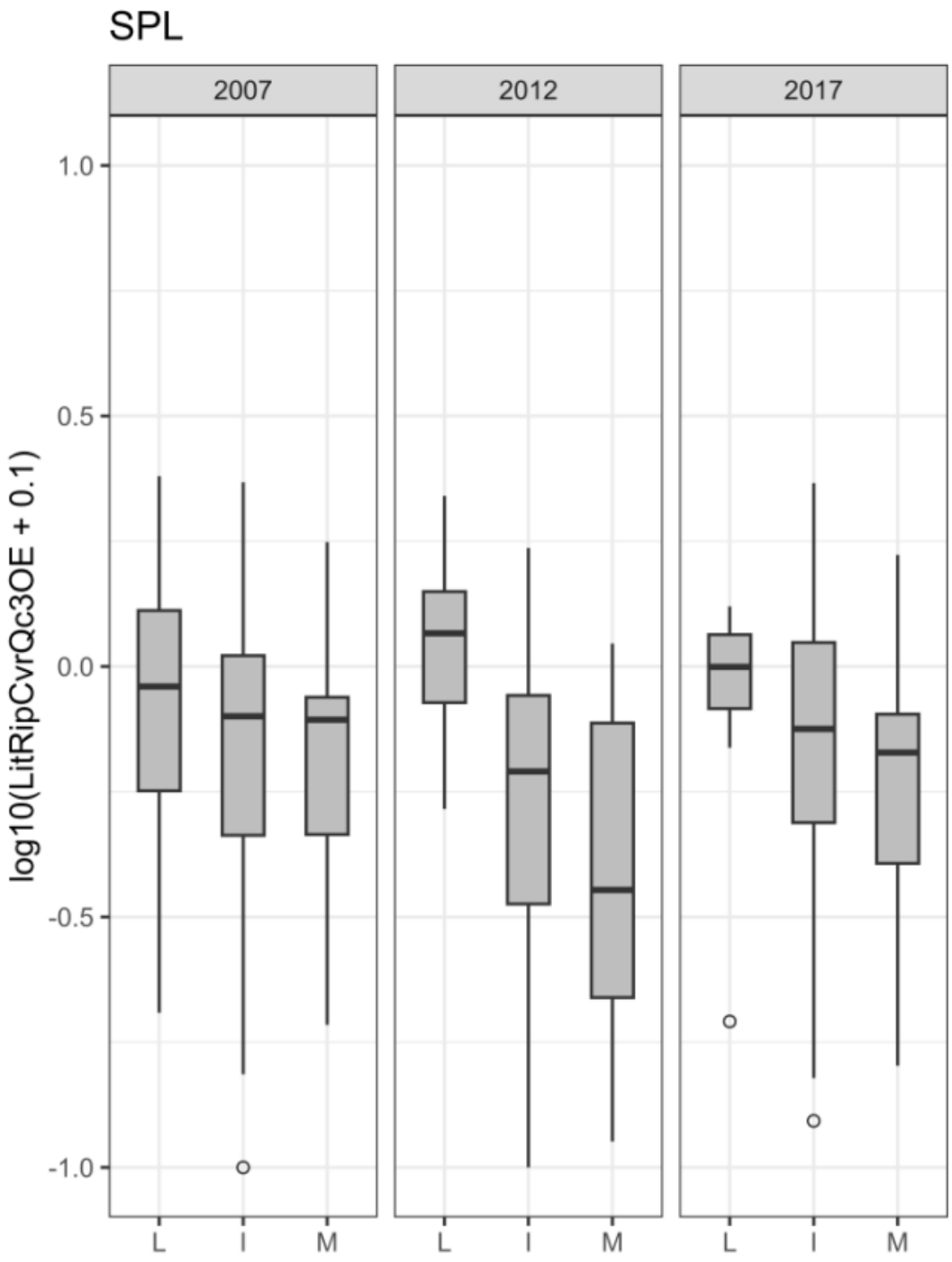

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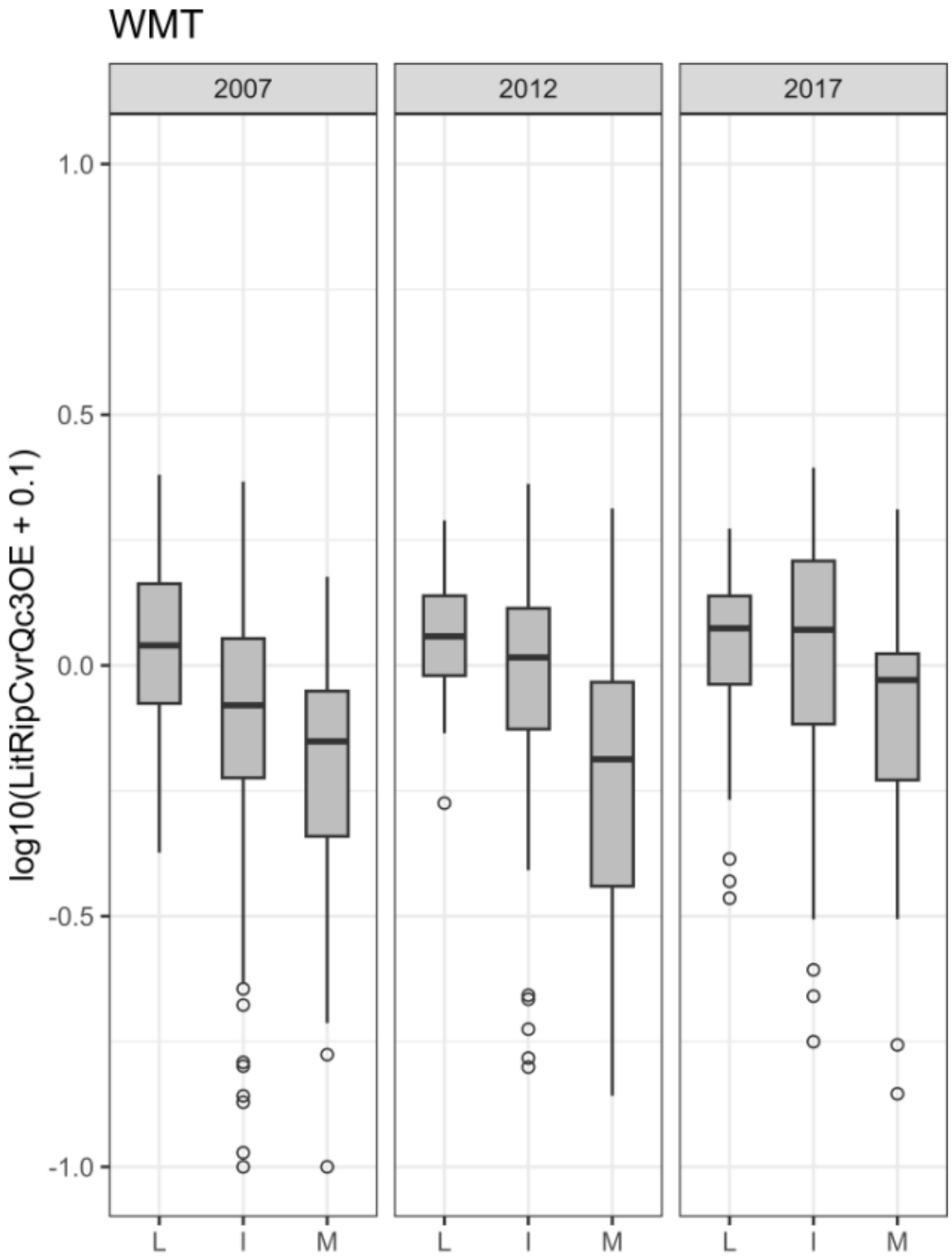
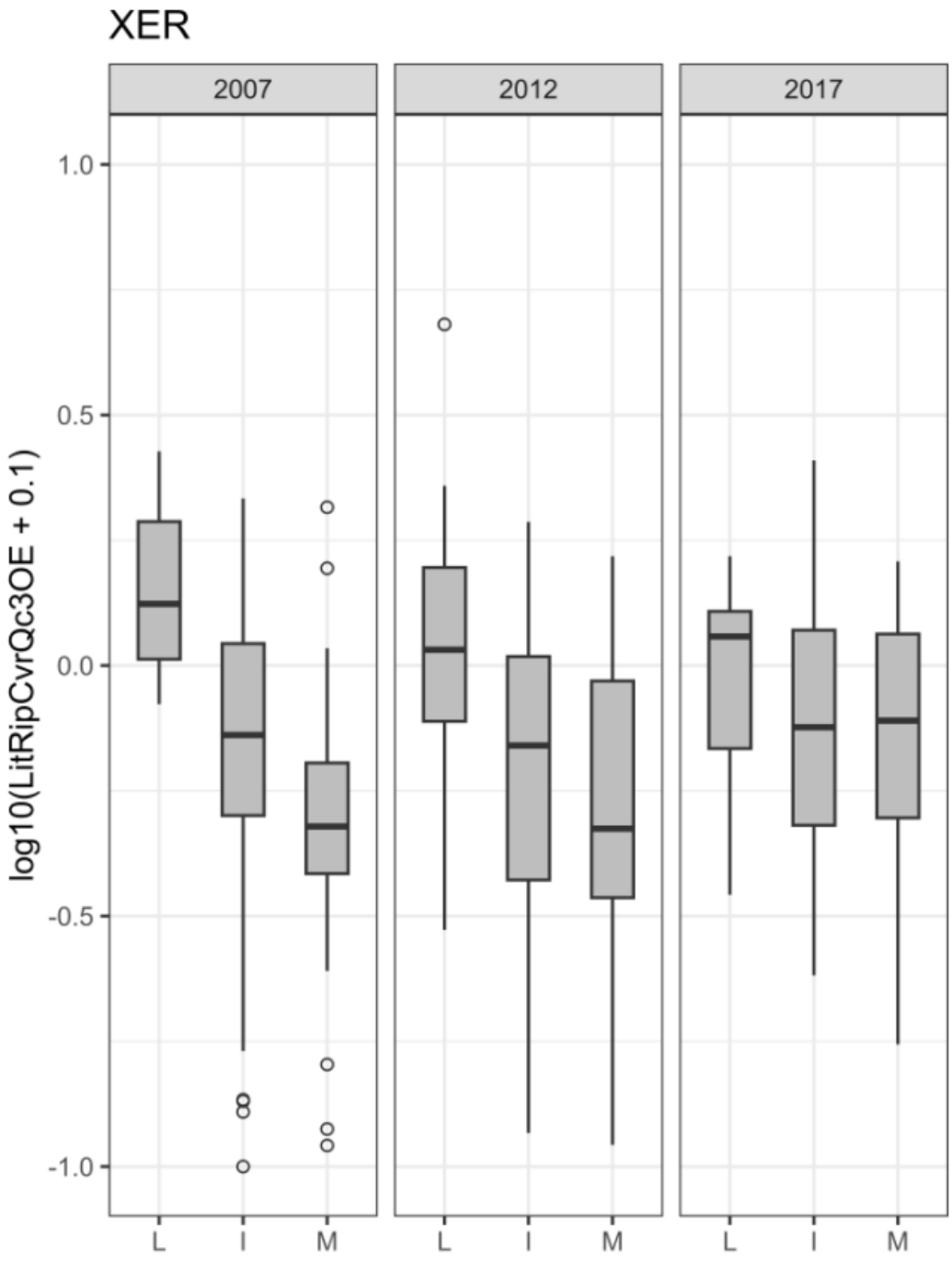


Littoral-Riparian Complexity:



Appendix B: Survey Design and Estimated Extent Summary for NLA 2007, 2012, 2017 and 2022

Category	Characteristic	Description	2022	2017	2012	2007
sampling frame	sampling frame	source	NHDPlus HR	NHDPlus and NHDPlus HR for 1-5 ha lakes	NHDPlus, version 2	NHD
sampling frame	sampling frame	total number of lake objects in source (NHD)	6,512,454 (waterbody polygons)	586,678 (lake objects)	378,858 (lake objects)	389,005 (lake objects)
sampling frame	sampling frame	lake objects included in the sampling frame	497,840	465,901	277,886	123,369
sampling frame	sampling frame exclusions	lake objects excluded because they are not expected to meet the target population definition	6,014,614	120,777	100,972	265,636 (of which 233,627 were 1-4ha)
survey design	survey design		GRTS stratified by state and unequal probability by lake size within state	GRTS stratified by state and unequal probability of selection by lake size within state	GRTS with stratification and unequal probability of selection by lake size within state	GRTS with stratification and unequal probability of selection by lake size within state
survey design	restriction	minimum lakes per state	8	7	7	7
survey design	restriction	maximum lakes per state	50	50	43	none
survey design	stratification	stratification	by state	by state	by state and NLA12_CLS	None

Category	Characteristic	Description	2022	2017	2012	2007
survey design	lake area categories	description (ha)	(1-4], (4-10], (10-50], >50	(1-4], (4-10], (10-20], (20-50], >50	(1-4], (4-10], (10-20], (20-50], >50	(4-10], (10-20], (20-50], (50-100], >100
survey design	lake area categories	minimum (ha)	1 ha	1 ha	1 ha	4 ha
survey design	expected unique lakes	total lakes	904	904	904	909
survey design	expected sample size	total visits	1,000	1,000	1,000	1,000
survey design	expected split	new/previously sampled	50/50	50/50	62/38	NA
survey design	revisits	number of lakes	96	96	96	91
survey implementation	survey design lakes sampled	total lakes samples (used in population estimates)	981	1,005	1,038	1130
survey implementation	survey design lakes sampled	Size class: 1-4 ha	216	204	87	0
survey implementation	survey design lakes sampled	Size class: 4-10 ha	195	179	142	73
survey implementation	survey design lakes sampled	Size class: 10-50 ha	293	NA	NA	NA
survey implementation	survey design lakes sampled	Size class: 10-20 ha	NA	192	173	162
survey implementation	survey design lakes sampled	Size class: 20-50 ha	NA	164	225	211
survey implementation	survey design lakes sampled	Size class: >50 ha	277	266	411	684

Category	Characteristic	Description	2022	2017	2012	2007
estimated extent	estimated lake population	target population: All lakes ≥ 1ha (LCB95Pct-UCB95Pct)	268,018 (256,329-279,706)	224,916 (194,076-255,755)	126,113*	NA
estimated extent	estimated lake population	target population: Large lakes ≥ 4 ha	98,519	76,177	68,777	65,259*
estimated extent	estimated lake population	target unknown	NA	3,290	3,538	--
estimated extent	estimated lake population	non-target lakes	229,822	237,695	114,695	55,146
estimated extent	estimated lake population	sampled population	124,309	109,701	111,818	49,546
estimated extent	estimated lake population	NLA report result representation	target population	target population	sampled population	sampled population

*Upper and lower confidence intervals (CI) are not provided since reporting changed from the sampled population to the target population in 2017. Estimated target population values for 2012 and 2007 were updated in 2017. Weights for all survey years can be found in the "Data for Population Estimate" files on the NARS Data page.

Appendix C: NLA 2022 Indicator Benchmark Summary

Physical habitat benchmarks are not included since regionally relevant lake-specific benchmarks are modeled.

Metric Category	Indicator	Benchmark Description	National/ Ecoregion	Condition class	Value	Units	General Assessment Notes
Biological	Benthic Macroinvertebrate	NLA-derived regionally specific benchmark	Coastal Plains	Good	≥51.8	--	Sample collected from the lake bottom at 10 shoreline locations and composited for each lake. Organisms were usually identified to genus and an index was developed based on life history characteristics and tolerance to environmental conditions.
Biological	Benthic Macroinvertebrate	NLA-derived regionally specific benchmark	Eastern Highlands	Good	≥44.5	--	
Biological	Benthic Macroinvertebrate	NLA-derived regionally specific benchmark	Plains	Good	≥39.5	--	
Biological	Benthic Macroinvertebrate	NLA-derived regionally specific benchmark	Upper Midwest	Good	≥51.4	--	
Biological	Benthic Macroinvertebrate	NLA-derived regionally specific benchmark	Western Mountains	Good	≥47.6	--	
Biological	Benthic Macroinvertebrate	NLA-derived regionally specific benchmark	Coastal Plains	Poor	<44.1	--	
Biological	Benthic Macroinvertebrate	NLA-derived regionally specific benchmark	Eastern Highlands	Poor	<31.4	--	
Biological	Benthic Macroinvertebrate	NLA-derived regionally specific benchmark	Plains	Poor	<26.6	--	
Biological	Benthic Macroinvertebrate	NLA-derived regionally specific benchmark	Upper Midwest	Poor	<37.2	--	
Biological	Benthic Macroinvertebrate	NLA-derived regionally specific benchmark	Western Mountains	Poor	<32.6	--	
Biological	Zooplankton	NLA-derived regionally specific benchmark	Coastal Plains	Good	≥59.42	--	Sample collected from the water column at the open-water site. Organisms were usually identified to genus and an index was developed based on life history characteristics and tolerance to environmental conditions.
Biological	Zooplankton	NLA-derived regionally specific benchmark	Eastern Highlands	Good	≥73.595	--	
Biological	Zooplankton	NLA-derived regionally specific benchmark	Plains	Good	≥36.72	--	

Metric Category	Indicator	Benchmark Description	National/ Ecoregion	Condition class	Value	Units	General Assessment Notes
Biological	Zooplankton	NLA-derived regionally specific benchmark	Upper Midwest	Good	≥63.68	--	
Biological	Zooplankton	NLA-derived regionally specific benchmark	Western Mountains	Good	≥60.78	--	
Biological	Zooplankton	NLA-derived regionally specific benchmark	Coastal Plains	Poor	<53.77	--	
Biological	Zooplankton	NLA-derived regionally specific benchmark	Eastern Highlands	Poor	<60.03	--	
Biological	Zooplankton	NLA-derived regionally specific benchmark	Plains	Poor	<28.17	--	
Biological	Zooplankton	NLA-derived regionally specific benchmark	Upper Midwest	Poor	<52.03	--	
Biological	Zooplankton	NLA-derived regionally specific benchmark	Western Mountains	Poor	<51.32	--	
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	CPL	Good	≤12.7	ug/L	Sample collected from a vertically integrated water column at the open-water site. Measured concentrations were compared to benchmarks.
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	NAP	Good	≤4.52	ug/L	
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	NPL	Good	≤10.9	ug/L	
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	SAP	Good	≤5.54	ug/L	
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	SPL-manmade	Good	≤8.97	ug/L	
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	SPL-natural	Good	≤118	ug/L	
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	TPL	Good	≤13.9	ug/L	
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	UMW	Good	≤6.7	ug/L	

Metric Category	Indicator	Benchmark Description	National/ Ecoregion	Condition class	Value	Units	General Assessment Notes
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	WMT	Good	≤1.83	ug/L	
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	XER	Good	≤5.92	ug/L	
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	CPL	Poor	>28	ug/L	Sample collected from a vertically integrated water column at the open-water site. Measured concentrations were compared to benchmarks.
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	NAP	Poor	>8.43	ug/L	
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	NPL	Poor	>19.3	ug/L	
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	SAP	Poor	>13.1	ug/L	
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	SPL-manmade	Poor	>12.6	ug/L	
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	SPL-natural	Poor	>219	ug/L	
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	Temperate Plains	Poor	>19.8	ug/L	
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	UMW	Poor	>14.6	ug/L	
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	WMT	Poor	>3.86	ug/L	
Biological	Chlorophyll *a*	NLA-derived regionally specific benchmark	XER	Poor	>9	ug/L	
Chemical	Acidity	Nationally consistent, literature benchmark described in Herlihy et al. (1991)	National	Good	ANC > 50 ueq/L	ueq/L	ANC (corrected for DOC) measured from a vertically integrated water column at the open-water site. Measured concentrations were compared to benchmarks.
Chemical	Acidity	Nationally consistent, literature benchmark described in Herlihy et al. (1991)	National	Poor	ANC ≤ 0 µeq/L and DOC values < 6 mg/L	ueq/L	

Metric Category	Indicator	Benchmark Description	National/ Ecoregion	Condition class	Value	Units	General Assessment Notes
Chemical	Atrazine	EPA aquatic plant concentration equivalent level of concern (CE-LOC); click here	National	Above Benchmark = Poor	> 3.4 ppb	ppb	Sample collected from a vertically integrated water column sample at the open-water site. Measured concentrations were compared to benchmark.
Chemical	Atrazine	Atrazine minimum detection level (MDL)	National	Detected	> or = 0.046	ppb	
Chemical	**Cylindrospermopsin	EPA recreational water qualtiy criteria and swimming advisory recommendation. USEPA 2019. EPA 822-R-19-001.	National	Above Benchmark = Poor	>15	ppb	Sample collected from a vertically integrated water column sample at the open-water site. Measured concentrations were compared to benchmark.
Chemical	**Cylindrospermopsin	Cylindrospermopsin minimum detection level (MDL)	National	Detected	> or = 0.05	ppb	
Chemical	Microcystins	EPA recreational water qualtiy criteria and swimming advisory recommendation. USEPA 2019. EPA 822-R-19-001.	National	Above Benchmark = Poor	>8	ppb	Sample collected from a vertically integrated water column sample at the open-water site. Measured concentrations were compared to benchmark.
Chemical	Microcystins	Microcystin minimum detection level (MDL)	National	Detected	> or = 0.1	ppb	
Chemical	**Enterococci	EPA Statistical Threshold Value USEPA 2012. EPA 820-F-12-058	National	Above Benchmark = Poor	>1,280	CCE/ 100 mL	Sample collected from last littoral station or the launch site in an area that was approximately 1 m deep at about 0.3 m (12 inches) below the water.
Chemical	Oxygen (Dissolved)	Nationally consistent, literature benchmark; warmwater daily minimum for "other life stages"; US EPA 1986. Quality Criteria for Water ("Gold Book")	National	Poor	<= 3 ppm	ppm	Measures were collected from the in-situ oxygen measure from the top 2m of the profile at the index site. The mean of all measurements between 0 and 2 meters was compared to the benchmark.
Chemical	Oxygen (Dissolved)	Nationally consistent, literature benchmark; warmwater daily minimum for “early life stages"; US EPA 1986. Quality Criteria for Water ("Gold Book")	National	Good	>= 5 ppm	ppm	
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	CPL	Good	≤659	ug/L	Sample collected from a vertically integrated water column at the

Metric Category	Indicator	Benchmark Description	National/ Ecoregion	Condition class	Value	Units	General Assessment Notes
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	NAP	Good	≤428	ug/L	open-water site. Measured concentrations were compared to benchmarks.
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	NPL	Good	≤849	ug/L	
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	SAP	Good	≤266	ug/L	
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	SPL-manmade	Good	≤650	ug/L	
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	SPL-natural	Good	≤7840	ug/L	
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	TPL	Good	≤865	ug/L	
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	UMW	Good	≤766	ug/L	
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	WMT	Good	≤253	ug/L	
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	XER	Good	≤605	ug/L	
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	CPL	Poor	>923	ug/L	Sample collected from a vertically integrated water column at the open-water site. Measured concentrations were compared to benchmarks.
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	NAP	Poor	>655	ug/L	
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	NPL	Poor	>1620	ug/L	
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	SAP	Poor	>409	ug/L	
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	SPL-manmade	Poor	>830	ug/L	
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	SPL-natural	Poor	>11100	ug/L	

Metric Category	Indicator	Benchmark Description	National/ Ecoregion	Condition class	Value	Units	General Assessment Notes
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	TPL	Poor	>1350	ug/L	
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	UMW	Poor	>926	ug/L	
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	WMT	Poor	>429	ug/L	
Chemical	Total Nitrogen	NLA-derived regionally specific benchmark	XER	Poor	>954	ug/L	
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	CPL	Good	≤43	ug/L	Sample collected from a vertically integrated water column at the open-water site. Measured concentrations were compared to benchmarks.
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	NAP	Good	≤16	ug/L	
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	NPL	Good	≤63	ug/L	
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	SAP	Good	≤18	ug/L	
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	SPL-manmade	Good	≤30	ug/L	
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	SPL-natural	Good	≤486	ug/L	
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	TPL	Good	≤38.4	ug/L	
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	UMW	Good	≤24.8	ug/L	
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	WMT	Good	≤23.4	ug/L	
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	XER	Good	≤44	ug/L	

Metric Category	Indicator	Benchmark Description	National/ Ecoregion	Condition class	Value	Units	General Assessment Notes
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	CPL	Poor	>59.5	ug/L	Sample collected from a vertically integrated water column at the open-water site. Measured concentrations were compared to benchmarks.
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	NAP	Poor	>27.9	ug/L	
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	NPL	Poor	>82	ug/L	
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	SAP	Poor	>33	ug/L	
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	SPL-manmade	Poor	>43	ug/L	
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	SPL-natural	Poor	>839	ug/L	
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	TPL	Poor	>57.5	ug/L	
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	UMW	Poor	>40	ug/L	
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	WMT	Poor	>43	ug/L	
Chemical	Total Phosphorus	NLA-derived regionally specific benchmark	XER	Poor	>84.8	ug/L	
Chemical	Trophic State	Nationally consistent, NLA-derived benchmark	National	Oligotrophic	≤2	ug/L	Sample collected from a vertically integrated water column at the open-water site. Trophic state was based on measured chlorophyll *a* concentrations.
Chemical	Trophic State	Nationally consistent, NLA-derived benchmark	National	Mesotrophic	>2 and ≤7	ug/L	
Chemical	Trophic State	Nationally consistent, NLA-derived benchmark	National	Eutrophic	>7 and ≤30	ug/L	
Chemical	Trophic State	Nationally consistent, NLA-derived benchmark	National	Hypereutrophic	>30	ug/L	

** identifies new or updated benchmarks for NLA 2022

Appendix D: Zooplankton

11.1 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. Tables D.1 through D.5 list each metric by its variable name, which of the six metric categories it was assigned to (see Section 7.4.3), 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, ad S:N value for repeatability).

Table D. 1. List of candidate metrics used to develop the zooplankton MMI for the Coastal Plains bioregion.

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)	14.7	50.2	-1,67	1.2
Abundance/ Biomass/ Density	ZOFN_BIO	Biomass represented by individuals collected in fine mesh net (50-um)	20.5	67.2	-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)	5.6	22.9	--1.30	1.9
Richness/Diversity	FAM_NAT_NTAX	Number of families represented by distinct native taxa (coarse and fine net samples combined)	11.9	9.3	2.66	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)	15.3	11.9	2.29	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)	0.22	0.02	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)	1.0	0.15	1.8	7.2
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

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	HERB_NTAX	Number of distinct herbivore taxa (coarse and fine net samples combined)	11.9	8.8	2.22	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
Trophic	OMNI_PDEN	Percent of total density represented by omnivorous individuals (coarse and fine net samples combined)	18.12	39.82	-2.37	1.7
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	4.8	35.0	-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	17.82	39.27	-2.36	1.7
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)	11.6	34.6	-1.73	1.0
Cladoceran	BOSM300_PTAX	Percent of distinct taxa in the 300-count subsamples that are in the family Bosminidae (coarse and fine net samples combined)	7.98	4.07	2.94	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)	85.21	79.61	0.86	1.9
Richness/Diversity	GEN300_NTAX	Number of genera represented by distinct taxa (coarse and fine net samples combined)	14.1	11.1	2.16	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
Richness/Diversity	GEN300_NAT_NTAX	Number of genera represented by distinct native taxa (coarse and fine net samples combined)	14.1	11.0	2.24	1.5
Richness/Diversity	FAM300_NTAX	Number of families represented in 300 count subsamples (coarse and fine net samples combined)	10.9	8.6	2.61	2.2
Richness/Diversity	FAM300_NAT_NTAX	Number of native families represented in 300 count subsamples (coarse and fine net samples combined)	10.9	8.6	2.72	2.1
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	1.4
Rotifer	COLLO300_BIO	Biomass represented by individuals of the rotifer order Collothecaceae in the 300-count subsamples (coarse and fine net samples combined)	0.08	0.01	1.81	7.0
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)	0.46	0.14	2.45	1.5
Trophic	HERB300_NTAX	Number of distinct taxa that are herbivores in 300 count subsamples (coarse and fine net samples combined)	10.9	7.8	2.58	1.8
Trophic	OMNI300_PIND	Percent of omnivorous individuals in 300 count subsamples (coarse and fine net samples combined)	15.54	28.43	-1.85	1.4
Trophic	OMNI300_PTAX	Percent of distinct taxa that are omnivores in 300 count subsamples (coarse and fine net samples combined)	23.75	37.16	-2.91	4.1
Trophic	OMNI300_PBIO	Percent of biomass represented by omnivorous individuals in 300 count subsamples (coarse and fine net samples combined)	27.14	33.99	-0.79	1.2
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

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	ROT_PRED300_BIO	Biomass represented by rotifer individuals that are predators in 300 count subsamples (coarse and fine net samples combined)	0.46	0.14	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)	6.0	3.7	2.45	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)	18.35	30.18	-3.06	3.6

Table D. 2. 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 D. 3. 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 D. 4. 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 Floscullariaceae (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 D. 5. 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

11.2 Non-target taxa in zooplankton samples that are excluded from enumeration

TAXA ID	TAXON NAME	PHYLUM	CLASS	SUBCLASS	ORDER	SUBORDER	FAMILY	GENUS	SPECIES
1026	AMPHIPODA	ARTHROPODA	MALACOSTRACA	EUMALACOSTRACA	AMPHIPODA				
1030	APPENDICULARIA	CHORDATA	APPENDICULARIA						
1051	BIVALVIA	MOLLUSCA	BIVALVIA						
1217	HEMIGRAPSUS SANGUINEUS	ARTHROPODA	MALACOSTRACA	EUMALACOSTRACA	DECAPODA		VARUNIDAE	HEMIGRAPSUS	SANGUINEUS
1359	MYSIDAE	ARTHROPODA	MALACOSTRACA	EUMALACOSTRACA	MYSIDA		MYSIDAE		
1389	PECTINARIIDAE	ANNELIDA	POLYCHAETA	PALPATA	CANALIPALPATA	TEREBELLIDA	PECTINARIIDAE		
1390	PHYLLODOCIDAE	ANNELIDA	POLYCHAETA	PALPATA	ACICULATA		PHYLLODOCIDAE		
1410	POLYCHAETA	ANNELIDA	POLYCHAETA						
1461	TREMATODA	PLATYHELMINTHES	TREMATODA						
1495	UCA	ARTHROPODA	MALACOSTRACA	EUMALACOSTRACA	DECAPODA	PLEOCYEMATA	OCYPODOIDAE	UCA	
5033	MYSIS RELICTA	ARTHROPODA	MALACOSTRACA	EUMALACOSTRACA	MYSIDA		MYSIDAE	MYSIS	RELICTA
5049	GAMMARIDAE	ARTHROPODA	MALACOSTRACA	EUMALACOSTRACA	AMPHIPODA	GAMMERIDEA	GAMMARIDAE		
5491	CORBICULA	MOLLUSCA	BIVALVIA	HETERODONTA	VENEROIDA		CORBICULIDAE	CORBIUCLA	
5497	DECAPODA	ARTHROPODA	MALACOSTRACA	EUMALACOSTRACA	DECAPODA				
5501	GAMMARUS	ARTHROPODA	MALACOSTRACA	EUMALACOSTRACA	AMPHIPODA	GAMMERIDEA	GAMMARIDAE	GAMMARUS	
5503	HYALELLA	ARTHROPODA	MALACOSTRACA	EUMALACOSTRACA	AMPHIPODA	GAMMERIDEA	HYALELLIDAE	HYALELLA	HYALELLAGAMMARUS
5504	HYALELLA AZTECA CMPLX	ARTHROPODA	MALACOSTRACA	EUMALACOSTRACA	AMPHIPODA	GAMMERIDEA	HYALELLIDAE	HYALELLA	AZTECA CMPLX
5505	HYDRA	CNIDARIA	HYDROZOA		ANTHOATHECATAE		HYDRIDAE	HYDRA	
5521	MONOCOROPHIUM	ARTHROPODA	MALACOSTRACA	EUMALACOSTRACA	AMPHIPODA	GAMMERIDEA	COROPHIIDAE	MONOCOROPHIUM	
5522	NEOMYSIS MERCEDIS	ARTHROPODA	MALACOSTRACA	EUMALACOSTRACA	MYSIDA		MYSIDAE	NEOMYSIS	MERCEDIS
5525	PALAEMONETES	ARTHROPODA	MALACOSTRACA	EUMALACOSTRACA	DECAPODA	PLEOCYEMATA	PALAEMONIDAE	PALAEMONETES	
5526	PALAEMONIDAE	ARTHROPODA	MALACOSTRACA	EUMALACOSTRACA	DECAPODA	PLEOCYEMATA	PALAEMONIDAE		
5543	ANISOPTERA	ARTHROPODA	INSECTA		ODONATA			ANISOPTERA	

TAXA ID	TAXON NAME	PHYLUM	CLASS	SUBCLASS	ORDER	SUBORDER	FAMILY	GENUS	SPECIES
5544	CHAETONOTUS	GASTROTRICHA			CHAETONOTIDA		CHAETONOTIDAE	CHAETONOTUS	
5545	COLLEMBOLA	ATRHROPODA	INSECTA		COLLEMBOLA				
5546	CORIXIDAE	ARTHROPODA	INSECTA	PTERYGOTA	HEMIPTERA	HETEROPTERA	CORIXIDAE		
5548	DIPTERA	ARTHROODA	INSECTA		DIPTERA				
5549	DYTISCIDAE	ARTHROPODA	INSECTA	PTERYGOTA	COLEOPTERA	ADEPHAGA	DYTISICIDAE		
5550	EPHEMEROPTERA	ARTHROPODA	INSECTA		EPHEMEROPTERA				
5551	GASTROPODA	MOLLUSCA	GASTROPODA						
5552	GASTROTRICHA	GASTROTRICHA							
5554	INSECTA	ARTHROPODA	INSECTA						
5555	NEMATODA	NEMATODA							
5556	NEOGOSSEA	GASTROTRICHA			CHAETONOTIDA		NEOGOSSEIDAE	NEOGOSSEA	
5557	NOTONECTIDAE	ARTHROPODA	INSECTA	PTERYGOTA	HEMIPTERA	HETEROPTERA	NOTONECTIDAE		
5558	ODONATA	ARTHROPODA	INSECTA		ODONATA				
5559	OLIGOCHAETA	ANNELIDA	CLITELLATA	OLIGOCHAETA					
5562	PLECOPTERA	ARTHROPODA	INSECTA	PTERYGOTA	PLECOPTERA				
5563	TARDIGRADA	TARDIGRADA							
5564	TRICHOPTERA	ARTHROPODA	INSECTA	PTERYGOTA	TRICHOPTERA				
5565	UNIONOIDA	MOLLUSCA	BIVALVIA	PALAEOHETERODONTA	UNIONOIDA				