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A Decade of Change: Crustacean Zooplankton Communities in Nine Minnesota Large Lakes

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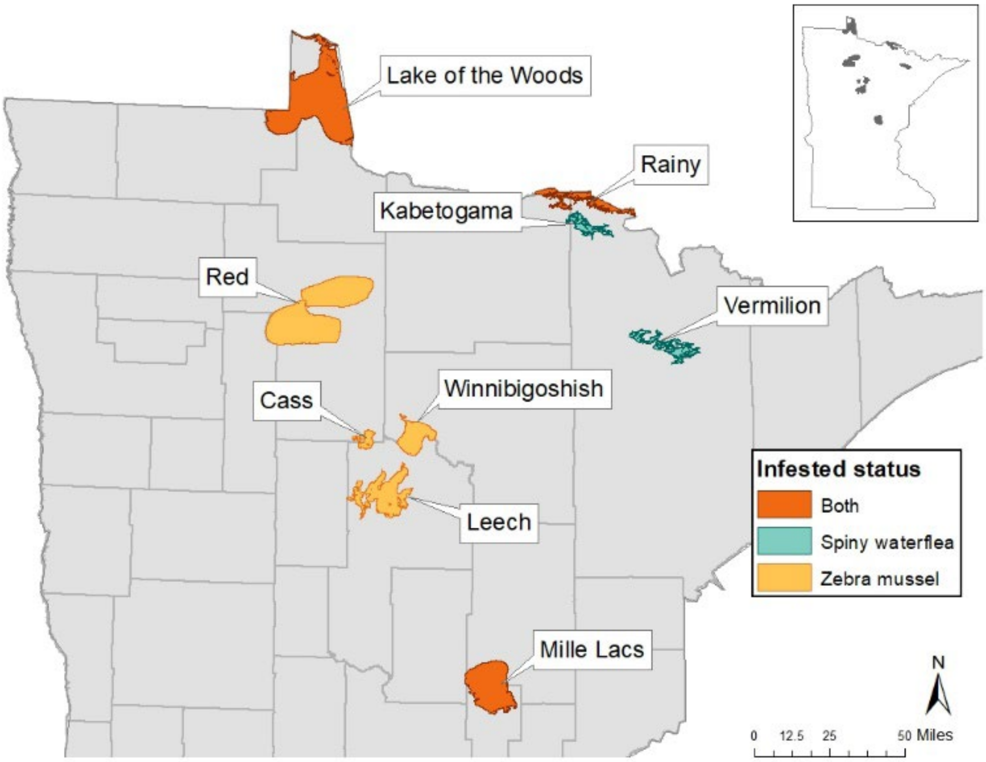
EXECUTIVE SUMMARY

The purpose of this report is to summarize the zooplankton community in the nine largest walleye lakes in Minnesota (hereafter, Large Lakes). Standardized zooplankton sampling was conducted consistently in the Large Lakes since 2012. Since that time, aquatic invasive species (AIS) such as spiny waterflea (*Bythotrephes cederströmii*, synonym *Bythotrephes longimanus*) and/or zebra mussels (*Dreissena polymorpha*), made their way into all nine lakes. This report examines the impacts of those invasive species on zooplankton community structure and function, and we provide broad recommendations to the zooplankton monitoring program on the frequency and intensity of data collection for another decade.



Highlights/Insights. —We observed major and progressive impacts of spiny waterflea and zebra mussel on native zooplankton biomass, community composition, and secondary production. These impacts varied among lakes based on which taxon/taxa invaded, characteristics of invasion (timing and magnitude), and the zooplankton assemblage. We found that the impacts of AIS varied, both between spiny waterflea and zebra mussels and among the four major groups of native zooplankton (small cladocerans, large *Daphnia* spp., calanoid copepods, and cyclopoid copepods). In all cases, however, AIS were related to declines in zooplankton population and community metrics. Additionally, declines in native zooplankton varied throughout the summer, and in the case of communities invaded by spiny waterflea, persisted even early in the season before the invasive predator was detected in our samples.

Recommendations. —We determined appropriate sample efforts that are lake-specific (Table A.1). We also recommend limiting to 30 individual nauplii and copepodite measurements in each sample to improve efficiency. We will continue to refine and standardize protocols (whenever possible) so that the data continue to advance our understanding of AIS impacts to Minnesota waters. We recommend that monitoring of zooplankton continues in the Section of Fisheries Large Lakes program. The data are important for understanding impacts to food webs, serving as an indicator of system change. We further recommend that studies related to the effects of AIS on lake ecosystems and fisheries continue to be supported, beyond the work of the Large Lake program. Data and samples from this program have been used by researchers to quantify the impacts of zebra mussels and spiny waterflea on these lakes (Kerfoot et al. 2016; Rantala et al. 2022; Bethke et al. 2023). Additionally, there are many unexplored applications of zooplankton data that will increase understanding of these important ecosystems.

INTRODUCTION

Minnesota's nine largest natural Walleye lakes (>10,000 ha) are some of the most precious freshwater resources in the state. Historically, they contribute to ~ 40% of the statewide Walleye harvest and act as important fishing waters to Minnesota recreationalists (MNDNR 1997). In 1983, the Minnesota Department of Natural Resources (MNDNR) Section of Fisheries established the Large Lake Monitoring Program (LLP) which consists of annual collection, analysis, and reporting of fish population data to document long-term population trends, develop management recommendations, and engage in public outreach (MNDNR 1997). The sampling protocols were standardized to meet the LLP objectives and are further described in the Large Lake Sampling Guide (Wingate and Schupp 1984). The LLP has primarily focused on Walleye (*Sander vitreus)*, Yellow Perch (*Perca flavescens)*, and Northern Pike (*Esox lucius)* populations, but the Section continues to enhance fisheries assessments by including innovative methods and additional species in their programs.

As the LLP evolved, information from other (non-fish) trophic levels were incorporated to support the fish population monitoring. Crustacean zooplankton (hereafter zooplankton), for example, were collected periodically from some of the Large Lakes for over 20 years. Zooplankton are aquatic microorganisms that link lower (e.g., phytoplankton) and higher (e.g., fish) trophic levels in lakes. Zooplankton are useful indicators of lake productivity as their populations respond to algal dynamics and are a conduit of energy transfer from the lower to upper compartments in aquatic food webs (Rudstam et al. 1993; Johnson and Kitchell 1996, Gamble et al. 2011). Zooplankton are also subject to top-down forces, as fish species are zooplanktivorous either in early stages or throughout their life history (e.g., Cisco *Coregonus artedi*, Yellow Perch *Perca flavescens*, Walleye *Sander vitreus*; Mathias and Li 1982; Mills et al. 1986; Johnson and Kitchell 1996; Hoxmeier et al. 2004; Gamble et al. 2011; Uphoff et al. 2019; Gatch et al. 2021). Zooplankton are highly responsive to environmental change due to their size and short generation time (Rusak et al. 2002; Olden et al. 2006), so they serve as good predictors for stress-induced changes in fish abundance, growth, and survival.

Aquatic invasive species (hereafter AIS) were introduced to the Laurentian Great Lakes in the early 1800s and have dispersed inland to Minnesota's lakes and rivers within the last four decades (Mills et al. 1993; Escobar et al. 2017). Numerous studies demonstrated the threat of AIS to freshwater ecosystems and their ability to alter trophic interactions with zooplankton. For example, spiny waterflea (*Bythotrephes cederströmii,* synonym *Bythotrephes longimanus)* is known to directly predate on cladocerans, reducing native zooplankton biomass and diversity (Yan et al. 2002, Strecker et al. 2006). Zebra mussels (*Dreissena polymorpha*) are also associated with reductions in native zooplankton abundance and biomass (Karatayev et al.

1997; Higgins and Vander Zanden 2010). With that in mind, a zooplankton monitoring component was established by the LLP when invasive zebra mussels were discovered in Lake Mille Lacs in 2006 and spiny waterflea were discovered in Lake of the Woods in 2007. Systematic data collection and zooplankton monitoring documented AIS-induced change among mid-trophic levels and may potentially detect change in other food web compartments. As a result, a formal zooplankton monitoring program was implemented for nine of the Large Lakes beginning in 2012 (Table 1). The zooplankton program excludes Lake Pepin, which is part of the Upper Mississippi River Long Term Resource Monitoring program and has its own zooplankton monitoring component.

The main goals of monitoring zooplankton in the LLP were to 1) develop a baseline of native zooplankton communities prior to AIS invasion, 2) monitor for the presence of AIS in tow samples, and 3) document the impacts of AIS on native zooplankton over time (Hirsch 2014). In general, fisheries managers and researchers use the zooplankton communities as sentinels to detect ecosystem-level changes to these waterbodies (Burdis and Hirsch 2005, Kerfoot et al. 2016, Burdis and Hirsch 2017, Rantala et al. 2022).

The zooplankton component of the LLP is a collaborative effort between MNDNR Fisheries-Large Lakes Specialists, who collect the samples, and the MNDNR Ecological and Water Resources (EWR) Lake Ecology Unit, which analyzes the samples. Additionally, biologists from Voyageurs National Park and Red Lake Nation DNR are partners on these efforts.

TABLE 1.—Characteristics of nine the Large Lakes in Minnesota. Infestation year of zebra mussels (ZM) and/or spiny waterflea (SWF) is based on the MNDNR infested waters list. Data sourced from MNDNR Infested Waters List [*date accessed Dec 2022*], Fisheries Survey Module, and MPCA. Chl-a = chlorophyll-a, P = total phosphorus.

Lake name	Zooplankton sampling	Infestation year (species)	Area (ha)	Depth (m)		Secchi (m)	Chl-a (µg/L)	P (µg/L)
				Mean	Max			
Cass	2012–2021	2014 (ZM)	6,458	8	37	3.5	7.2	22.6
Kabetogama	2001–2003, 2007–2010, 2013–2021	2007 (SWF)	9,726	9	24	2.4	7.8	37.9
Lake of the Woods [a]	1994–1999, 2007–2021	2007 (SWF), 2019 (ZM)	123,666	8	64	1.1	7.4	29.7
Leech	2012–2021	2016 (ZM)	41,700	6	46	3.1	3.9	16.8
Mille Lacs	2006–2021	2005 (ZM), 2009 (SWF)	51,902	9	13	3.1	3.8	26.4
Rainy [a]	2001–2003, 2007–2010, 2013–2021	2007 (SWF), 2021 (ZM)	18,046	10	49	2.5	3.8	22

				Depth (m)				
Lake name	Zooplankton sampling	Infestation year (species)	Area (ha)	Mean	Max	Secchi (m)	Chl-a (µg/L)	P (µg/L)
Red (Upper only)	1999–2000, 2012–2021	2019 (ZM)	19,425	4	5	1.8	13.8	45.3
Vermilion	2011–2021	2015 (SWF)	15,893	6	23	2.6	6	25.9
Winnibigoshish	2012–2021	2013 (ZM)	22,854	5	21	2.5	5.4	22

[a]Minnesota waters only.

Justification and significance

This report synthesizes zooplankton data collected from the LLP over the last 10 years (2012–2021), when annual sampling was conducted consistently across all lakes. When summarizing individual lakes, we examined historic records prior to 2012 if data were available. In total there have been ~3,730 samples collected and analyzed for zooplankton within the LLP, providing a robust dataset for assessing community changes within each lake and among lakes. Historically, the zooplankton data were summarized in MNDNR Large Lakes annual reports (e.g., Nelson 2022) to complement fish population trends and used in several publications to answer research-guided questions (Burdis and Hirsch 2005; Kerfoot et al. 2016; Burdis and Hirsch 2017; Staples et al. 2017; Hansen et al. 2020; Rantala et al. 2022; Bethke et al. 2023). However, to date there is no syntheses evaluating the long-term dataset of zooplankton for all the Large Lakes within the program. Some topics of interest that we investigated for the current synthesis include:

- How have AIS altered zooplankton community structure?
- Are the current methods sufficient to detect change?
- Which lakes are important in terms of continued zooplankton monitoring?

The goal of this effort is to generally summarize a decade of data, explore the implications of AIS on zooplankton communities, and develop recommendations to maximize the utility of zooplankton sampling for the LLP.

Objectives

1. We described trends in zooplankton production, community structure, and biomass among lakes exposed to one or more AIS (i.e., zebra mussel, spiny waterflea, or both).
2. We examined seasonal variability in zooplankton biomass and compared trends among taxonomic groups and lake invasion status.
3. We described lake-specific trends in zooplankton communities.
4. We estimated minimum sample sizes needed for detecting trends and changes in the zooplankton biomass.
5. For each zooplankton species, we estimated the minimum number of individuals to measure for detecting changes in biomass.

METHODS

Field Protocol. — Whole water column vertical tows were collected by MNDNR Large Lake Specialists and local partners for each lake. All samples were collected using a 30-cm opening, 80-µm mesh plankton net. Vertical zooplankton tows were taken at pre-selected sites within a lake, depending upon lake size and morphology. Sites were chosen based on the deepest locations in the main body of lake and in other significant bays. *Refer to* Table A.2— *Metadata for sampling effort by lake and Figure A.1–A.8 — Site maps by lake.*

At each site, a zooplankton net was lowered from an anchored boat until the cod end (end with sample container) was approximately 0.5 m from the lake bottom. The net was then hauled vertically at 0.5 to 1 m/s until it reached the surface. Samples were rinsed into a plastic Nalgene bottle and preserved with 95–100% reagent-grade denatured ethyl alcohol. Tows were typically collected every two weeks in May and June and monthly from July through October. There were some exceptions to the timing of this sampling, which are noted in the individual lake results.

Laboratory Protocol. — Samples were processed by taxonomists in the MNDNR EWR Biology Laboratory. Sample volumes were adjusted to a known volume (typically between 100–2000 mL) by filtering through 80-*µ*m mesh netting and rinsing specimens into a graduated beaker. Water was added to the beaker to a volume that provides at least 150–200 organisms per 5-ml aliquot. The beaker was then swirled in a figure-eight motion to ensure thorough mixing. A 5-ml aliquot was drawn from each sample using a bulb pipet and transferred to a counting grid. Individual zooplankton specimens were identified, counted, and measured using a dissecting microscope (at 25x magnification) and a computerized image analysis system (called "ZOOPS", developed by the MNDNR). A compound microscope was used to aid in species identification (or the lowest practical taxonomic group, 10–63x magnification). Immature copepods were identified and counted as nauplii or copepodites. Additionally, due to the difficulty associated with identifying diaptomids to the genus level*, Leptodiaptomus minutus* and *Skistodiaptomus oregonensis* were identified and reported at the family level, Diaptomidae. We used taxonomic keys from Brooks (1957), Edmondson (1959), Balcer et al. (1984), Hudson et al. (2003), and Haney et al. (2013).

The samples were also examined for the presence and abundance of spiny waterflea (*B. cederströmii,* synonym *B. longimanus*), zebra mussel (*D. polymorpha*) veligers and a large predatory zooplankton, *Leptodora kindtii*, prior to zooplankton processing as described above. In most cases, the whole sample was examined due to the low abundance and/or larger size for all three species. Total counts were made using a gridded petri dish or counting chamber. All veliger analyses were done using a dissecting microscope (at 20x magnification) with a cross polarized light (Johnson 1995).

Data processing. — Density (number/L), biomass (µg/L), percent composition by number and weight, mean length (mm), mean weight (µg), and total count of each taxon identified in each sample was generated by the ZOOPS analysis system and was recorded in the EWR zooplankton database. Mean weight and biomass estimates were calculated using published length-weight regression coefficients (Dumont et al. 1975; Culver et al. 1985). Individual sample reports (PDF files) and Excel summary reports were submitted to the LLP annually and were generated from this system. The data are archived in the EWR zooplankton database.

We aggregated taxa into 5 major functional groups based on Heiskary et al. (2016): small cladocerans, large *Daphnia* spp. (*Daphnia pulicaria* and *Daphnia galeata mendotae*), *Holopedium*, adult calanoid copepods, and adult cyclopoid copepods (Table A.3). In certain instances, immature copepods were included in the analyses as a sixth group since they are potential food sources for predacious zooplankton. The larger zooplankton, *L. kindtii,* and spiny waterflea were not included in the functional groups or total zooplankton biomass or density estimates because they were inconsistently reported and measured over time. To date, 3 staff members have analyzed the samples, providing a highly consistent and dependable dataset. We used a PERMANOVA (permutational multivariate analysis of variance) to test for an "analyst effect" in the dataset related to species and life stage identification, specifically for differences between the 2 main analysts (Jodie Hirsch and Kylie Cattoor) in the taxonomic resolution of organisms. There was a significant difference between analysts in the identification of adult and immature copepods that explained some of the differences in abundance and biomass among years in the dataset (p <0.01*; Figure 1). The analyst effect, however, was not observed for cladocerans (density; P = 0.94, biomass; P = 0.52). To account for these discrepancies, we applied a correction to copepod density and biomass data from 2020 and 2021 by using estimates based on a linear mix effects model (Table A.4).

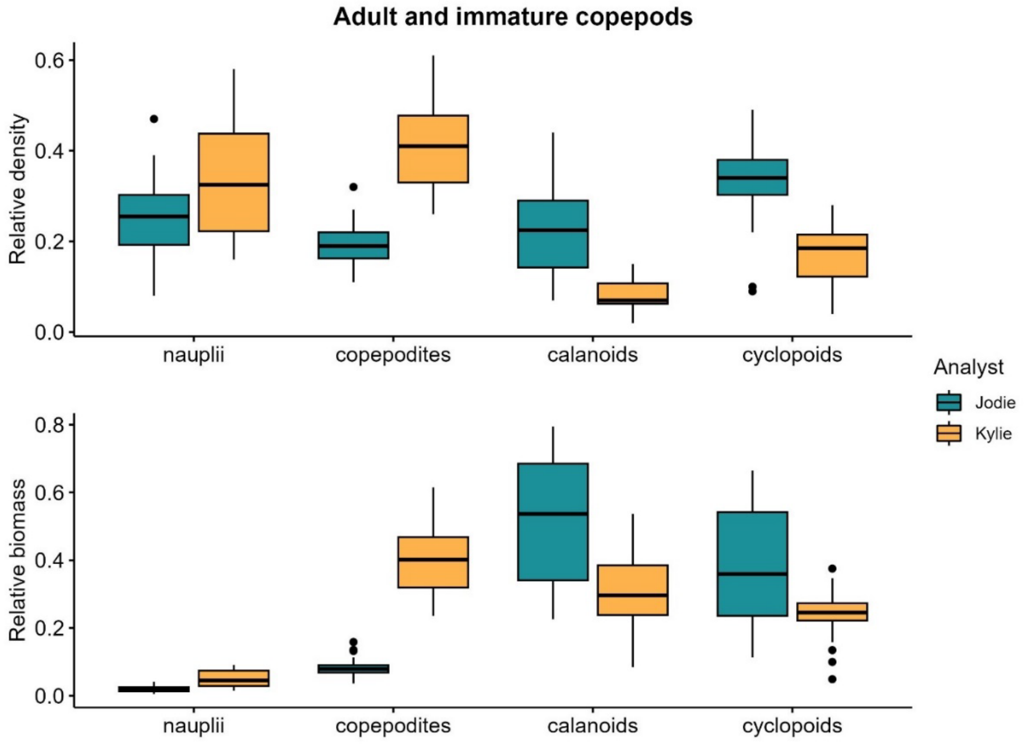


FIGURE 1.—Boxplots comparing relative densities and biomass (proportions of total) of immature (nauplii and copepodites) and adult copepods (calanoid and cyclopoid) by analyst. Blue color represents data analyzed by Jodie Hirsch collected in 2018 and 2019. Yellow color represents data analyzed by Kylie Cattoor collected in 2020 and 2021. Each box represents the interquartile range, with the median represented by a bold horizontal line. Whiskers represent the range of the data, and outliers are plotted as black circles.

Zooplankton trends. —We used data from 2012–2021 (n = 10 years) to compare zooplankton trends across all lakes. We used all available data when examining changes pre- and post-invasion of spiny waterflea and/or zebra mussels for both individual lakes and when comparing invasion scenarios. Annual averages were calculated by taking the mean for each sample date, the mean for each month, and then summarized into year. We restricted our analysis to samples collected from May to October, unless otherwise noted.

In several of the analyses we compared zooplankton metrics among different invasion scenarios related to zebra mussels and spiny waterflea (i.e., uninvaded, zebra mussel invaded, spiny waterflea invaded, or both zebra mussel and spiny waterflea invaded). We assigned invasion status by using information from the MNDNR infested waters list (https://www.dnr.state.mn.us/invasives/ais/infested.html). We considered lakes infested with spiny waterflea the year its presence was confirmed in the lakes. We defined lakes to be ecologically impacted three years after zebra mussels were confirmed in-lake (i.e., three-year lag). We also considered a two-year lag for zebra mussels. These lag times were based on work summarizing zebra mussel population dynamics from multiple systems in the Northern Hemisphere (Strayer at al. 2019). Rainy Lake, listed as infested with zebra mussels in 2021, and Red Lake, listed in 2019, were treated as “spiny waterflea only” and “uninvaded”, respectively, lakes in the analyses. No zebra mussel veligers were found in the LLP samples from Rainy Lake in 2021, however we would expect to find veligers in the future if the zebra mussel population grows in this system. We analyzed the two basins of Lake Vermilion (East and West) separately because they differed in the timing of invasion.

We used non-metric multidimensional scaling (NMDS) to identify patterns in the zooplankton communities among lakes. Taxa were summarized by lake, and years were grouped as either pre- or post-invasion. We applied a 3-year lag before considering a lake invaded by zebra mussels, as mentioned above. We used zooplankton biomass data rather than density and applied the Wisconsin transformation (Oksanen et al. 2022). Biomass is considered a better representation of ecosystem-level effects on other trophic levels and would reduce the influence of abundant but low individual biomass of immature copepods. We used Bray-Curtis dissimilarity estimates to compare zooplankton community structure among lake and pre- and post-invasion groups in a pairwise approach. We then ran a cluster analysis and a permutational multivariate analysis of variance (PERMANOVA) test to identify groups or clusters of lake-pre/post invasion that were similar in community structure and included zooplankton taxa that contributed to the first 50%–75% these patterns. We used the R package *vegan* (Oksanen et al. 2022) for the multivariate analyses.

Secondary production is the formation of heterotrophic biomass through time. It integrates multiple metrics of population success, including birth rate, death rate, fecundity, growth rate, and reproduction (along with other measures), making it a holistic way to measure changes to populations and communities (Benke 1993). We modelled zooplankton production using the size-frequency method (Guerrero and Rodriguez 1994). Many zooplankton are parthenogenetic, and most can have multiple broods over the summer, so following a cohort is not possible. The size-frequency method uses population size-demographics to recreate an average cohort for each summer using length data from individual zooplankton taxa. Length data were converted to biomass as described in a previous section. We made a temperature-specific correction for generation time using a published model (Gillooly 2000) and used modelled daily temperature profile data (Corson-Dosch et al. 2023). We modified code from Cross et al. (2011) for the analyses, which uses bootstrapping (1,000 iterations) to estimate annual production and error for each taxon-lake-year combination by choosing random subsamples, with replacement, on each date. For within-lake trends in zooplankton production, we grouped cladocerans and calanoid copepods together as “grazing zooplankton” for one analysis and tested trends in cyclopoid copepods separately, as “predatory zooplankton”. We used a non-parametric, monotonic trend analysis (Mann-Kendall test, 1-sided) to test for declines in production and estimated the slope of the trend using a Sen’s slope estimate. We used the *trend* package in R for these analyses (Pohlert 2023).

We assessed the impact of zebra mussels and/or spiny waterflea on native cladoceran, calanoid, and cyclopoid copepod production with a generalized linear mixed-effects model. We included random variables for each lake and each year. The model was fit using the R package *lme4* (Bates et al. 2015). We used Akaike Information Criterion for model selection. We re-ran the selected model using restricted maximum likelihood (REML) to obtain unbiased estimates. We plotted the model results using simulated data from the final models, holding random variables constant. We estimated 95% confidence intervals via bootstrapping (250 iterations) using the bootMer() function in that same package. We log-transformed the response (production, $\mu g\ L^{-1}\ d^{-1}$) to fit the data to a Gaussian distribution.

Seasonal variability in zooplankton communities is an important consideration in the context of food web dynamics. To explore the impacts on seasonal patterns of native cladoceran (separated into Large *Daphnia* spp. and small cladocerans), calanoid, and cyclopoid copepod biomass, we used a generalized additive model. We estimated taxon-specific biomass using published length-mass relationships, as described above. To account for seasonal asynchrony related to latitudinal position of the lakes (46.226° N –49.020° N, Lake Milles Lacs and Lake of the Woods, respectively), we modelled time using both day-of-year and cumulative degree days. We used modelled water temperature data to estimate cumulative degree days in base 0 °C (Corson-Dosch et al. 2023). We defined invasion status as described above. We included a random effect for lake and a factor-smooth for a time-invasion status interaction. We log-transformed the response (biomass, µg/L) to fit the data to a Gaussian distribution. We used AIC and examination of modeling-checking functions to select the final model. We used the R package *mgcv* to build the GAM (Wood 2011, 2017; Wood et al. 2016). We re-ran the selected model using REML to obtain unbiased estimates. We plotted the results of the model by simulating the data using the REML models, holding the random variables constant, and presenting biomass estimates as a percentage of the modeled median biomass of uninvaded lakes through the time series. We plotted the model results this way to generalize the results for all lakes, instead of plotting each lake separately.

We examined lake-specific trends in zooplankton communities in several ways. We quantified annual zooplankton density and/or biomass (total or average) as a time series. We calculated the Theil-Sen slope based on daily zooplankton estimates and tested for a single change point in the time series by using the Pettitt’s test. Finally, we visualized zooplankton biomass and production by functional groups alongside spiny waterflea and/or zebra mussel annual densities. See above for more detail on secondary production calculations. We performed a similar NMDS for each individual lake. We used zooplankton density data and applied either the Wisconsin or square root transformation to the dataset. We used Bray-Curtis dissimilarity estimates to compare zooplankton community structure among years. In addition, taxonomic diversity for each lake was calculated using the Shannon diversity index. We then tested for changes in diversity across years using analysis of variance (ANOVA).

Data analysis to assess sample effort and efficiencies. —We restricted our analyses to data collected from 2012– 2021 (n = 10 years). To assess zooplankton measurement effort needed for biomass estimates, we used length measurements and dry weight estimates across all samples to explore variation in length and dry weight for individual taxa. The following formula was used to calculate the number of measurements needed (*n*) given an estimate of the standard deviation (σ) to have 95% confidence ($Z = 1.96$) that the observed average length was within 10% of the true average length (d = 0.1*mean length of all individuals in the dataset):

$$n = \frac{Z^2\sigma^2}{d^2}$$

Nauplii and copepodites made up ~ 38% of all zooplankton individuals counted in the LLP samples, indicating they made a large contribution to the time spent processing samples, and could, through their number, have significant impacts on estimates of total biomass. With this in mind, we explored total sample biomass estimates under three scenarios of measurement effort (n = 2,786 samples); 1) the same number of individuals measured in all taxa, 2) measuring a specified number of nauplii, plus all individuals of other taxa, and 3) measuring a specified number of nauplii and copepodites, plus all individuals of other taxa. The resulting estimates were then compared to the observed total sample biomass.

For investigating levels of sampling effort necessary for zooplankton monitoring in the future, we conducted power analyses for detecting changes in average annual total biomass across all samples from May to September within each lake. To quantify uncertainty in annual average total biomass estimates, the biomass data were log-transformed and modeled as a function of month and site with a random year effect, representing the average annual zooplankton biomass. The analytic standard error estimates for the year effects from the mixed effect model were used to represent uncertainty in annual biomass estimates (Figure 2). We then ran a power analysis with the model to estimate how uncertainty in annual total biomass estimates changes with reduced or increased sampling effort.

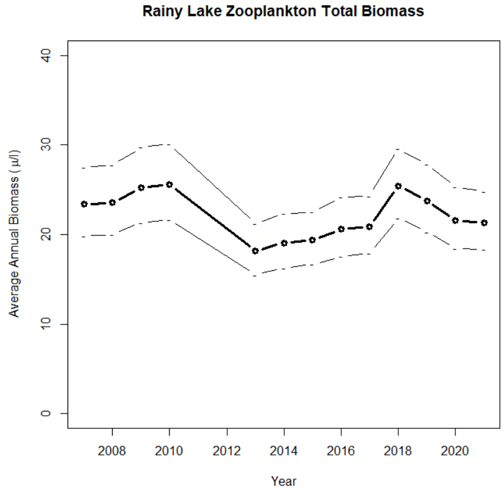


FIGURE 2.—Example showing temporal changes in total biomass for Rainy Lake 2007–2021 with 85% confidence intervals representing model precision (non-overlapping confidence intervals are different at the α = 0.05 level).

To evaluate how reduced sampling within each month affected the power to detect changes in biomass, the data were bootstrapped 2000 times by taking *n* samples at random from each month/year combination for *n* = [3, 5, 7, 9 samples] representing varying levels of monthly sampling effort. For each level of *n*, this generated 2000 data sets at each sampling effort level to which the model was re-fit, and the Year Effect estimates and their estimated SEs were recorded. The precision of Year Effect estimates for reduced sampling effort levels was calculated by averaging the analytically derived SE estimates from each of the 2000 model fits, which was then used to calculate power for detecting a 30–50% decrease in biomass in a 1-sided test (Figure 3).

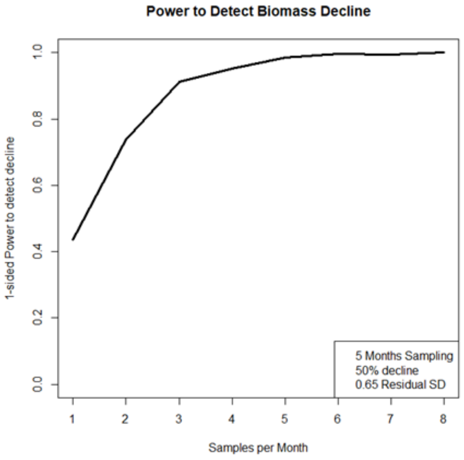


FIGURE 3.—Example output of a power analysis to determine the number of samples per month needed in individual lakes to detect a 50% decrease in zooplankton biomass between years.

All analyses were done in the statistical software program R (R Development Core Team 2009), and use of additional R packages was mentioned above where relevant. Figures were produced using the package ggplot2 (Wickham 2016).

RESULTS & DISCUSSION

Programmatic Summary of Zooplankton Communities

A total of 33 zooplankton taxa (including 1 invasive) were identified in the nine Large Lakes with an average of 21 taxa/lake (Table A.3). Cyclopoid copepods in Lake Mille Lacs were identified only to order (Order Cyclopoida) for continuity with previous time series data. We consolidated certain taxa into larger groups when we found redundancy (e.g. *Alona* spp. and *Alona setulosa* were all considered *Alona* spp.). A detailed summary of the number of samples and individuals collected in the Large Lake Program can be found in Table A.2 and A.3.

From 2012–2021, zooplankton density and biomass varied both within and among lakes (Table 2). Red Lake, Lake Mille Lacs, and Rainy Lake had the lowest average densities (<15 individuals/L) and biomass (<41 µg/L) in the time series. All lakes are primarily dominated by copepods and their immature forms (up to 89% relative abundance). Interestingly, Red Lake with the lowest density and biomass, had the largest proportion of cladocerans (~46% relative abundance).

TABLE 2.—Summary of native zooplankton collected from 2012–2021 in Minnesota's nine Large Lakes. Lakes are ordered by increasing mean density.

	Density (#/L)		Biomass (µg/L)		
Lake	Range	Mean	Range	Mean	n
Red	0.3–55.7	7.9	1.1–104.4	17.8	100
Mille Lacs	0.8–63.7	13.5	2.7–160.8	34.5	74
Rainy	2.4–43.9	13.8	6.8–173.8	40.3	73
Cass	8.3–55.4	22.7	16.7–126	69.0	68
Lake of the Woods	3.3–68.6	23.6	4–183.6	66.1	67
Kabetogama	3.3–193	25.0	9.9–1793.6	98.9	61
Leech	13.3–49.5	27.6	25.5–169.3	78.1	73
Vermilion	16.7–123.8	40.7	25.2–276.1	111.1	73
Winnibigoshish	12–117.2	58.2	18.1–370.6	139.9	68

Impacts of AIS

Since 2007, spiny waterflea invasions were confirmed in 5 of the Large Lakes (Figure 4). We determined qualitative levels of spiny waterflea abundance based on a univariate cluster analysis (with the number of clusters; k=3). We designated lake infestation status as follows; critical (1–27 number/m^3), severe (28–121 number/m^3), and extreme (>121 number/m^3). All spiny waterflea lakes reached *severe* level densities during year 0–4 of known infestation. Lake of the Woods and Rainy Lake reached *extreme* level densities by year 8–11. The spiny waterflea densities are undoubtably underestimates because plankton samples were collected during daylight hours. Like many zooplankton, spiny waterflea exhibit diel vertical migration behaviors, and predatory zooplankton densities have been documented to be ~775% greater at night compared to daytime densities in freshwater lake systems (Doubek et al. 2020).

Given that the timing of detection and magnitude of spiny waterflea densities varied over time, it is not surprising that native zooplankton impacts also varied among lakes. In general, there is an inverse relationship between spiny waterflea and native zooplankton densities and biomass (Figure 5a, 5b). We observed a significant decline in native zooplankton density and biomass post-invasion in spiny waterflea lakes (Table A.5). These included declines in the major functional groups (i.e. cladocerans, cyclopoid, and calanoid copepods). Spiny waterflea consume small-bodied, slow, herbivorous cladocerans and are known to reduce species richness, biomass, and abundance of this group (Yan et al. 2001; Boudreau and Yan 2003; Strecker and Arnott 2008). As reported from other studies (Kerfoot et al. 2016, Cutter et al. 2023), spiny waterflea impacts on copepods were varied, with declines in cyclopoids and immature copepods demonstrated in some systems.

As of 2022, zebra mussels were confirmed in five of the Large Lakes based on presence of veligers, adults, or both. Veligers are monitored by collection in zooplankton tows, which indirectly provide information on the in-lake zebra mussel populations. In general, we observed a wide range of densities that vary among year and lake especially when densities are low (Figure 6). Veliger abundances typically peak in mid-June thru July and are temperature dependent (water temperatures reach ≥ 60°F before veligers appear; Montz et al. 2021). Lake of the Woods and Red Lake had extremely low densities of veligers (~ 0.001–0.10 individuals/L) and were only found in select locations and during some sample events in their respective lakes. To date, the location and presence of adult zebra mussels in Lake of the Woods and Red Lake are unknown. Only the population in Lake Mille Lacs is surveyed regularly as part of the Large Lake Monitoring program. Jones and Montz (2020) noted that while veligers initially increased with adult density, veliger density was not a reliable predictor of adult abundance over time. We cannot infer the long-term dynamics of zebra mussel adults from veliger densities, which makes understanding their impacts challenging (Strayer et al. 2019).

In general, zebra mussel-infested lakes in this study are still in their 'early’ invasion years, so the impacts of zebra mussels vary among lakes. Lakes Winnibigoshish, Mille Lacs, and Cass have the greatest densities of zebra mussel veligers (~10–266/L) and data show inverse relationships between veliger and native zooplankton densities (Figure 7a, 7b). The other lakes, such as Lake of the Woods, Leech Lake, and Red Lake, will likely require more time to detect noticeable patterns, given the short length of time zebra mussels have been present in those lakes. We observed a significant increase in overall zooplankton density post-invasion by zebra mussels, but no change in overall zooplankton biomass (Table A.5), when we pooled data from all the lakes. For calanoid copepods, we detected a significant increase in both density and biomass post-invasion in lakes with zebra mussels.

Interestingly, the post-invasion zooplankton communities in all the lakes (except for Lake Winnibigoshish) are changing in similar ways (shifting towards the northwest quadrant of the ordination, Figure 8) with reductions in *D. galeata mendotae*, diaptomid, and *Diacyclops bicuspidatus thomasi* biomass. The cluster analysis identified two zooplankton community clusters from the Bray-Curtis dissimilarity matrix calculated from taxa biomasses for eight of the nine lakes (P <0.01; Figure 8), and pre- and post-invasion patterns are consistent among the lakes. Lake Mille Lacs was excluded from the analysis due to a lower resolution in adult cyclopoid taxonomy compared to the other lakes. Certain lakes show similar zooplankton communities, possibly related to their geographic proximity to one another (i.e. Leech and Cass). However, other lakes did not (e.g., Rainy and Kabetogama) and fell into distinctly different community clusters. Interestingly, Rainy and Kabetogama have similar slopes and trajectories from pre- to post-invasion. It isn't clear why lakes were grouped into the two clusters, i.e., why the communities of certain lakes more alike than others? It is likely that zooplankton communities are shaped by multiple lake characteristics, including habitat, food resources, predation, lake morphometry, and/or other factors (Brooks and Dodson 1965; Vanni 1987; Dodson et al. 2009). Changing climate and increased climate variability may also be a factor in the patterns we saw, even during this relatively short time series dataset. We will continue to monitor shifts in community structure overtime as more post-invasion data become available for each lake system. It is possible that zooplankton communities in these lakes will 'stabilize' or 'recover' once they have been exposed to the invasive species for long periods of time and possibly shift toward a community of zooplankton that is less sensitive or resistant to invasive species impacts (Strayer 2006; Karatayev et al. 2023).

Taxon-specific responses. —We found a varied response of the daily production of native zooplankton groups across the lakes under different invasion scenarios compared to production in lakes with neither zebra mussels or spiny waterflea (labeled as "Neither" in plots) after accounting for variability related to lake identity or annual differences (Figure 9a). Small cladoceran production, but not production of large *Daphnia* spp., was reduced in the presence of spiny waterflea and both spiny waterflea and zebra mussels (P <0.001; Figure 9b). Calanoid production was reduced in the presence of zebra mussels or spiny waterflea (P <0.05), and cyclopoid production declined when either invasive invertebrate was found, including together (P <0.05). We did not test for differences in production between lakes with different combinations of the two invasive invertebrates.

The varied relationships of native zooplankton production to the two invasive invertebrates are likely due to a couple of different processes. Declines may be due to competition with zebra mussels (Strayer et al. 1999; Miehls et al. 2009) or spiny waterflea (Kerfoot et al. 2016) for food, and/or predation, especially of small cladocerans, by spiny waterflea (Lehman and Cáceres 1993; Barbiero and Tuchman 2004; Kerfoot et al. 2016). Zebra mussels may also consume copepod nauplii, reducing population size (Vanderploeg et al. 1993; Schulz and Yurista 1999; Dumitru et al. 2001). Additionally, some taxa or early life stages may be vulnerable to consumption by zebra mussels due to their size (MacIsaac et al. 1991, 1995; Pace et al. 1998). Finally, it is possible that in lakes with spiny waterflea, mate limitation is reducing the production of overwintering life stages via sexual reproduction (Kerfoot et al. 2016).

Seasonal patterns of change. —We found that not only do zebra mussels and spiny water flea have impacts on native zooplankton production integrated across the summer, but there are also impacts on biomass that vary within the open water season (Figure 10, Table A.6). In general, zooplankton biomass was reduced in the presence of either or both invasive invertebrates relative to the average summer biomass in non-infested lakes. Reduction of cladoceran and cyclopoid biomass (inferred from effect size, Table A.7) was greater when both invasives were present relative to lakes with single-species invasions. There was also a time (measured in cumulative degree days, base 0 °C)-invasion status interaction, so that the magnitude of the effects of the invasives varied throughout the summer. At some points, there was no discernable effect of the presence of the invasive species. For example, in late spring/early summer, calanoid biomass is the same, regardless of invasion status of the lakes. It is worth noting that the best-fit model for seasonal calanoid biomass included time as represented by day-of-year, not cumulative degree days. We presented the calanoid model results with time as cumulative degree days, however, for consistency among the seasonal plots.

The seasonal pattern of the decline in biomass was similar among all zooplankton functional groups, with groups maintaining a smaller magnitude peak in biomass during early summer, although the timing may be shifted (e.g., calanoid plot, Figure 10). The modelled seasonal patterns of these groups indicate some increases in biomass nearing the end of the monitoring period. Presumably, the declines in biomass are via the same mechanisms that caused the decline in daily production, and in fact, biomass is an important component of production (Benke 1996). Interestingly, native zooplankton biomass was reduced in spiny water flea infested lakes in late spring and early summer, before the predatory zooplankton is detected in the zooplankton samples. It is possible that these populations are experiencing a predator-driven Allee effect, like other predator-prey systems (Gascoigne and Lipcius 2004; Kramer and Drake 2010), although this has not been documented where *Bythotrephes* is an invasive predator. However, more detailed analyses should be explored related to this effect.

Lake-Specific Summaries of Zooplankton Trends

Cass Lake (04003000) Beltrami Co.

There was an overall decline in zooplankton community density, biomass, and production over the last 10 years in Cass Lake (Figure 11; Thiel-Sen slope= -0.96 number L^{-1} y^{-1} and –5. μg L^{-1} y^{-1}, *P* <0.001* for density and biomass; -1.4 μg L^{-1} d^{-1} y^{-1} and -1.1 μg L^{-1} d^{-1} y^{-1}, *P* <0.05 for each, for grazing and predatory zooplankton production, respectively. Zebra mussel veligers were first detected in 2014 and were observed as high as ~45 veligers/L by 2016. After the first year of invasion, zooplankton biomass declined from 52 μg/L to 32 μg/L and has not recovered since (Figure 12; 34.2 μg/L as of 2021). This decline, however, was only marginally statistically significant (Pettitt's test for single change-point detection; *P* = 0.07). From 2012 through 2021, zooplankton community production ranged from 122–33.1 μg L^{-1} d^{-1}. Two temporal clusters were identified in the zooplankton community based on taxa and density (2012, 2020–2021 and 2013–2019; Figure 13). There was a significant increase in nauplii and *Tropocyclops prasinus mexicanus* in 2012, 2020–2021 compared to 2013–2019. D*iacyclops bicuspidatus thomasi* and diaptomids are the primary copepods in Cass Lake. Calanoid and cyclopoid copepods appear to be the most impacted from zebra mussel invasion. However, we did not observe a significant decrease in diversity overtime (Figure 14; *P* = 0.81).

Lake Andrusia (04003800), which is connected to Cass Lake, is sampled annually in May (2012–2021). While this waterbody tends to have higher zooplankton biomass than Cass Lake and is a targeted site for walleye fry stocking efforts, the most recent years a dramatic decline in the abundance (-62%) and biomass (-83%) of zooplankton was observed (Figure 15).

Sample Recommendations. —We will continue to sample Lake Andrusia in May on an annual basis. Historically, Cass Lake has been sampled at four sites from May to September and occasionally into October. Based on the power analysis, we suggest reducing sampling to two sites per visit with two visits during the months of May and June and one visit during the months July–September (refer to Table A.1and Figure A.9).

Lake Kabetogama (69084500) St. Louis Co.

We were able to include additional data collected from 2001–2003 and 2007–2010 in our analyses. There has been an overall significant decline in zooplankton density, biomass, and production in the last 20 years (Figure 16; Thiel-Sen slope= -0.35 number L^{-1} y^{-1} and -0.47 μg L^{-1} y^{-1}, $P < 0.001$* for density and biomass; –3.5 μg DM L^{-1} d^{-1} y^{-1}, $P < 0.05$*, and –2.6 μg DM L^{-1} d^{-1} y^{-1}, $P < 0.01$*, for grazing and predatory zooplankton production, respectively). Spiny waterflea was first detected in 2007 and has exhibited robust densities for more than a decade. While there are data gaps, both the zooplankton density and biomass declined following spiny waterflea invasion (Figure 17). Zooplankton production ranged from 9.7–148.8 μg L^{-1} d^{-1}. Four temporal clusters were identified in the zooplankton community based on taxa and density and showed significantly distinct communities pre-, during, and post- spiny waterflea invasion (Figure 18; $F = 4.25$, $P < 0.01$*). *Daphnia galeata mendotae*, copepodites, *Tropocyclops prasinus mexicanus, Diacyclops bicuspidatus thomasi* were the main taxa driving this pattern. While small cladocerans were not mentioned as strong contributors, community diversity declined following invasion, which included many of these taxa (Figure 19; $F = 10.29$, $P < 0.01$*). There are concerns zebra mussels will establish in Lake Kabetogama as they were recently reported in Black Bay of Rainy Lake in 2021. While the spiny waterflea-zooplankton interactions are considered 'stable' with no major shifts in the last 10 years, we are interested in tracking additional synergistic impacts related to spiny waterflea, zebra mussels, and zooplankton in the coming years.

Sample Recommendations. —We will continue to sample Lake Kabetogama on an annual basis. Historically, sample collection has been coordinated with Voyageurs National Park (VNP). Collection includes sampling at one site with two visits during the month of June and one visit in May and in July–September (Table A.1). VNP recently received project funding for a 'Zooplankton Vulnerability Assessment to AIS' to conduct more intensive sampling. Given the unknown status of the zebra mussel invasion and other projects in progress, we recommend no changes to sampling in Lake Kabetogama at this time.

Lake of the Woods (39000200) Lake of the Woods/ Roseau Co.

We were able to include additional data collected from 1994–1998 and 2007–2012 into our analysis. Samples collected pre-2007, 2010, and 2012 were not included in the trend analyses due to low sample effort (Table A.2; <10 samples/season). Additionally, samples collected from 2008–2012 used a 150-μm mesh plankton net that likely underestimated immature copepod abundance during the period.

There was an overall significant increase in zooplankton density and a significant decrease in zooplankton biomass since 2007 (Figure 20; Thiel-Sen slope= 1.06 number L^{-1} y^{-1} and –2.91 μg L^{-1} y^{-1}, r, $P < 0.001$*). The increase in total zooplankton density occurred within the last two years based on many immature copepods found in Muskeg Bay and Long Point. However, due to their size, the total zooplankton biomass did not change dramatically for those years and is the preferred metric for assessing long term trends in Lake of the Woods. Spiny waterflea was first detected in Lake of the Woods in 2007 with maximum densities >163 individuals/m^3 observed in August of 2018. The annual mean density ranged between ~4–20 individuals/m^3 over the last 10 years. The annual mean biomass of zooplankton was reduced from 145 to 62 μg/L within the first year of spiny waterflea invasion and has stayed between 32–73 μg/L annually since then (Figure 21). Zooplankton production was not estimated for Lake of the Woods, due to lack of available (observational or modelled) temperature data for the entire time series. Future work will use a combination of observational data and modelled temperature values utilizing surficial temperature collected by satellite. The zooplankton community shifted in the last two years due to the abundance of immature copepods, appearing more similar to pre-AIS invasion years Figure 22; apart from 1995). While two clusters were identified based on taxa and density, they were not significantly different ($F = 1.73$, $P = 0.13$). This was also true for diversity, based on the Shannon diversity index Figure 23; $P = 0.23$). Small cladocerans were significantly reduced following spiny waterflea invasion (Pettitt's test for single change-point detection in 2008, $P < 0.05$*), but have increased since 2019.

More recently, zebra mussel veligers were found in Lake of the Woods at very low densities (<0.36 veligers/L). If the zebra mussel population becomes established, we would expect to see an increase in veliger density overtime. Typically, this can take anywhere from three to five years before zebra mussel veligers become more abundant (Strayer et al. 2019). At this point, zooplankton do not show compounding impacts from both invasive species. Since zebra mussel invasion, native zooplankton (specifically Cladocera) density has increased, suggesting a potential recovery for the group over the last three years.

Sample Recommendations. —We will continue to monitor zooplankton in relation to AIS expansion on an annual basis. Based on the power analysis, we will maintain zooplankton collection at three sites May thru September. Samples will be collected twice monthly in

May/June and reduced to one sample monthly from July to September (refer to Table A.1 and Figure A.10).

Leech Lake (11020300) Cass Co.

There was an overall significant decline in zooplankton density, biomass, and production over the last 10 years (Figure 24; Thiel-Sen slope= -1.19 number L^{1} y^{-1} and -6.53 µg L^{-1} y^{-1}, *P* <0.001* for density and biomass; -1.6 µg DM L^{-1} d^{-1} y^{-1}, *P* <0.01* for grazing zooplankton production.) The trend for predatory zooplankton was not significant (*P* = 0.11). In 2016, zebra mussel veligers were first detected in Leech Lake at low abundance (<0.05 veligers/L). After five years, the population started to expand, and densities were observed as high as ~9 veligers L^{-1} in 2021. Zooplankton showed reductions in both density (22 to 14 number/L) and biomass (64 to 42 µg/L) within the first year of invasion (Figure 25). Since 2020, the mean biomass of zooplankton dropped below 28 µg/L with a significant increase in small-sized immature copepodites and a significant decrease in adult calanoid and cyclopoid copepods as well as large *Daphnia*. These include taxa such as diaptomids, *Diacyclops bicuspidatus thomasi* and *Daphnia galeata mendotae* (Figure 26). Zooplankton diversity has significantly decreased (Figure 27) and community production has ranged from 13.8–45.3 µg DM L^{-1} d^{-1} over the sampled years.

Sample Recommendations. — We will continue to sample Leech Lake on an annual basis. We will maintain zooplankton collection at five sites May thru October (Table A.1). We recommend sampling twice in May and June. The zebra mussel population continues to expand in Leech Lake and will be monitored seasonally in the coming years. A power analysis may be revisited once the zebra mussel population has stabilized.

Lake Mille Lacs (48000200) Mille Lacs Co.

The current analyses included data from 2006–2021, and we found a significant decline in zooplankton density, biomass, and production over the last 15 years (Figure 28; Thiel-Sen slope= -1.27 number L^{-1} y^{-1} and -2.68 µg DM L^{-1} y^{-1}, *P* <0.001* for density and biomass; -3.2 µg DM L^{-1} d^{-1} y^{-1}, *P* <0.01*, and -2.4 µg DM L^{-1} d^{-1} y^{-1}, *P* <0.001*, for grazing and predatory zooplankton production, respectively). Pettitt's test for single change-point detection showed a significant change occurring in 2011 for all three zooplankton community metrics ($P < 0.05^*$). Both zebra mussels (since 2005) and spiny waterflea (since 2009) are well established in Lake Mille Lacs. Jones and Montz (2020) describe the population growth of adult zebra mussels and the associated impacts over the course of the invasion. Their analyses show adult density increasing exponentially through 2010 before the population came to an equilibrium. The veliger data from the lake, however, were not strongly correlated to the patterns observed in adult population growth. In the current dataset, annual mean zooplankton biomass declined after 2011, but does not show a strong relationship with the veliger density data (Figure 29). It should be noted that zooplankton monitoring did not begin until 2006, so it is not clear if the "pre-invasion" state of the zooplankton community was captured in the early years of the monitoring. Zooplankton community production ranged from 4.0–99.8 µg DM L^{-1} d^{-1}over the sampled years.

Three main temporal clusters were identified in the zooplankton community based on taxa densities and showed significantly distinct communities pre- and post-invasion (Figure 30). Specifically, there was a significant reduction to several small cladoceran taxa (i.e. C*hydorus* sp*., Diaphanosoma, Ceriodaphnia* sp*., Daphnia retrocurva*) within the first years of spiny waterflea invasion which have yet to recover. In addition, cyclopoids and B*osmina* sp*.* were the next to decline, with significant reductions in 2012 and from 2014–2016. Overall, diversity significantly changed since the invasion of spiny waterflea (Figure 31), primarily due to a loss of taxa in the small cladoceran group, a preferred prey of spiny waterflea, and cyclopoid group.

A more comprehensive food web analysis that includes zooplankton, spiny waterflea, and zebra mussel interactions is found in a recent publication by Rantala et al. (2022).The paper examines trends in water clarity under three invasion scenarios: prior to invasion by either zebra mussels or spiny waterflea, the period after zebra mussels were found in the lake, and the current scenario, where both of these invasive invertebrate species are found in the lake. Zooplankton and zebra mussel data from 2006–2018 and water clarity data from 2000–2018 were used in the analyses. That work found that the simultaneous increase in the zebra mussel and spiny waterflea populations resulted in no net change in water clarity, as algal grazing by native zooplankton was replaced by the filtering of algae by zebra mussels.

Sample Recommendations. —Lake Mille Lacs has a large zooplankton dataset documenting complex food web interactions associated with the co-invasion of zebra mussels and spiny waterflea. While sample effort has fluctuated to accommodate other projects and adaptive management decisions, we suggest continuing to sample annually, but reducing the number of sites from nine per visit to five sites per visit at a frequency of two visits per month in May and June and one visit per month from July thru September (refer to Table A.1 and Figure A.11).

Rainy Lake (69069400) St. Louis Co.

We included additional data collected in 2001–2003 and 2007–2010 into our analysis of the Rainy Lake zooplankton community. There was a significant decline in zooplankton density and biomass over the last 20 years (Figure 32; Thiel-Sen slope = -0.15 number L^{-1} y^{-1} and -0.82 µg DM L^{-1} y^{-1}, P <0.001* for density and biomass; -0.8 µg DM L^{-1} d^{-1} y^{-1}, P <0.01*, and -0.5 µg DM L^{-1} d^{-1} y^{-1}, P <0.01*, for grazing and predatory zooplankton production, respectively). When data from 2003 were removed due to abnormally high values in density and biomass, the Theil-Sen slope decreased however the trend remained significant. Spiny waterflea was first detected in Rainy Lake in 2007. Apart from 2015, when maximum densities were up to 396 individuals/m^3, the annual mean density has been between ~5–13 individuals/m^3 for more than a decade. While there are some data gaps, zooplankton density and biomass were reduced during the initial years of invasion (Figure 33), although this decline was not significant (Pettitt's test for single change-point detection; P = 0.08 and 0.07). Zooplankton community production ranged from 5.1–82.1 µg DM L^{-1} d^{-1} over the sampled years. There was a distinct difference in the zooplankton community based on taxa and density pre- to post-invasion with significant reductions in small cladocerans (e.g. *Bosmina* sp., *Eubosmina coregoni*, *Daphnia retrocurva*; Figure 34). In addition, zooplankton diversity significantly decreased post-invasion (Figure 35).

Zebra mussels were discovered in Rainy Lake in the fall of 2021. While the spiny waterflea-zooplankton interactions have occurred since 2007, we will continue to track additional synergistic impacts related to spiny waterflea, zebra mussels, and zooplankton in the coming years.

Sample Recommendations. —We will continue to sample Rainy Lake on an annual basis in cooperation with Voyageurs National Park (VNP) biologists. Historically, samples were collected at five sites, one of which is sampled at 10-m and 30-m depths in the same vicinity (Table A.1). All sites are visited twice a month (except for site SE7) from May–June and once per month from July–September. VNP recently received funding for a project entitled 'Zooplankton Vulnerability Assessment to AIS' to conduct more intensive sampling. Given the unknown impacts of the zebra mussel invasion in the lake and other projects in progress, we recommend no changes to sampling in Rainy Lake.

Red Lake (04003500) Beltrami Co.

There was an overall increase in zooplankton density and biomass over the last 10 years (Figure 36; Thiel-Sen slope = 0.85 number $L^{-1}y^{-1}$ and 1.69 µg DM L^{-1} y^{-1}, P <0.001*). Grazing zooplankton (but not predatory zooplankton, P = 0.24) production in Upper Red Lake increased over the past decade (Thiel-Sen slope = 1.0 µg DM L^{-1} d^{-1} y^{-1}, P <0.05*), and but production in Lower Red Lake did not change (P = 0.08 and P = 0.19, for grazing and predatory zooplankton, respectively). Zebra mussel veligers were only detected in Red Lake in recent years. They were first found in the upper basin in 2018, so both basins were listed as infested in 2019. To date, veliger densities remain low (<0.11 veligers/L) and the veligers are not found lakewide. Compared to the other Large Lakes, the total zooplankton density and biomass were low in Red

Lake (Figure 37; Table 2). Zooplankton biomass dramatically declined in 2013 and 2014 for unknown reasons and has increased since that time. Zooplankton community production ranged from 1.3–25.0 µg DM L^{-1} d^{-1} in Upper Red Lake and 1.3–21.8 µg DM L^{-1} d^{-1} in Lower Red Lake from 2012–2021. The zooplankton community analysis also detected low species densities in 2013– 2014 (Figure 38). Specifically, there was a significant reduction in *Bosmina* sp., *Chydorus* sp., *Diacyclops bicuspidatus thomasi* and *Daphnia retrocurva* after 2012, but these have slowly recovered since that time. In the last two years, there was an increase in copepodites and *Bosmina* sp. Diversity did change over time, with up to 18 taxa observed in 2021 (Figure 39; F = 2.57, P = 0.15). Calanoids and cyclopoids continue to thrive in Red Lake but should be monitored closely as these two groups can be impacted after zebra mussel invasion.

Sample Recommendations. —We will continue to sample Red Lake annually in cooperation with the Red Lake Nation. The MNDNR and the Red Lake Nation have held a strong working relationship since the 1990s. Based on the power analyses, we will continue to sample at three sites per basin with two visits per month in May and June. It is possible to reduce the number of visits to one per month in July– September (refer to Table A.1 and Figure A.12). DNR will work closely with the Red Lake Nation before implementing any changes. There are additional veliger sampling sites coordinated with the Red Lake Nation and the MNDNR aquatic invertebrate biologist that will continue to be used in the assessment of zooplankton dynamics.

Lake Vermilion (69037800) St. Louis Co.

East Basin. — There was an overall decrease in zooplankton density, biomass, and predatory zooplankton production over the last 10 years (Figure 40; Thiel-Sen slope= -1.31 number L^{-1} y^{-1} and -11.61 µg DM L^{-1} y^{-1}, $P < 0.001$* for density and biomass; -1.9 µg DM L^{-1} d^{-1} y^{-1} , $P < 0.001$* for production). The trend for grazing zooplankton production was not significant (P = 0.07). Spiny waterflea were first detected in the East Basin of Lake Vermilion in 2015. In 2017, maximum densities were observed at 53 individuals/m^3. Annual mean zooplankton biomass declined from 99 to 46 µg/L in 2016 (Figure 41). Zooplankton community production ranged from 8.7–99.0 µg DM L^{-1} d^{-1}. Small cladoceran were most impacted by spiny waterflea invasion. Biomass was <1 µg/L for this group from 2017–2018 and 2020–2021. There was a significant increase ($P < 0.05$*) in *Tropocyclops prasinus mexicanus* and immature copepods in 2020–2021 (Figure 42), but in general, diversity did not change over time (Figure 43, P = 0.08).

West Basin. — There was an overall increase in zooplankton density and biomass over the last 10 years (Figure 40; Thiel-Sen slope= 1.87 number $L^{-1}y^{-1}$ and 1.51 µg DM L^{-1} y^{-1}, $P < 0.001$*). Production declined through time (range 13.8–90.6 µg DM L^{-1} d^{-1}), but the trend was not significant (Figure 41; P = 0.08 and P = 0.24, for grazing and predatory zooplankton, respectively). Although spiny waterflea were found in the East Basin of Lake Vermilion in 2015, it was not until 2018 that they were detected in the West Basin. Since the 2018 detection, spiny waterflea dynamics have been similar in both basins. Maximum spiny waterflea densities were observed at 46 individuals/m^3 in 2021. While there appears to be a 3-year lag between the two basins, the West Basin will likely achieve similar densities and potentially progress more quickly in the next few years. Recently, there were no large shifts in zooplankton density or biomass in the West Basin (Pettitt's test, $P = 0.49$). During the last year included in the time series, 2021, *Tropocyclops prasinus mexicanus* and immature copepods increased, however these trends were not significant (Figure 42; $P = 0.08$). This pattern was observed in the East Basin and might be due to seasonal variability rather than spiny waterflea dynamics. Diversity significantly increased, especially in the last year (Figure 43, $P < 0.05$*). We expect to see a decline in small cladoceran abundance and biomass as the spiny waterflea population grows. Historically, the West Basin has had higher densities of small cladocerans compared to the East Basin, so the impacts of spiny waterflea in the West Basin may be greater by comparison.

Sample Recommendations. —We will continue to sample Lake Vermilion annually at two sites per basin. The West Basin could benefit from an additional sample site if time and resources allow. There will be two visits per month in May–June and one visit per month in July thru September (refer to Table A.1 and Figure A.13). If spiny waterflea become well established in both basins and the zooplankton community does not change, it may be possible to reduce sample collection and/or analyses to every two to three years.

Lake Winnibigoshish (11014700) Cass Co.

The results summarize data collected from Lake Winnibigoshish and Lake Cut Foot Sioux (hereafter included as part of Lake Winnibigoshish). There was a significant decline in the density and biomass of zooplankton over the last 10 years (Figure 44; Thiel-Sen slope= -2.47 number L^{-1} y^{-1} and -12.51 µg DM L^{-1} y^{-1}, $P < 0.001$*). Zebra mussel veligers were first detected in Lake Winnibigoshish in 2012, and the waterbody was listed as infested in 2013. During the initial years of invasion, the annual mean density of veligers was low (< 2 veligers/L; from 2012–2016). Starting in 2017, veliger densities began to increase and were observed as high as ~38 veligers/L by 2021. In 2017, zooplankton biomass declined from 114 to 43 µg DM/L (Figure 45). The zooplankton community production ranged from 33.3–89.3 µg DM L^{-1} d^{-1} over the sampled years, with no trend through time detected ($P = 0.08$ and $P = 0.11$, for grazing and predatory zooplankton, respectively). Two temporal clusters were identified in the zooplankton community based on taxa and density (Figure 46). While the clusters do not align with pre- and post-zebra mussel invasion periods, there appears to be several episodic shifts in the zooplankton community structure that deserve further investigation. Immature copepods, *Diacyclops bicuspidatus thomasi, Daphnia galeata mendotae*, and *Bosmina* sp. represented ~ 70% of the community in Lake Winnibigoshish over the last 10 years. While there was some annual variability, diversity did not significantly changed over time (Figure 47; $P = 0.75$). Calanoids appeared to be impacted the most by zebra mussel invasion. Unlike other functional groups (i.e.

large *Daphnia* spp. and cyclopoids) that have rebounded, calanoid biomass remained below 30 µg/L since 2017.

Sample Recommendations. —Given the zebra mussel population is well established and no large changes to the zooplankton community were observed over the last five years, we recommend shifting to a two- or three-year sample rotation starting in 2023. We also recommend reducing from five sites to four sites in Lake Winnibigoshish (refer to Table A.1 and Figure A.14). We will continue to maintain the site in Cut Foot Sioux Lake. Cut Foot Sioux is a deeply stratified waterbody that flows directly into Lake Winnibigoshish. Samples should be collected during two visits per month in May and June and one visit per month in July–September.

Sample Effort and Efficiencies

Some taxa vary considerably in their measured length and estimated dry weight (Figure 48). Dry weight estimates were relatively more variable than length measurements. Taxa required on average ~30 measurements to be confident (95%) that the average measured length is within 10% of the actual average (95% of all estimates of n are between 13 and 49; Table A.8).

Simulations of total sample biomass where nauplii length estimates were replaced with the taxonomic averages across all samples in the analyses revealed that variation in nauplii length has an extremely small effect on estimates of total sample biomass (Figure 49). Relative to other taxa, variation in nauplii length (median = 191 µm, 95% range between 115 and 316 µm) and dry weight (median = 0.18 µg, 95% range between 0.08 and 0.42 µm) was extremely low. Copepodites were larger and more variable in measured length (median = 434 µm, 95% range between 254 and 799 µm) and estimated dry weight (median = 0.83 µg, 95% range between 0.45 and 3.1 µg) than nauplii. Nauplii are small, with an extremely low dry weight, but also abundant. On average, 37 nauplii were counted per sample. Reducing the maximum number measured to 30 could save on processing time with almost no effect on estimates of total sample biomass (0.1% average deviation with 95% of samples between 0–1.2% deviation). Copepodites also have high counts per sample (29 times on average) but are larger and their length is more variable than nauplii. Simulations revealed that variation in copepodite length has a larger effect on estimates of total sample biomass than nauplii (Figure 49c). Reducing the maximum number of nauplii and copepodites measured to 30 each per sample would have a slightly larger effect on sample biomass (0.9% average deviation with 95% of samples between 0–8%). Using the long-term average for both copepodites and nauplii (i.e., rather than measuring individuals in each sample) also had a larger effect on estimates of sample biomass (3.6% average deviation with 95% of samples between 0–14%).

We therefore recommend limiting the number of nauplii and copepodites measured to 30 individuals in each sample.

SUMMARY & RECOMMENDATIONS

In this report, we synthesized zooplankton data through October 2021 for nine Large Lakes in Minnesota. This work indicates that while both zebra mussels and spiny waterflea impact native zooplankton communities in this group of lakes, the effects are likely driven by complex interactions between food web components and influenced by physicochemical characteristics of the individual lakes. Both invasive invertebrates reduced zooplankton biomass and production for all functional groups (Figures 9a, 10), although differences among lakes increased variability when generalizing the impacts of different invasion status (Figure 9b). The magnitude of the impacts to native zooplankton also varied through the open water season for both invaders (Figure 10). Lake Mille Lacs was the only Large Lake where an abrupt change in zooplankton metrics occurred following invasion (i.e. 2011, Pettitt test, Figure 29). Multiple metrics indicated that the other Large Lakes were impacted, although the change was not as abrupt to be detected the Pettitt test (Table 3). We used comparisons of biomass and production estimates to demonstrate that not only do these invasive invertebrates impact the native zooplankton community, but likely have impacts on other trophic levels. Length of invasion, timing of monitoring in relation to the invasion and complex biotic and abiotic interactions are unique to each lake system and likely influenced the observed effect sizes. We identified efficiencies and provided recommendations to the field-laboratory process using statistical analyses. We provided suggestions to streamline the monitoring program with AIS status in mind for each lake. In general, we find value in continuing to monitor for spiny waterflea and zebra mussel veligers with zooplankton. Additional data and analyses are needed to better explain the dynamics of zooplankton and AIS across and among the Large Lakes.

TABLE 3. — Summary table of Theil-Sen slopes for zooplankton density, biomass, and production in Minnesota's Large Lakes. All values presented were significant ($P<0.05$). NS indicates no significant change. Arrows denote an overall increase or decrease in diversity. Production was not estimated in Lake of the Woods.

		Theil-Sen slope			
Lake name	AIS status	Density (number $L^{-1}y^{-1}$)	Biomass (μg DM L^{-1} y^{-1})	Production (μg DM L^{-1} d^{-1} y^{-1})	Shannon diversity
Cass	ZM	-1.0	-5.5	-2.0	NS
Kabetogama	SWF	-0.4	-0.5	-5.6	↓
Lake of the Woods	Both	1.1	-2.9	NA	NS
Leech	ZM	-1.2	-6.5	-2.7	↓
Mille Lacs	Both	-1.3	-2.7	-6.0	↓
Rainy	SWF*	-0.2	-0.8	-1.4	↓
Red	ZM	0.9	1.7	1.4 (Upper), NS (Lower)	NS
Vermilion (East)	SWF	-1.3	-11.6	-4.2	NS
Vermilion (West)	SWF	1.9	1.5	NS	↑
Winnibigoshish	ZM	-2.5	-12.5	NS	NS

*considered ‘Both’ as of fall 2021

Zebra mussels can drastically change lake ecosystems by altering complex food webs (Karavayev et al. 1997; Higgins and Vander Zanden 2010; Walsh et al. 2016). While we focused solely on pelagic veliger-zooplankton relationships, data from other trophic levels and habitats, including adult zebra mussels, may further explain the temporal and spatial dynamics of zooplankton in zebra mussel lakes. Additionally, we acknowledge the veliger data are not good predictors for assessing adult populations in lakes, congruent with other work (Strayer et al. 2019; Jones and Montz 2020). Interpreting the zooplankton data in the context of invasion impacts could benefit from first, determining if zebra mussels are fully established in the system by including additional monitoring of other life stages and secondly, including lower and higher trophic level information to determine other direct and indirect impacts of zebra mussels in the Large Lakes.

Understanding middle trophic levels provides additional lines of evidence, beyond the fish population surveys, in these lakes of large-scale system change. Data from the Large Lake program were included in multiple studies aimed at better understanding the dynamics of these lakes, including the effects of invasive species on fish habitat, including structural and light habitat (Jones and Montz 2020; Rantala et al. 2022). The data also informed work about AIS impacts on fish population dynamics (Staples et al. 2017; Hansen et al 2020; Honsey et al. 2020) and food web dynamics in these systems (Kerfoot et al. 2016; Rantala et al. 2022; Bethke et al. 2023). Future work will help us better understand the links between invertebrate communities and fish populations.

There are several opportunities to increase the value of the zooplankton monitoring component of the Large Lake program to better support fisheries managers. While it is generally agreed upon that zooplankton are critical to the survival of juvenile Walleye (Krabbenhoft et al. 2023) relatively little work has been done in Minnesota to characterize how influential zooplankton are to Walleye or other key fishes in the LLP (although see Hansen et al. 2020 and Bethke et al. 2023). Work conducted in other large lakes have established these connections between zooplankton and Walleye (e.g. Graham et al. 1992; May et al. 2021) and Yellow Perch (e.g. Dettmers et al. 2003). Additional research should explore the role zooplankton plays in Walleye and Yellow Perch recruitment in the large lakes, particularly given the observed broadscale declines in zooplankton biomass and anticipated increases in phenological mismatches due to climate change.

Additionally, collecting more information about spiny waterflea and *Leptodora* (i.e. length measurements) would enable us to understand the roles of these predators in food web energy dynamics. Confirming and/or monitoring of adult zebra mussel populations for comparison with veliger densities from the zooplankton tows, would allow for us to understand the potential for veliger data to be used as an index of population impacts. There is an opportunity to use zooplankton data to continue learning about the links among environmental and biotic components of the lakes, including fishes. Finally, archiving zooplankton tows, which is not currently done, would allow for animals collected in the present to be used in future work.

North Temperate lakes around the world are rapidly changing from multiple stressors, including climate change, eutrophication, invasive species, and land use change (Williamson et al. 2008; Gallardo et al. 2016; Grant et al. 2021; Jane et al. 2021; Vitense and Hansen 2023), and freshwater systems are experiencing higher rates of biodiversity loss than either terrestrial or marine systems (Jenkins 2003). These disturbances make long-term monitoring programs essential for documenting and understanding ecosystem change (Lindenmayer et al. 2012), as well as evaluating management activities as an important component of adaptive management of fisheries (Walters and Hilborn 1976; Hansen et al. 2015). Robust monitoring programs include flexibility to adapt to new questions, technologies, or methodologies (Lindenmayer and Likens 2009), and zooplankton are an important component of these programs, as they serve as sentinels of change (Papa and Briones 2014).

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DATA AVAILABILITY

Data that support the findings of this study are available from the corresponding author upon reasonable request.

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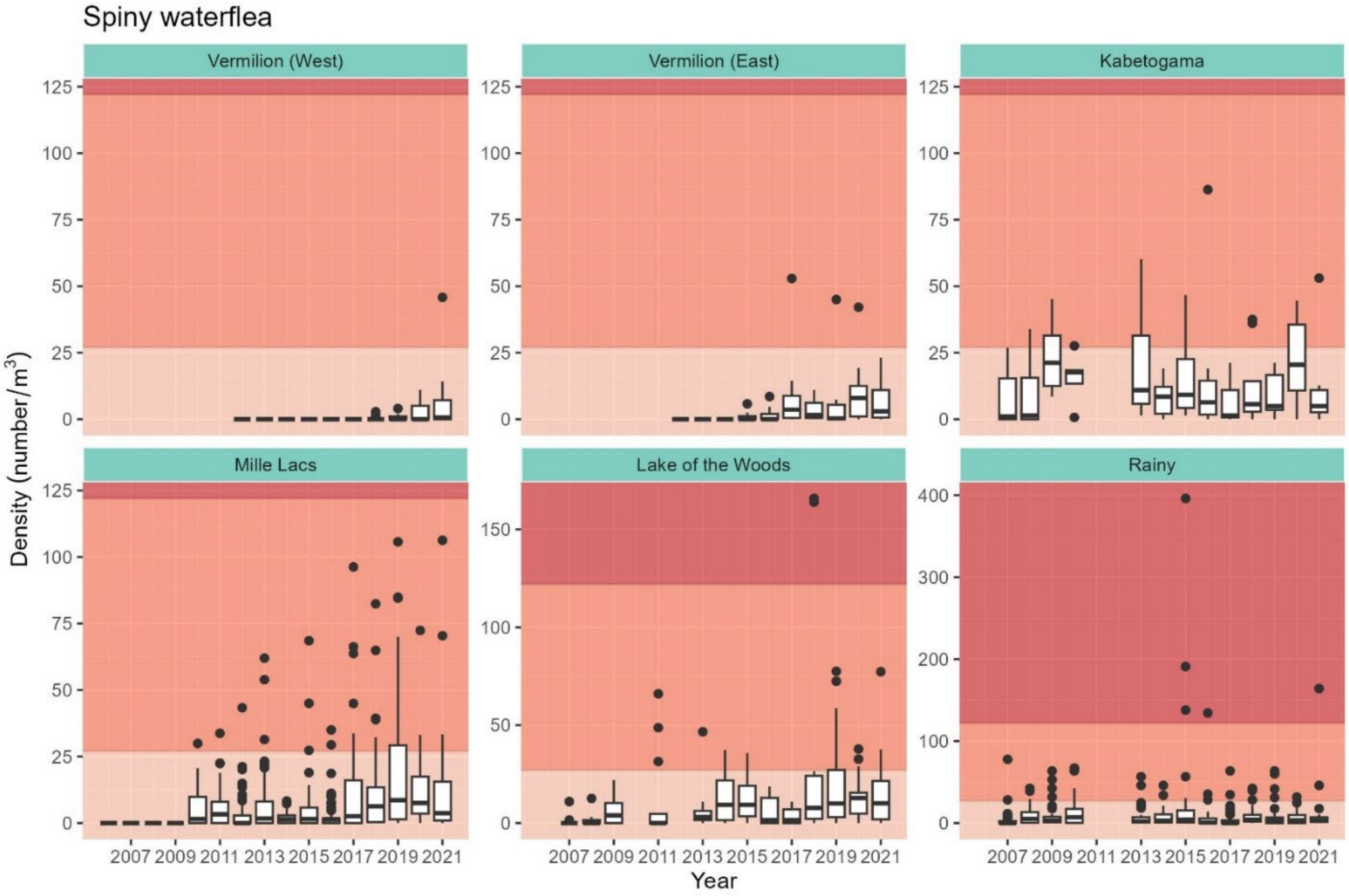


FIGURE 4.—Boxplots comparing daily densities (number/m^3) of spiny waterflea from five large lakes in Minnesota. Lakes are arranged from lowest to highest densities (top left to bottom right) and Y-axis varies by lake. Red shades indicate lake infestation status as either critical (1–27 number/m^3), severe (28–121 number/m^3), or extreme (>121 number/m^3). Data were collected annually from May-October when possible. Lake Vermilion is summarized by basin (East and West). Samples collected from Lake of the Woods in 2008–2012 used a 150 µm sized mesh net (vs. the standard 80 µm mesh). Samples from Lake Kabetogama and Rainy used a 153 µm sized mesh net.

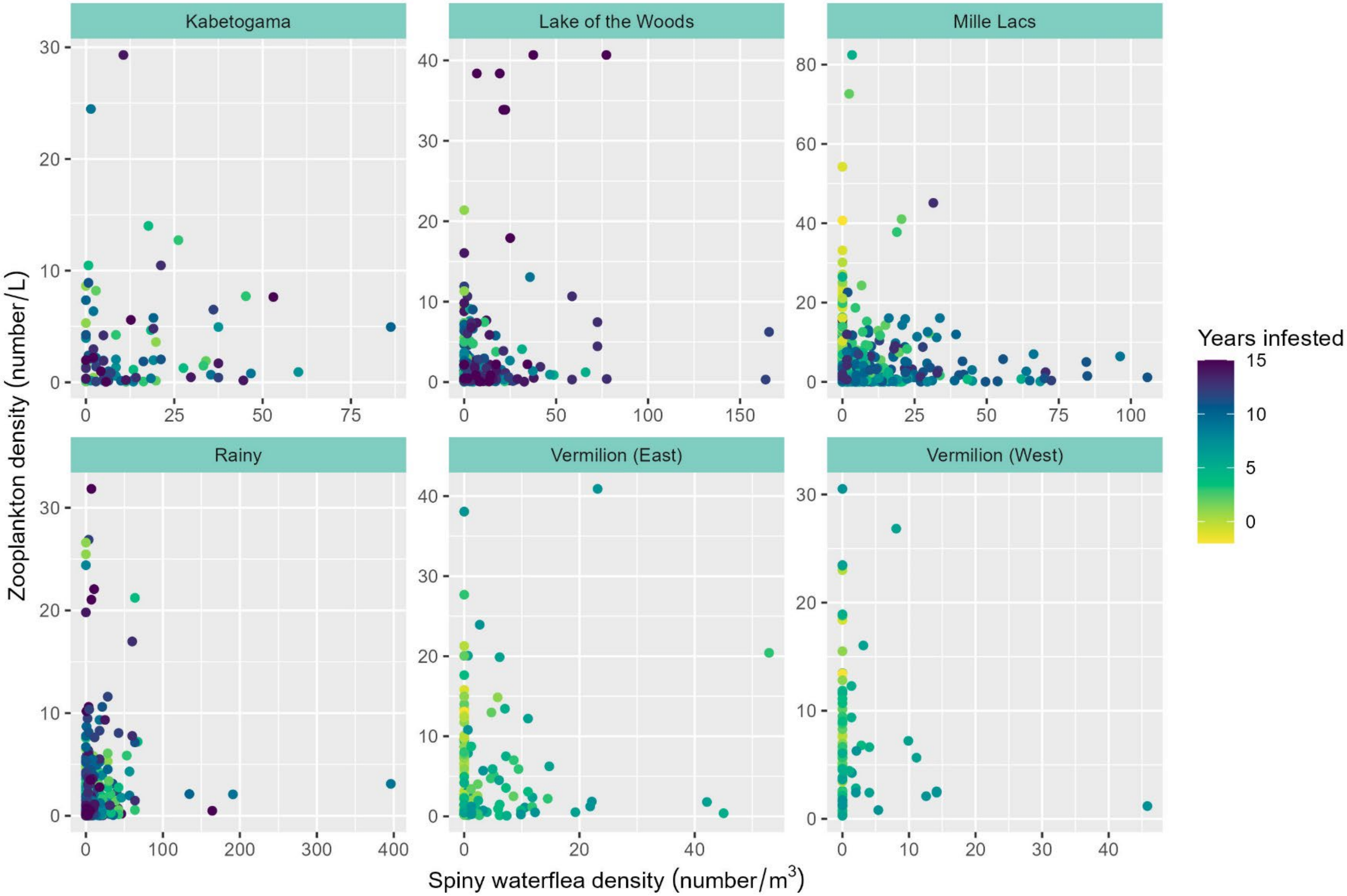


FIGURE 5a.—Relationships between total native zooplankton density (number/L) and spiny waterflea density (number m^{-3}). Color gradiant represents the number of years a particular lake has been infested with spiny waterflea (1= year listed as infested). Total samples by lake; Kabetogama (n=83), Lake of the Woods (n=82), Mille Lacs (n=98), Rainy (n=91), Vermilion- East (n=71), Vermilion-West (n=70).

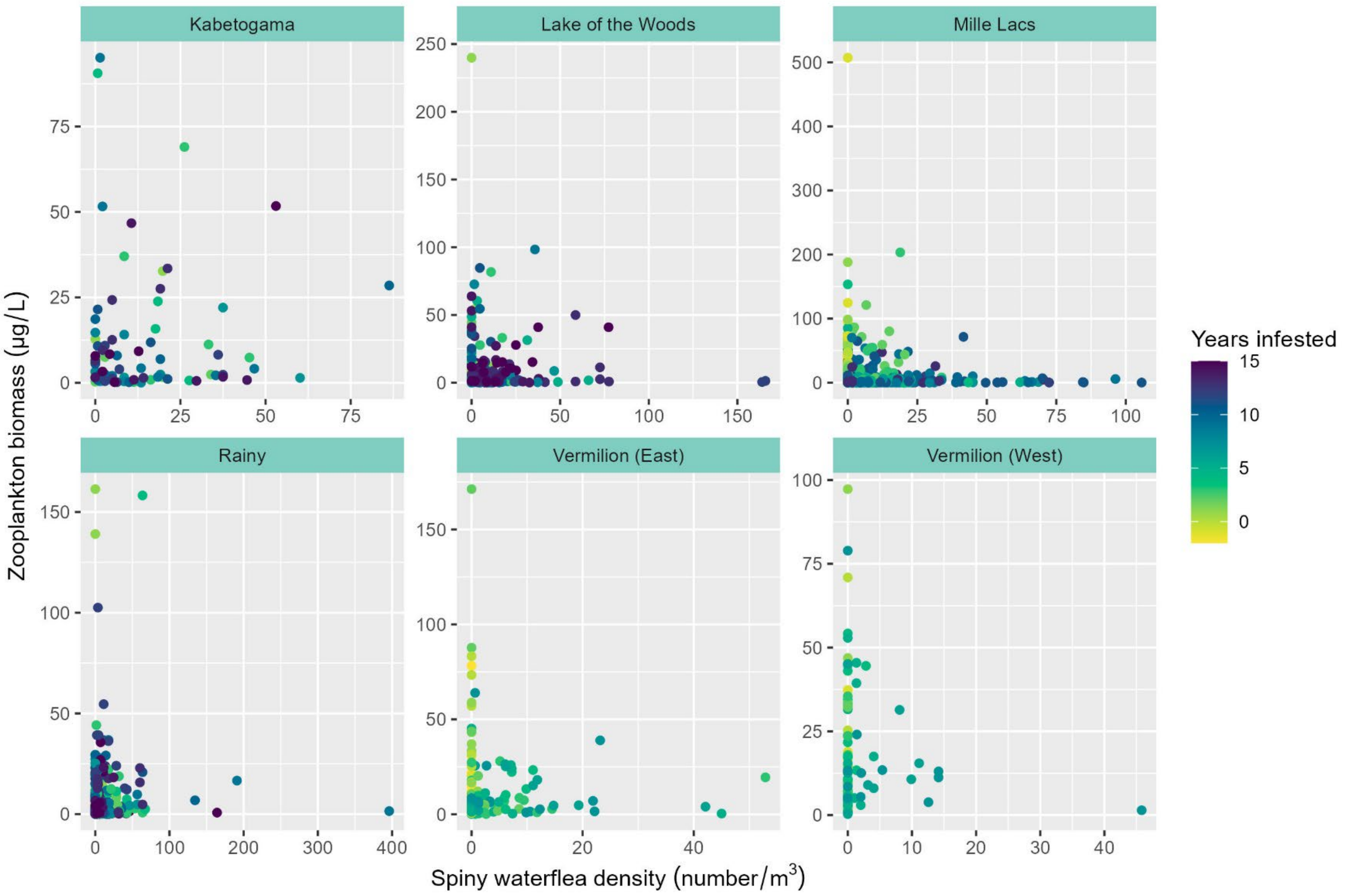


FIGURE 5b.—Relationships between total natve zooplankton biomass (µg/L) and spiny waterflea density (number/m^3). Color gradiant represents the number of years a particular lake has been infested with spiny waterflea (1= year listed as infested). Total samples by lake; Kabetogama (n=83), Lake of the Woods (n=82), Mille Lacs (n=98), Rainy (n=91), Vermilion- East (n=71), Vermilion-West (n=70).

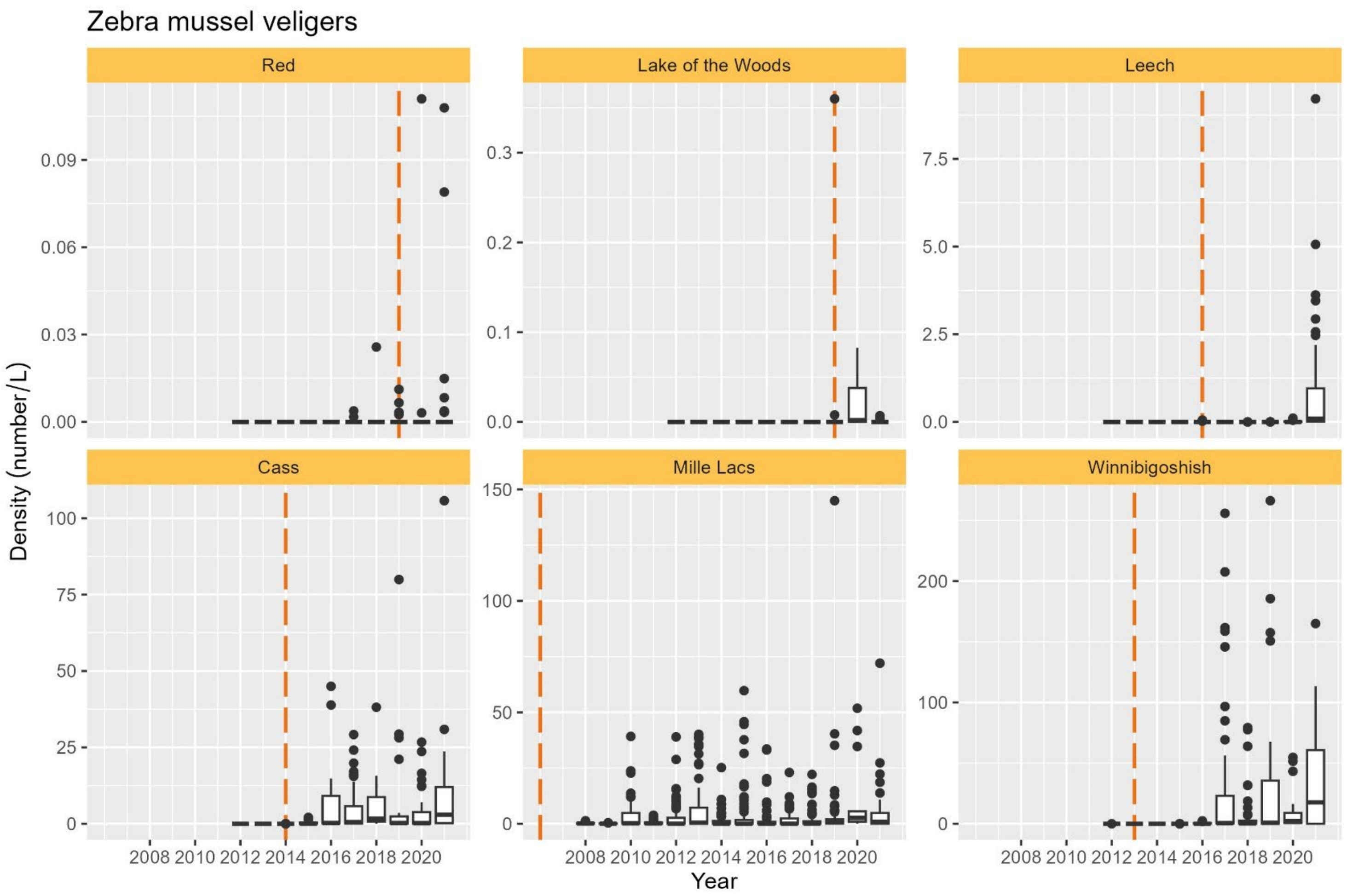


FIGURE 6.—Boxplots comparing daily densities (number/L) of zebra mussel veligers from six large lakes in Minnesota. Orange dashed line indicated year of unknown infestation. Lakes are arranged from lowest to highest densities (top left to bottom right) and Y-axis varies by lake. Data were collected annually from May-October when possible. Density data are also presented in the individual lake section (e.g. Figure 12).

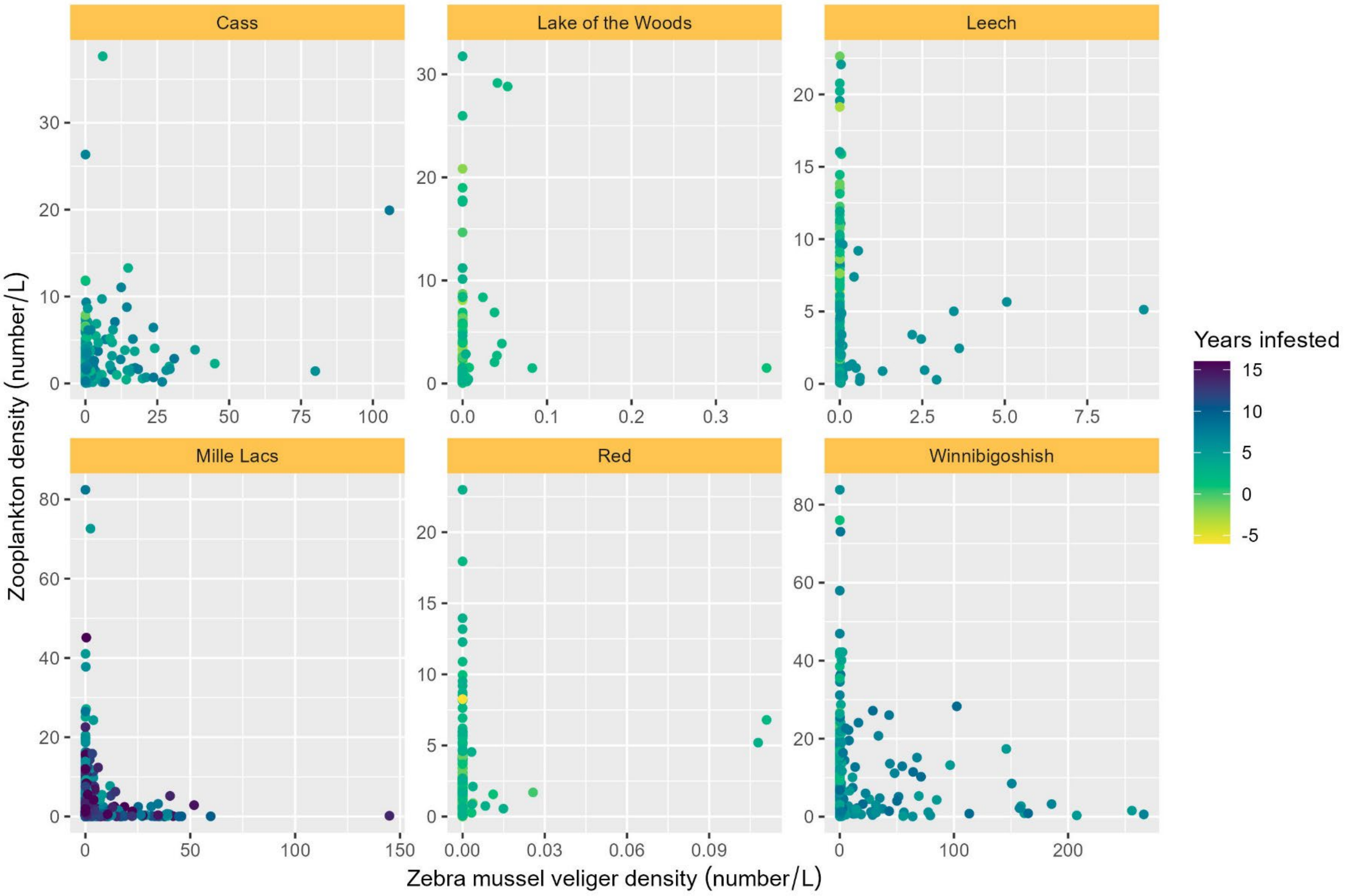


FIGURE 7a.—Relationships between total native zooplankton and zebra mussel veliger density (number/L). Color gradiant represents the number of years a particular lake has been infested with zebra mussels (1= year listed as infested). Total samples by lake; Cass (n=51), Lake of the Woods (n=52), Leech (n=56), Mille Lacs (n=90), Red (n=73), Winnibigoshish (n=48).

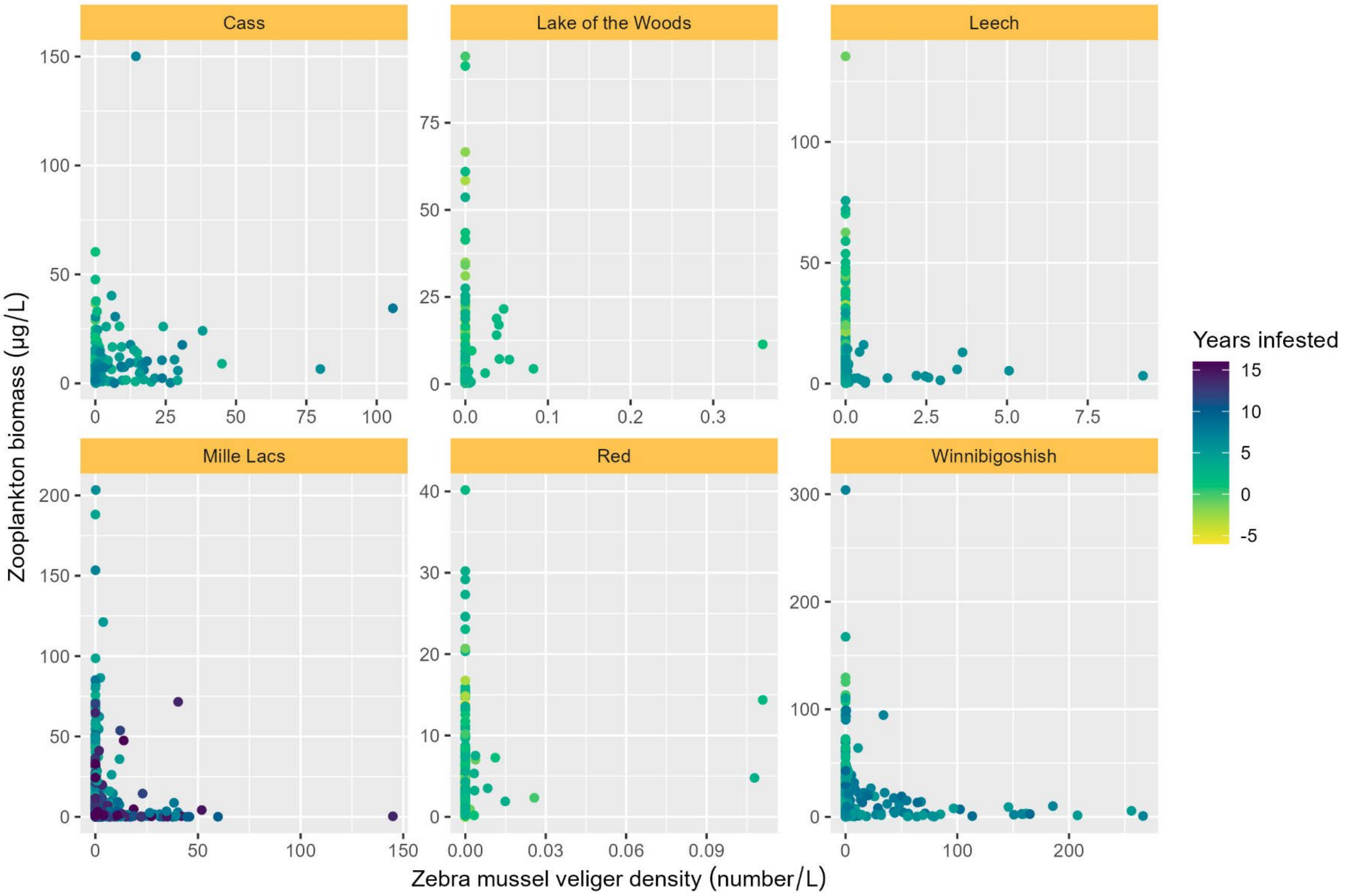


FIGURE 7b.—Relationships between total native zooplanton biomass (µg/L) and zebra mussel veliger density (number/L). Color gradiant represents the number of years a particular lake has been infested with zebra mussels (1= year listed as infested). Total samples by lake; Cass (n=51), Lake of the Woods (n=52), Leech (n=56), Mille Lacs (n=90), Red (n=73), Winnibigoshish (n=48).

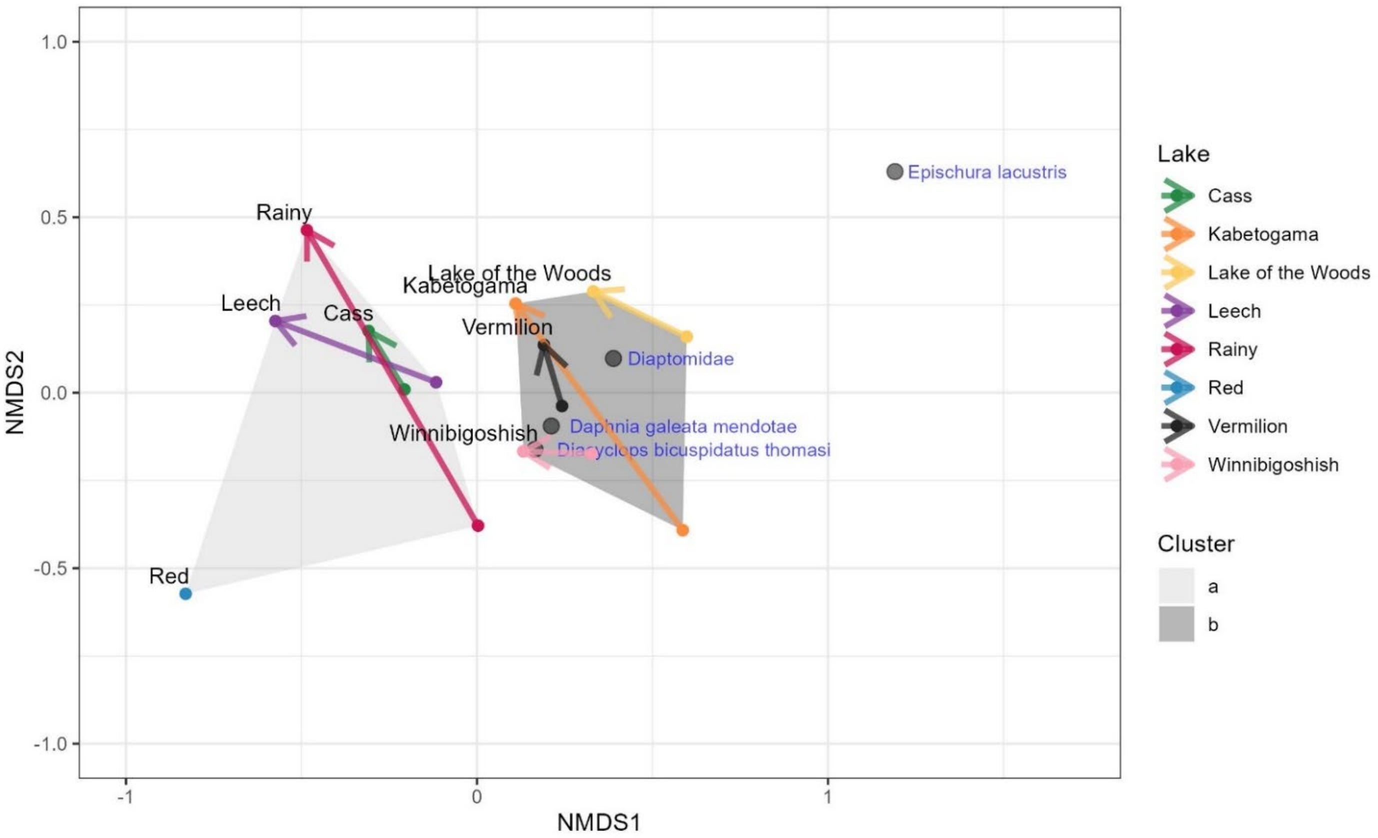


FIGURE 8.—A non-metric multidimensional scaling (NMDS) plot showing differences in zooplankton community structure in eight of the nine monitored Large Lakes (2-D stress = 0.07). Lake Mille Lacs was excluded due to differences in taxonomic resolution for cyclopoid copepods. Vectors show the trajectories of the lake zooplankton communities from pre-invasion to post-invasion years. Similar communities based on taxa and mean biomass are in closer proximity. The main taxa contributing to community patterns are labeled in the direction of increasing biomass of those taxa (solid grey dot with taxa name in blue). A cluster analysis identified two distinct types of zooplankton communities, an and b, each associated with four of the Large Lakes.

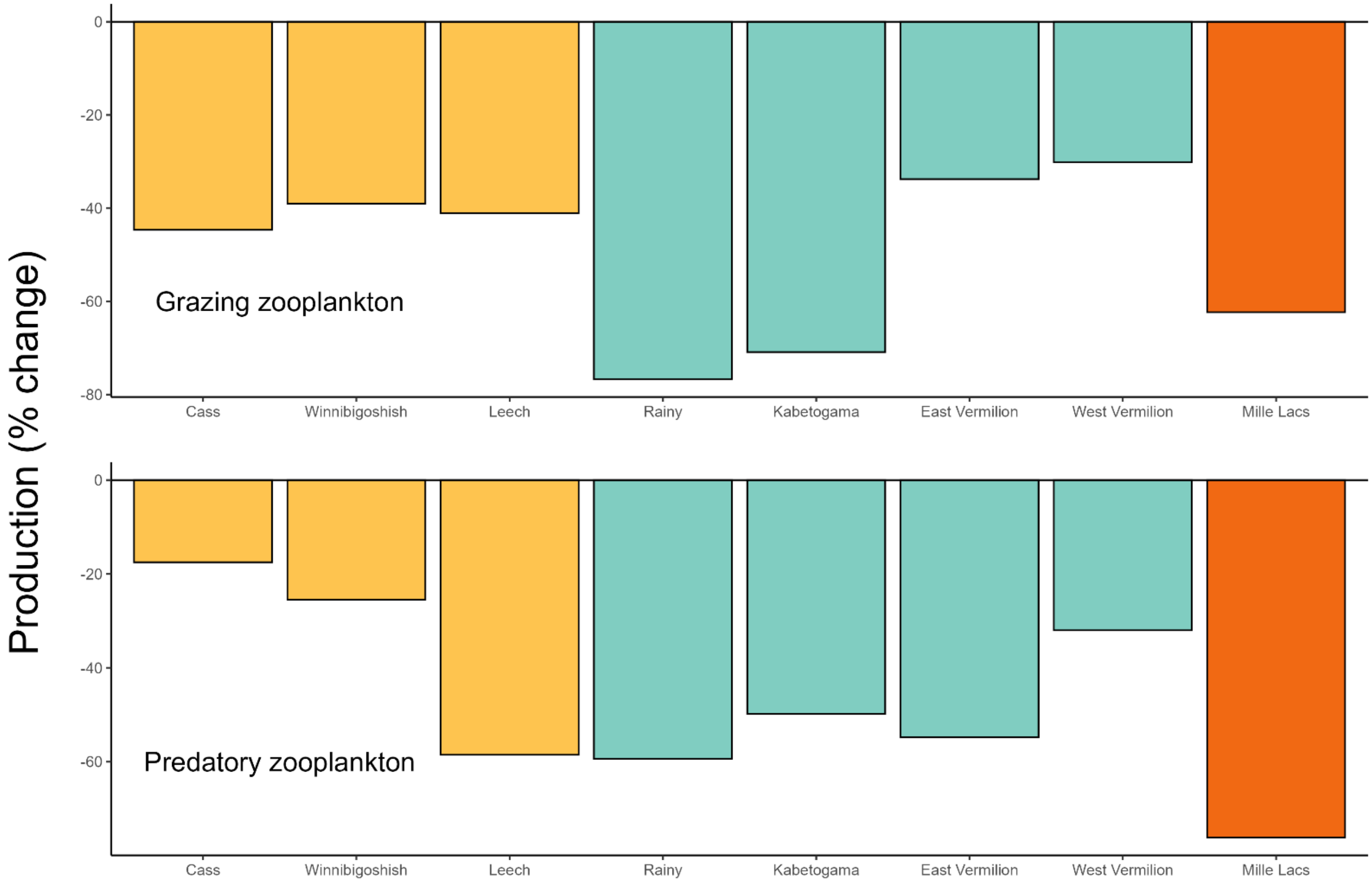


FIGURE 9a.—Plot of change in native zooplankton production in individual study lakes for grazing (top; cladocerans and calanoid copepods) and predatory (bottom, cyclopoids) taxa. We used a three-year lag time to assign invasion status for zebra mussel-invaded lakes. Estimates are color-coded for invasion status, yellow = zebra mussel only, blue = spiny waterflea only, orange = zebra mussel and spiny waterflea. Upper and Low Red lakes were excluded from the analyses, as we had <3 years of post-invasion data.

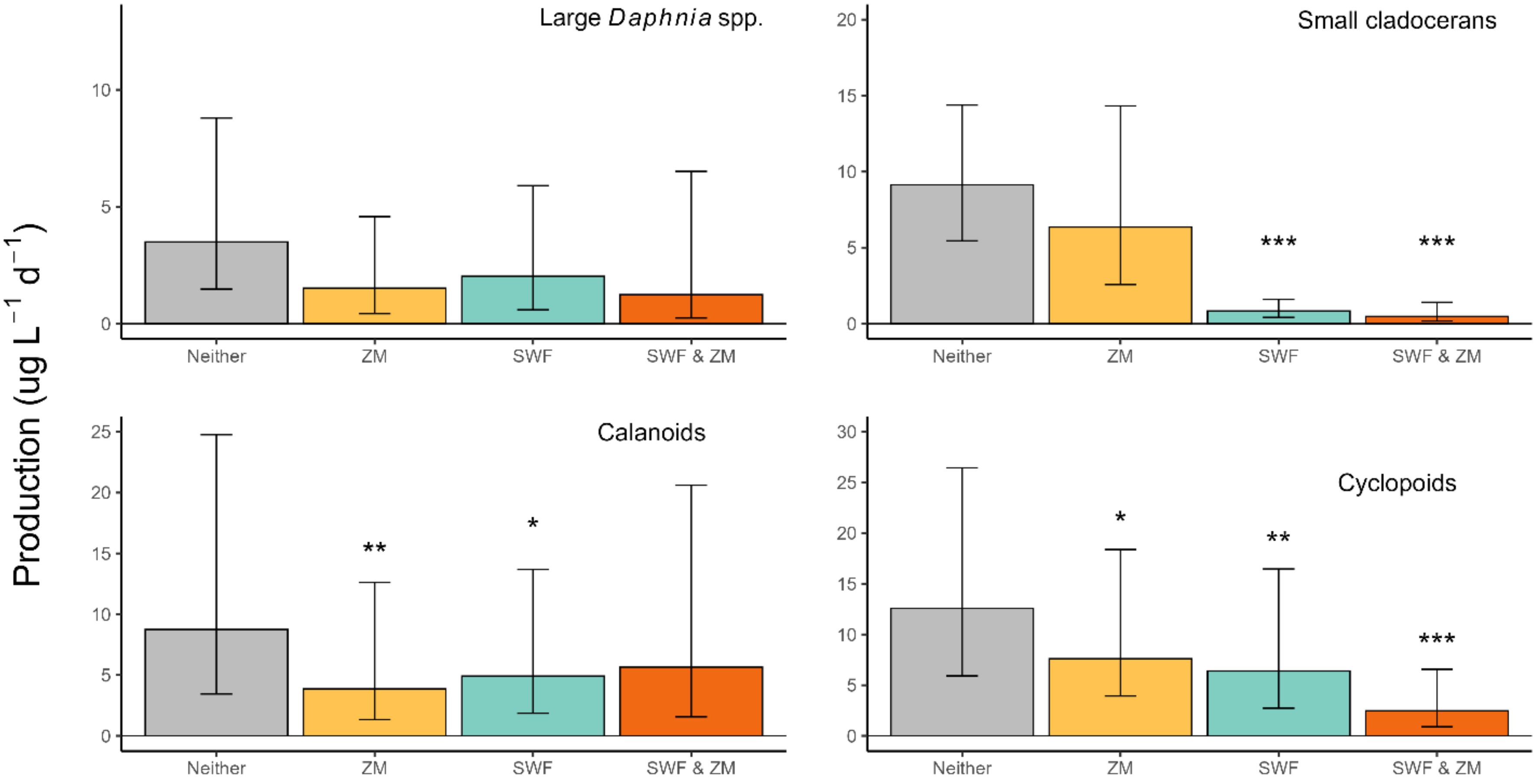


FIGURE 9b.—Median daily summer native zooplankton production (ug L^{-1} d^{-1}) in the study lakes when infested with spiny waterflea (SWF), zebra mussels (ZM), or both (SWF & ZM), and results of generalized linear mixed effects models relative to lakes without either of those invasive invertebrates (Neither). Impacts were estimated for large *Daphnia* spp., small cladocerans, and adult cyclopoid and calanoid copepods. Models included random effects for individual lakes and years (n = 121 for each analysis). Lake of the Woods was not included; Lake Vermilion was split into East and West basins. Error bars represent bootstrapped 95% confidence intervals. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

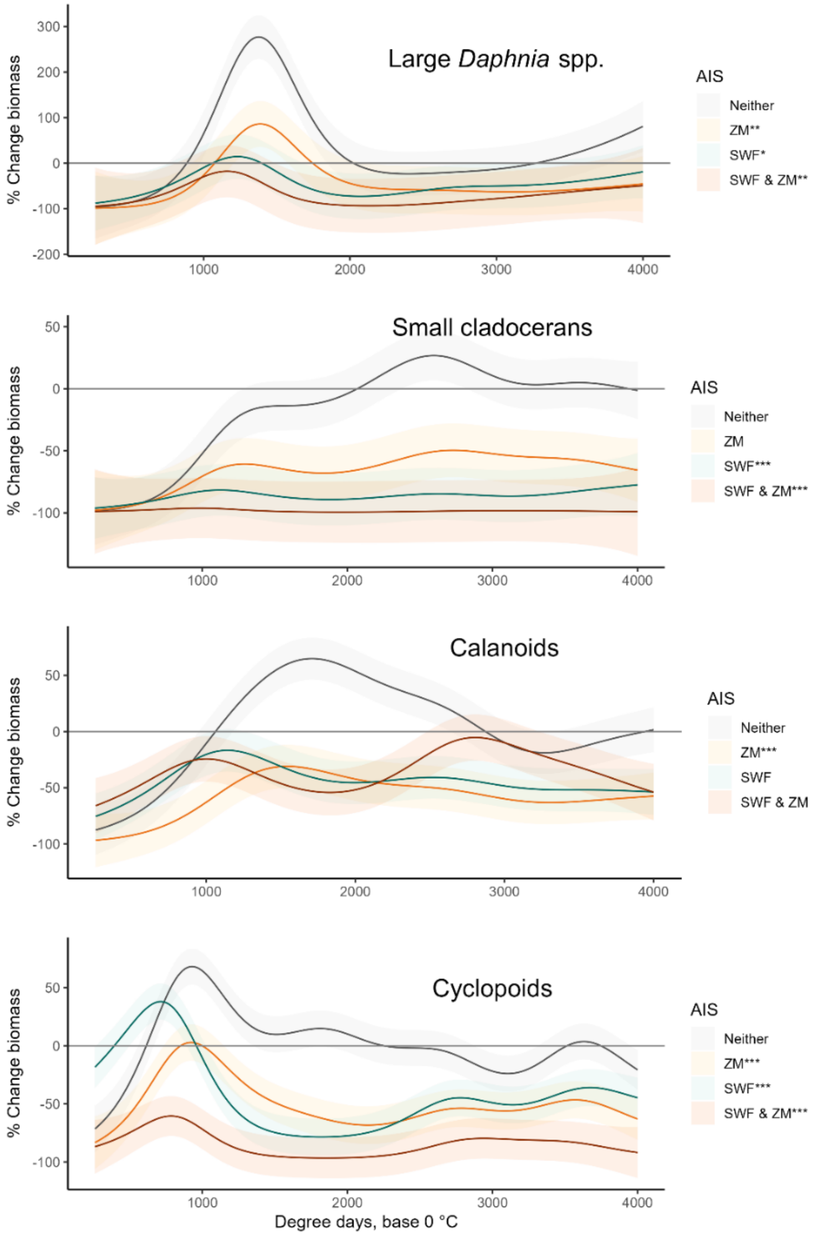


FIGURE 10.— Percent change of native zooplankton biomass (relative to average summer biomass in uninvaded lakes, 0 % on y-axis) in the presence of zebra mussels (ZM, yellow), spiny waterflea (SWF, blue), both (SWF & ZM, pink), or without either of those invasive invertebrates (Neither, gray). We estimated the effects of the different invasion scenarios relative to uninvaded lakes using generalized additive models (GAMs) of large *Daphnia spp.,* small cladocerans, adult copepods (calanoids and cyclopoids separately) through the summer from the study lakes. Time was represented by cumulative degree days (dd, base 0°C, Corson-Dosch et al. 2023). The effects of individual lakes and years were estimated by including random 'lake' and 'year' variables in the GAMs. We included a spline for time (dd) and a smoothing factor for the time*invasion status interaction. * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$. For context, in these lakes, median cumulative dd (2011–2021) were: May 15 = 686 dd; July 1 = 1553 dd; and September 30 = 3598.

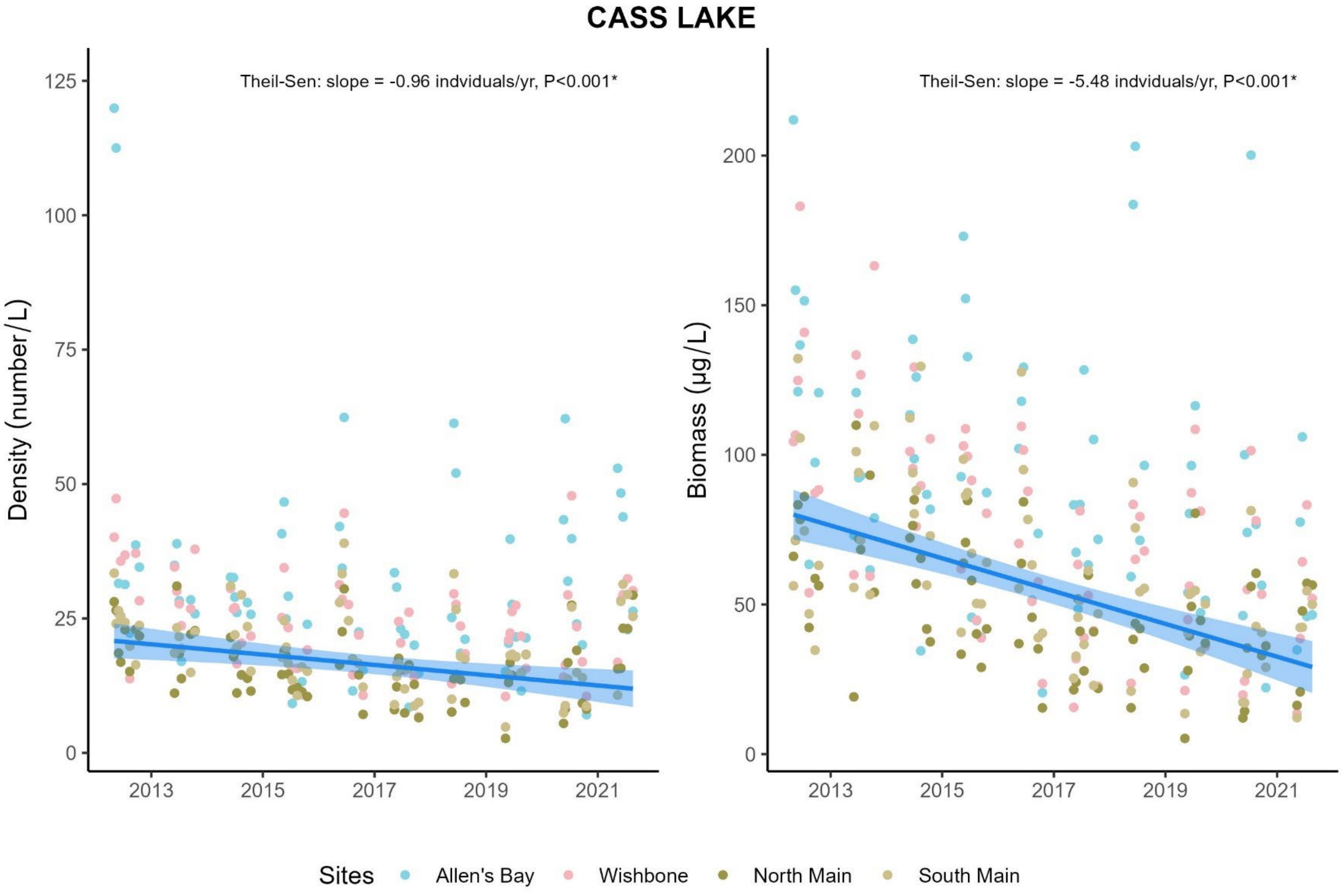


FIGURE 11. —Trend analyses show the total density (number/L) and biomass (µg/L) of zooplankton in Cass Lake. Colored dots represent one sampling event and correspond to four collection sites. The Theil-Sen slope shows a significant decrease in density and biomass from 2012–2021.

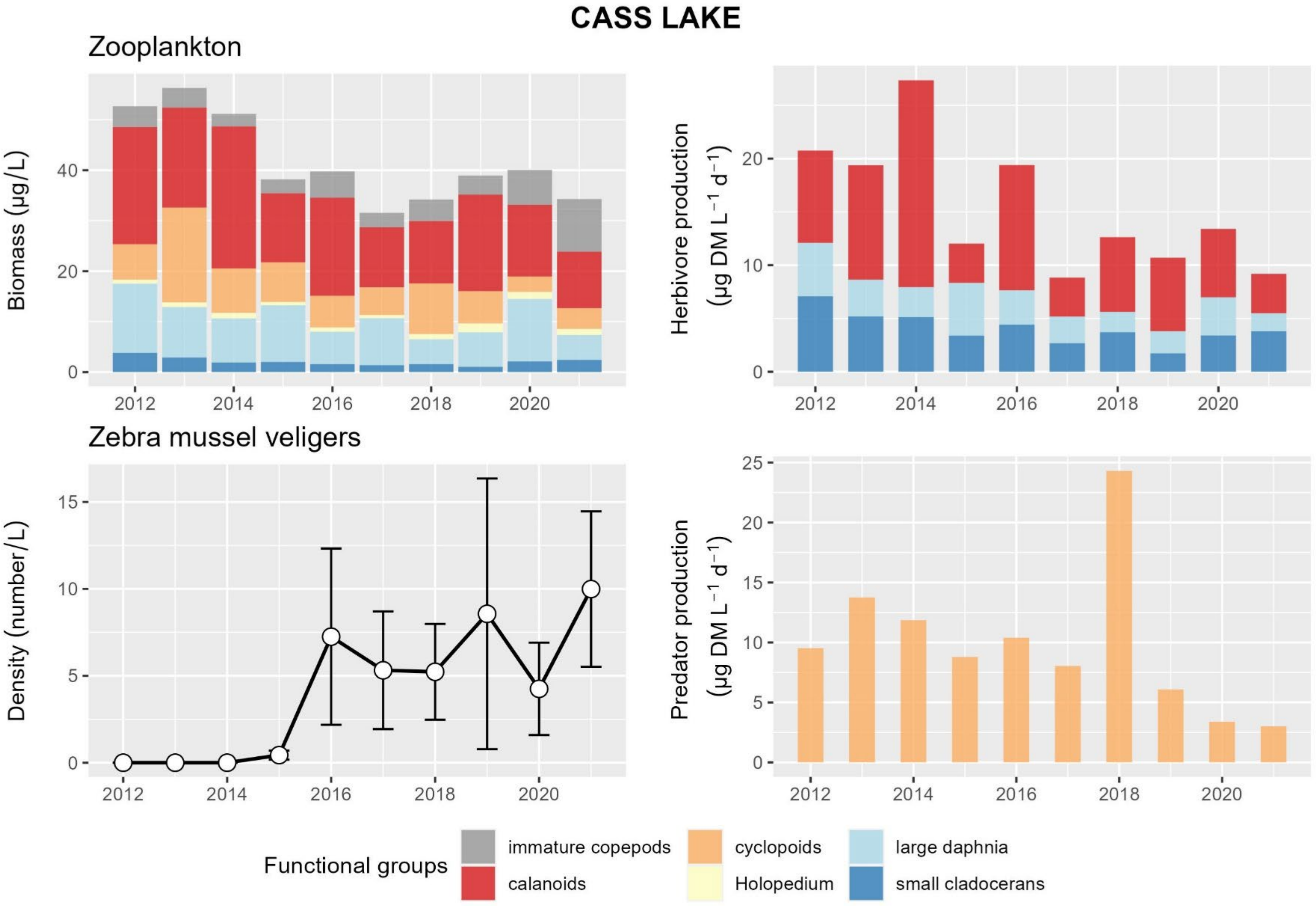


FIGURE 12.—Bar graphs show the annual mean biomass (µg/L) and secondary production (µg DM L^{-1} d^{-1}) of zooplankton in Cass Lake. Color bars represent different functional groups. Annual mean density (number L^{-1}) of zebra mussel veligers is from zooplankton samples. Error bars represent one standard error.

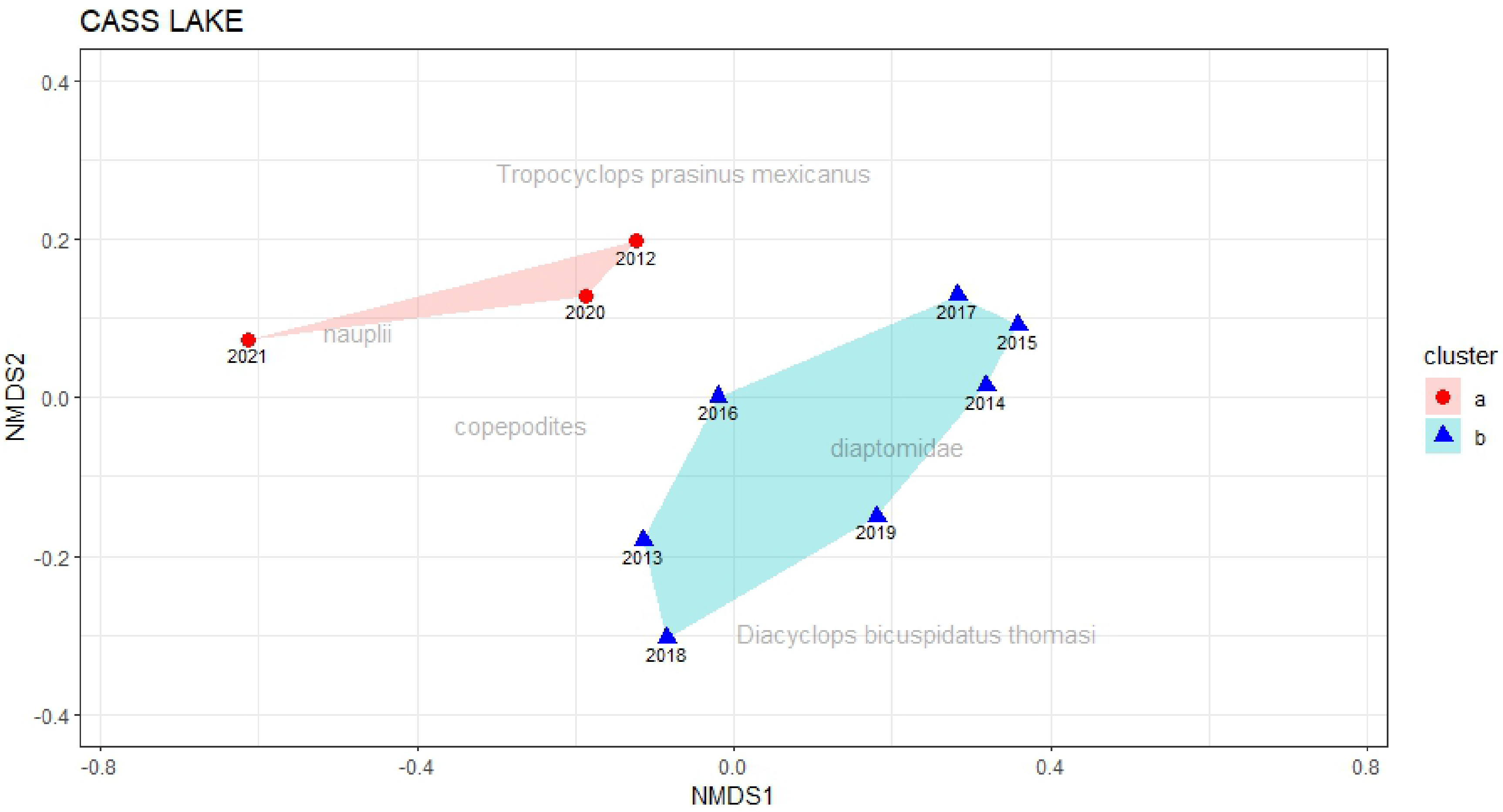


FIGURE 13.—A non-metric multidimensional scaling (NMDS) plot showing differences in zooplankton community structure in Cass Lake (2-D stress = 0.09). Symbols correspond to the zooplankton community in different years. Similar communities based on taxa and abundance are positioned closer together with the main taxa contributing to this pattern labeled in the direction of increasing abundance of those taxa.

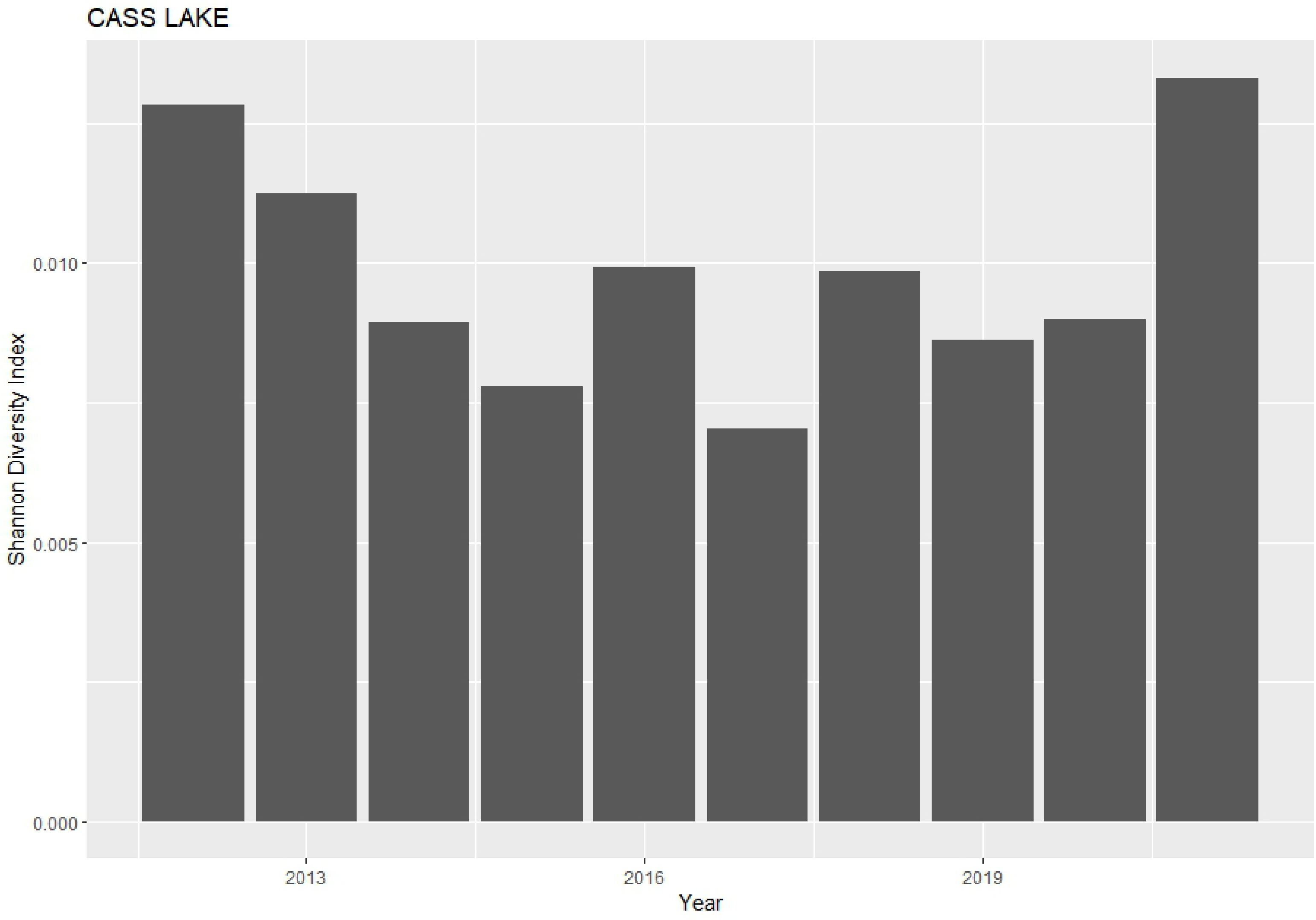


FIGURE 14.—Barplot summarizing the Shannon diversity index for the zooplankton community from 2012–2021 in Cass Lake.

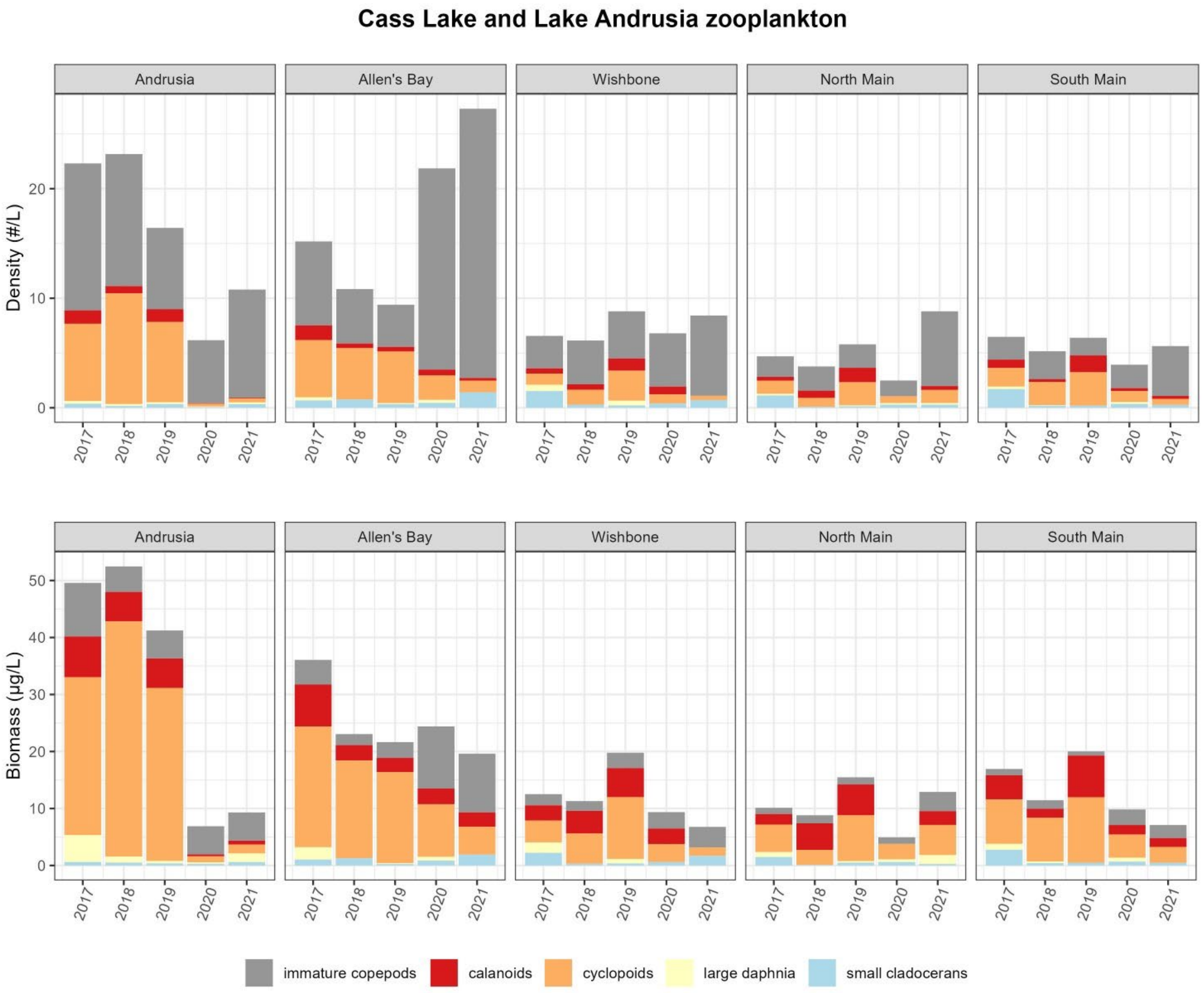


FIGURE 15. —Annual mean density (number/L) and biomass (µg/L) of zooplankton for five functional groups in Cass Lake (sites include Allen's Bay, Wishbone, North Main and South Main) and Lake Andrusia. Samples were collected in May from 2017–2021.

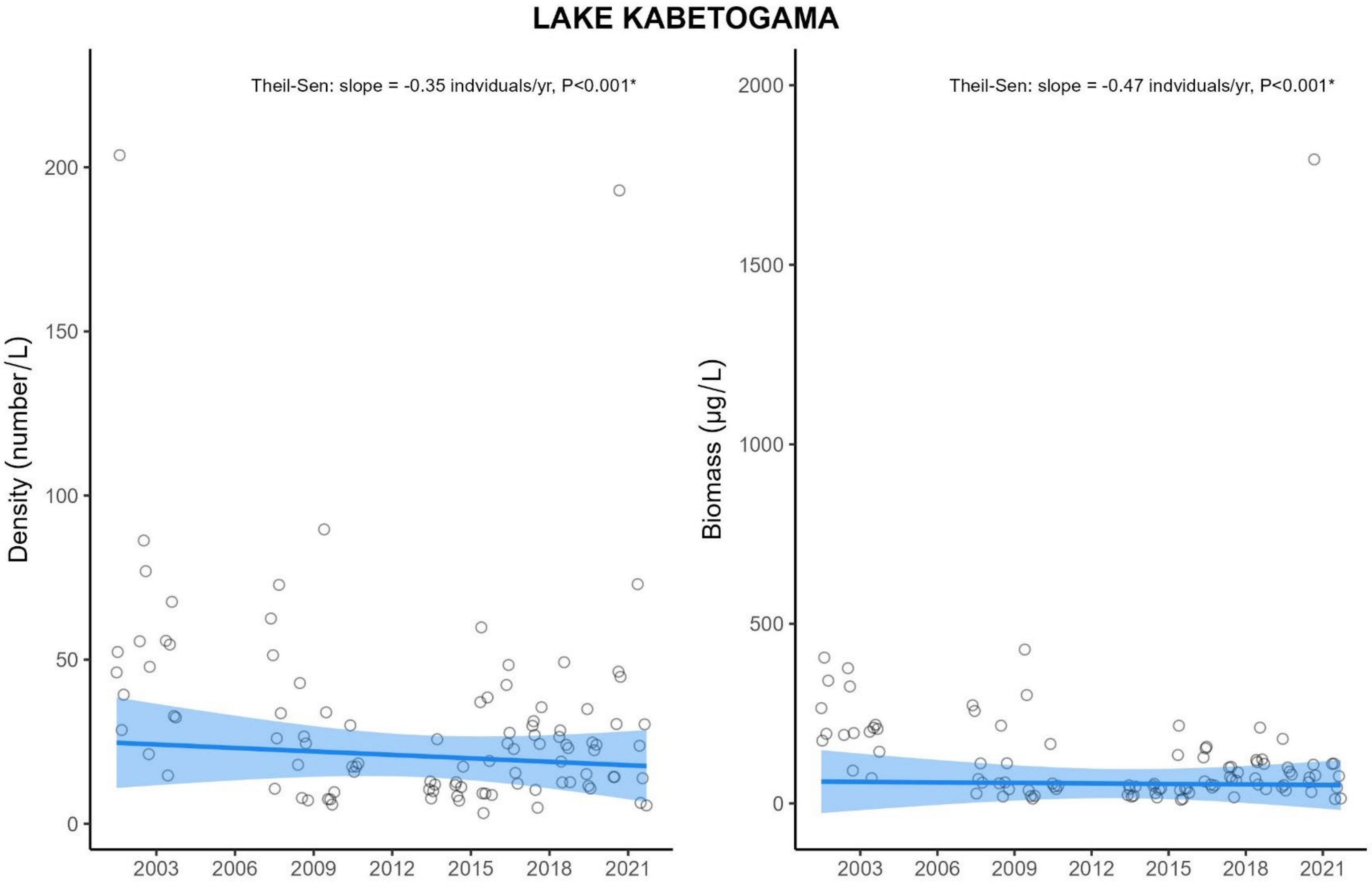


FIGURE 16.—Trend analyses show the total density (number/L) and biomass (µg/L) of zooplankton in Lake Kabetogama. Hollow circles represent one sampling event and correspond to site 1 only. The Theil-Sen slope shows a significant decrease in density and biomass from 2001–2021.

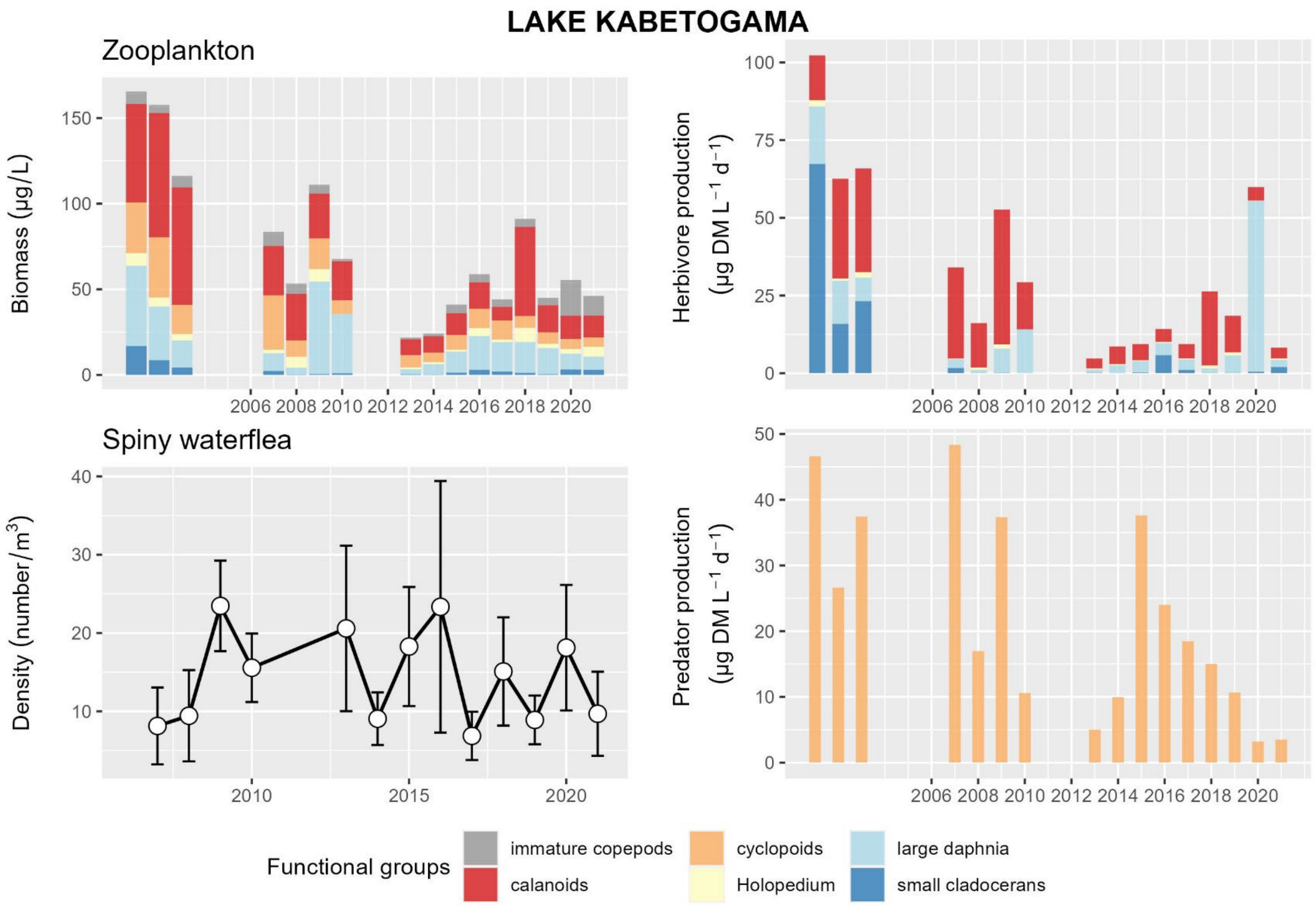


FIGURE 17.—Bar graphs show the annual mean biomass (µg/L) and secondary production (µg DM L^{-1} d^{-1}) of zooplankton in Lake Kabetogama. Color bars represent different functional groups. The data are from site 1 only and sampling event from August 3, 2020 was removed due to a large *Daphnia galeata mendotae* bloom that occurred. Annual mean density (number m^{-3}) of spiny waterflea is from zooplankton samples. Error bars represent one standard error.

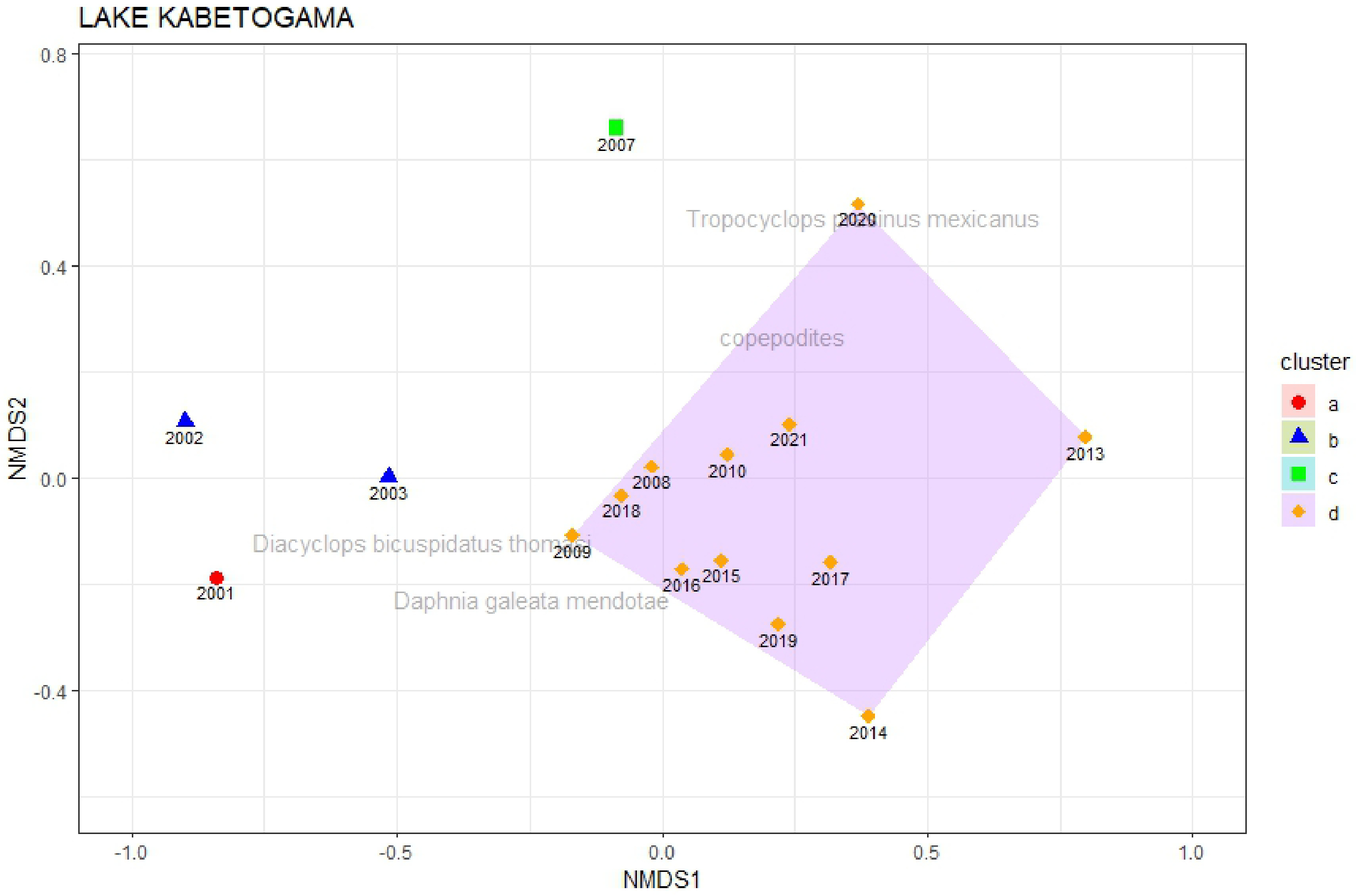


FIGURE 18.—A non-metric multidimensional scaling (NMDS) plot showing differences in zooplankton community structure in Lake Kabetogama (2-D stress = 0.05). Symbols correspond to the zooplankton community in different years. Similar communities based on taxa and abundance are positioned closer together with the main taxa contributing to this pattern labeled in the direction of increasing abundance of those taxa.

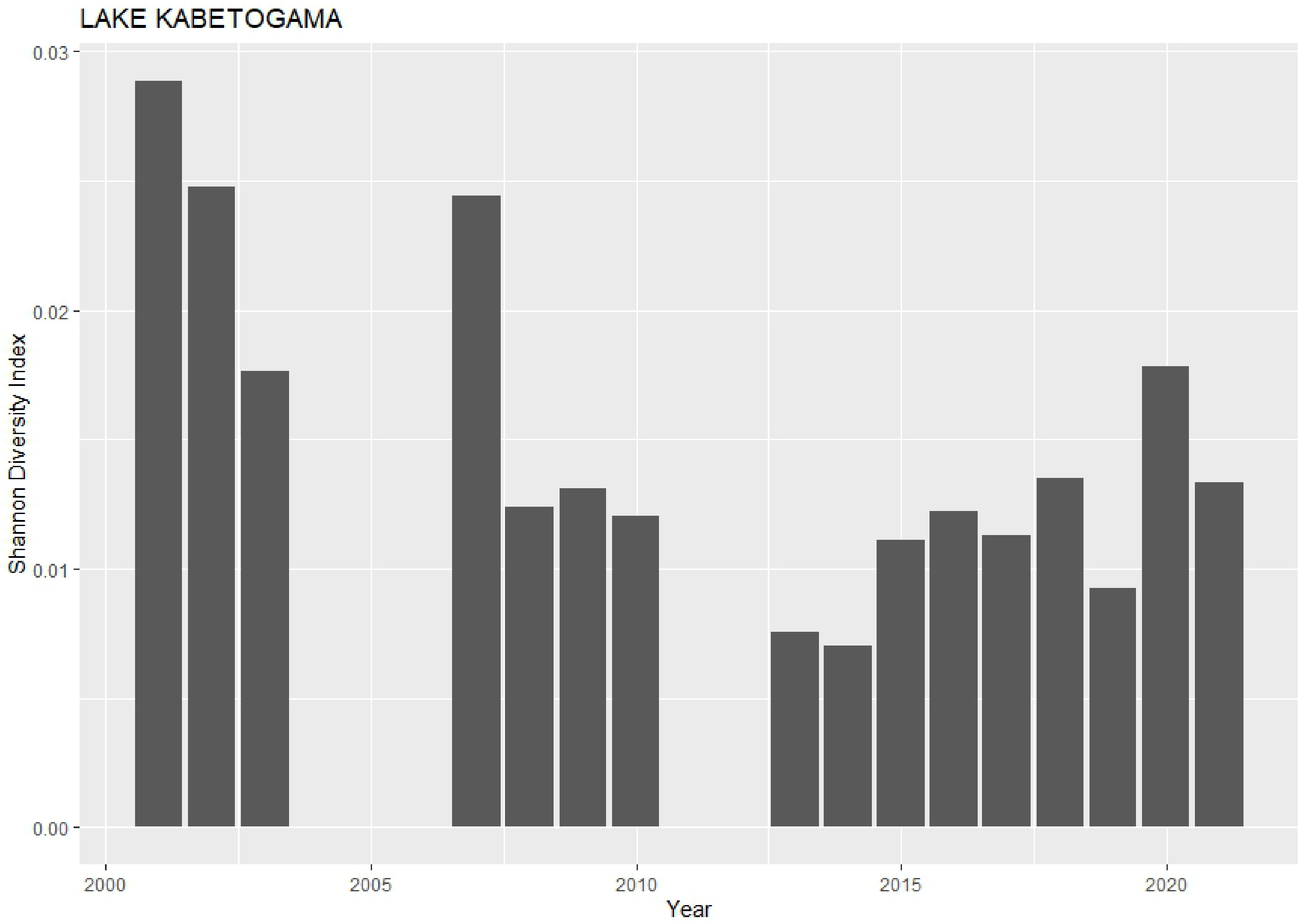


FIGURE 19.—Barplot summarizing the Shannon diversity index for the zooplankton community from 2001–2021 in Lake Kabetogama.

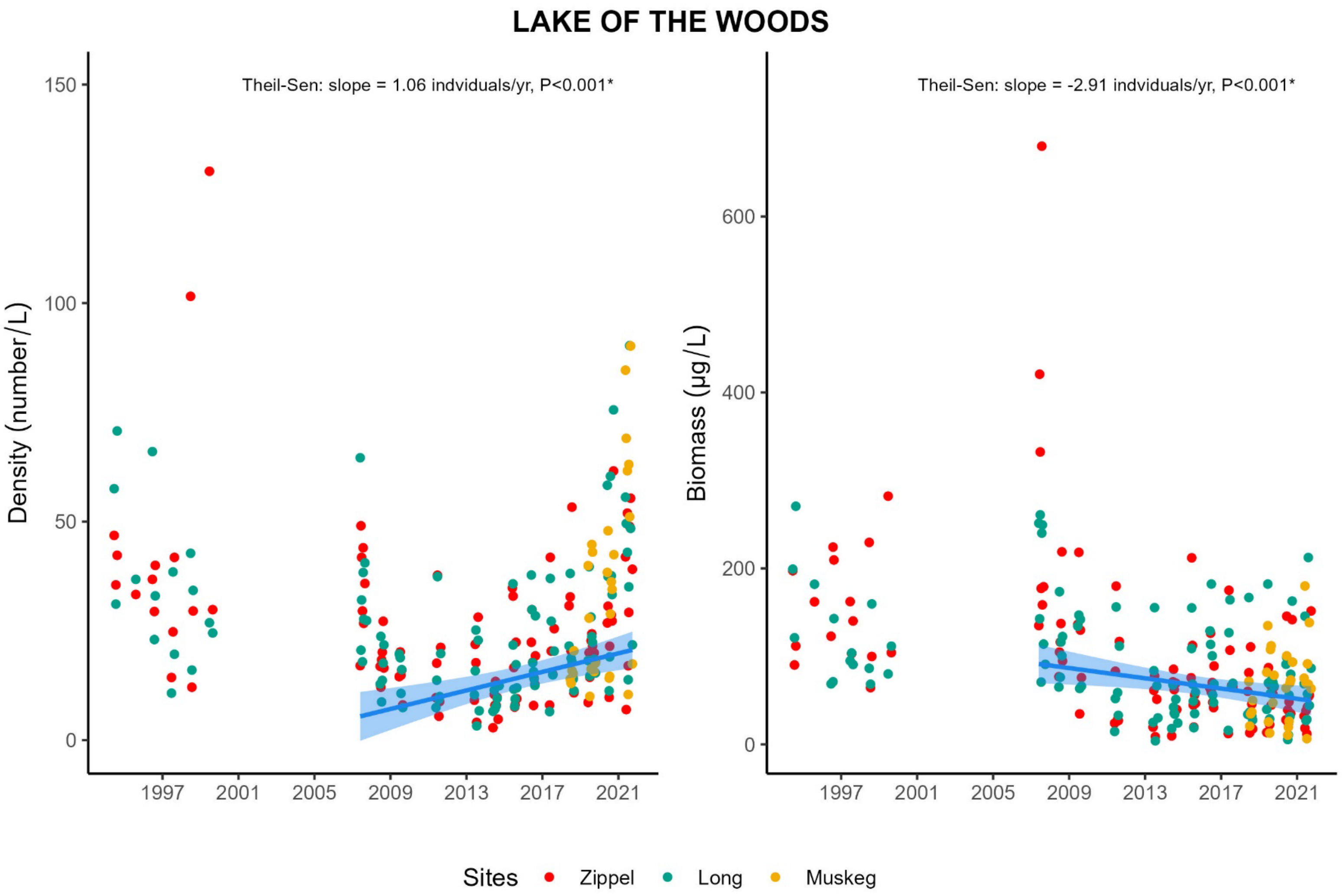


FIGURE 20.—Trend analyses show the total density (number/L) and biomass (µg/L) of zooplankton in Lake of the Woods. Colored dots represent one sampling event and correspond to three collection sites. The Theil-Sen slope shows a significant increase in density and decrease in biomass from 2007–2021. Data from 2010 and 2012 were excluded from the analysis and data collected from 2008–2012 used a 150 µm mesh plankton net.

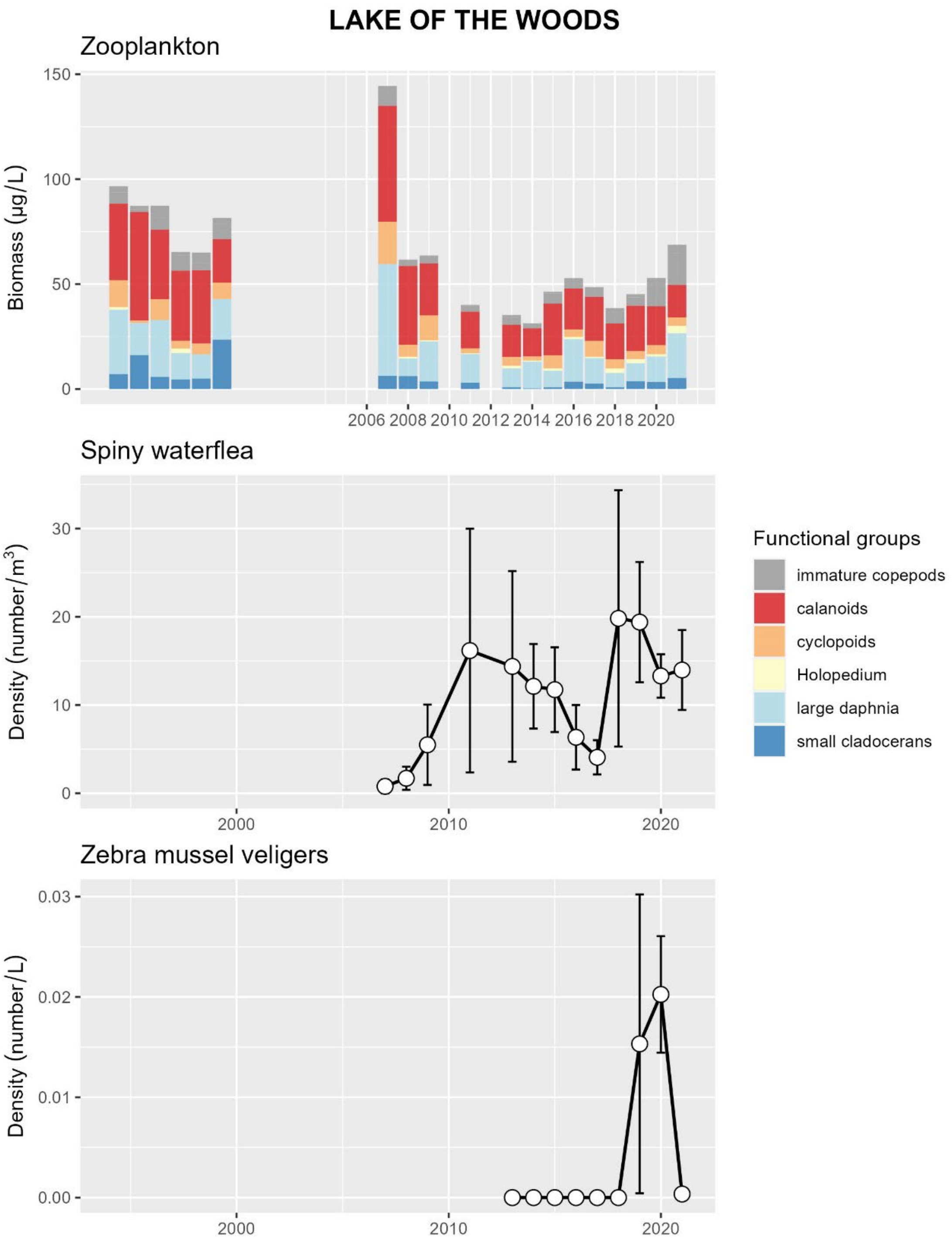


FIGURE 21.—Bar graphs show the annual mean biomass (µg/L) of zooplankton in Lake of the Woods. Color bars represent different functional groups. Data in 2010 and 2012 were collected in August only and are not summarized. Annual mean density of spiny waterflea (number m^{-3}) and zebra mussel veligers (number L^{-1}) are from zooplankton samples. Error bars represent one standard error.

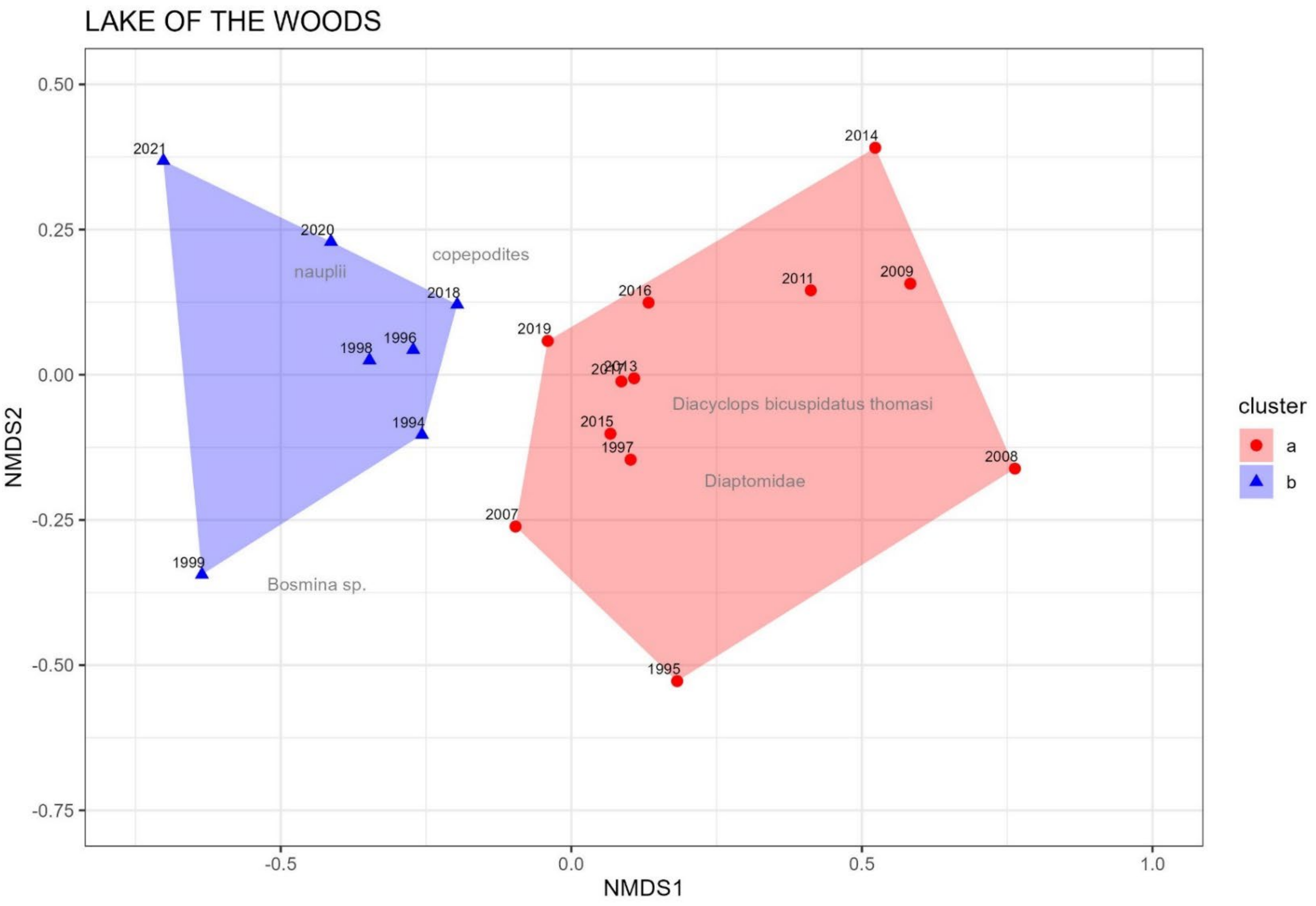


FIGURE 22.—A non-metric multidimensional scaling (NMDS) plot showing differences in zooplankton community structure in Lake of the Woods (2-D stress = 0.08). Symbols correspond to the zooplankton community in different years. Similar communities based on taxa and abundance are positioned closer together with the main taxa contributing to this pattern labeled in the direction of increasing abundance of those taxa.

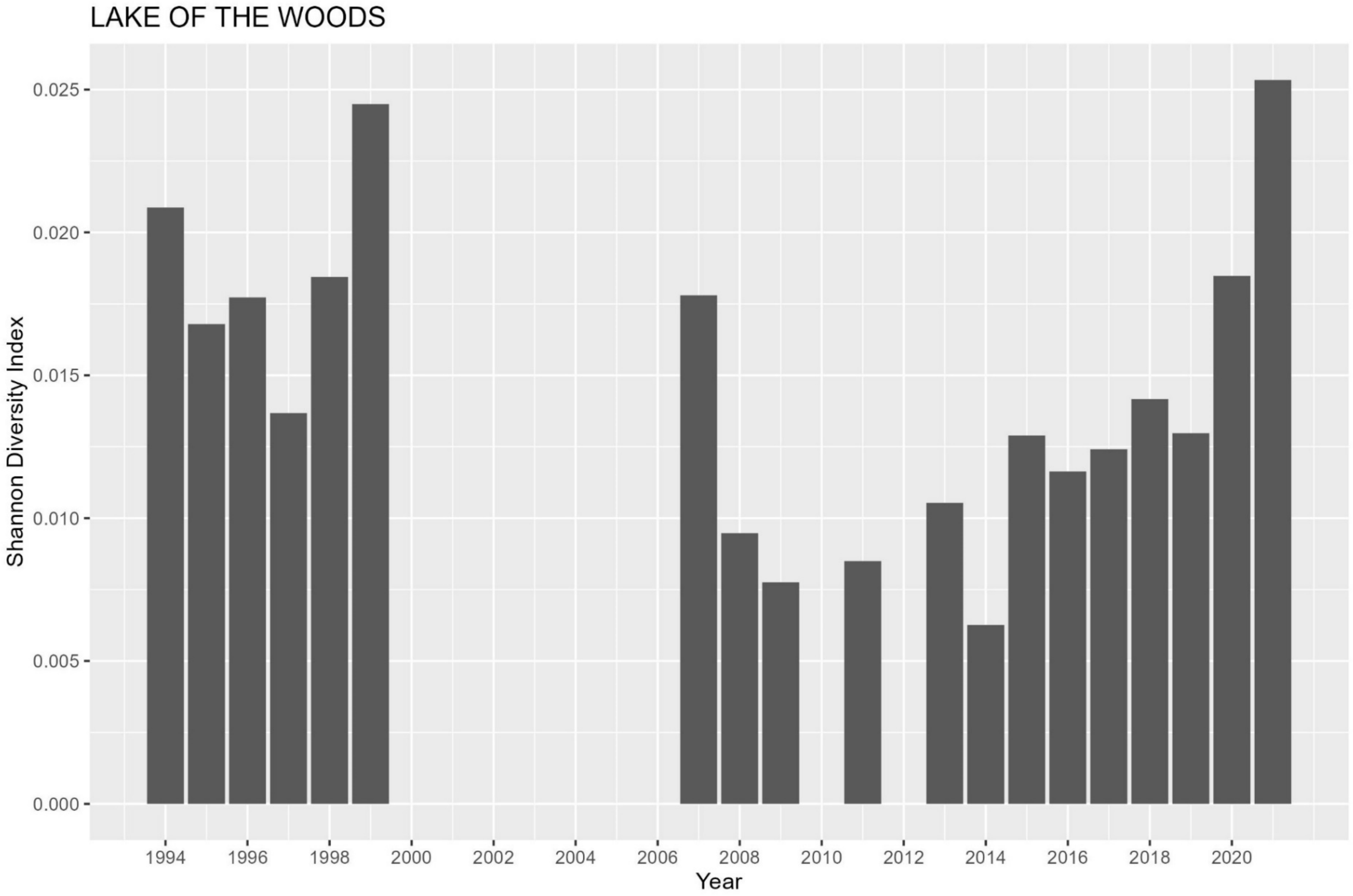


FIGURE 23.—Barplot summarizing the Shannon diversity index for the zooplankton community from 1994–2021 in Lake of the Woods.

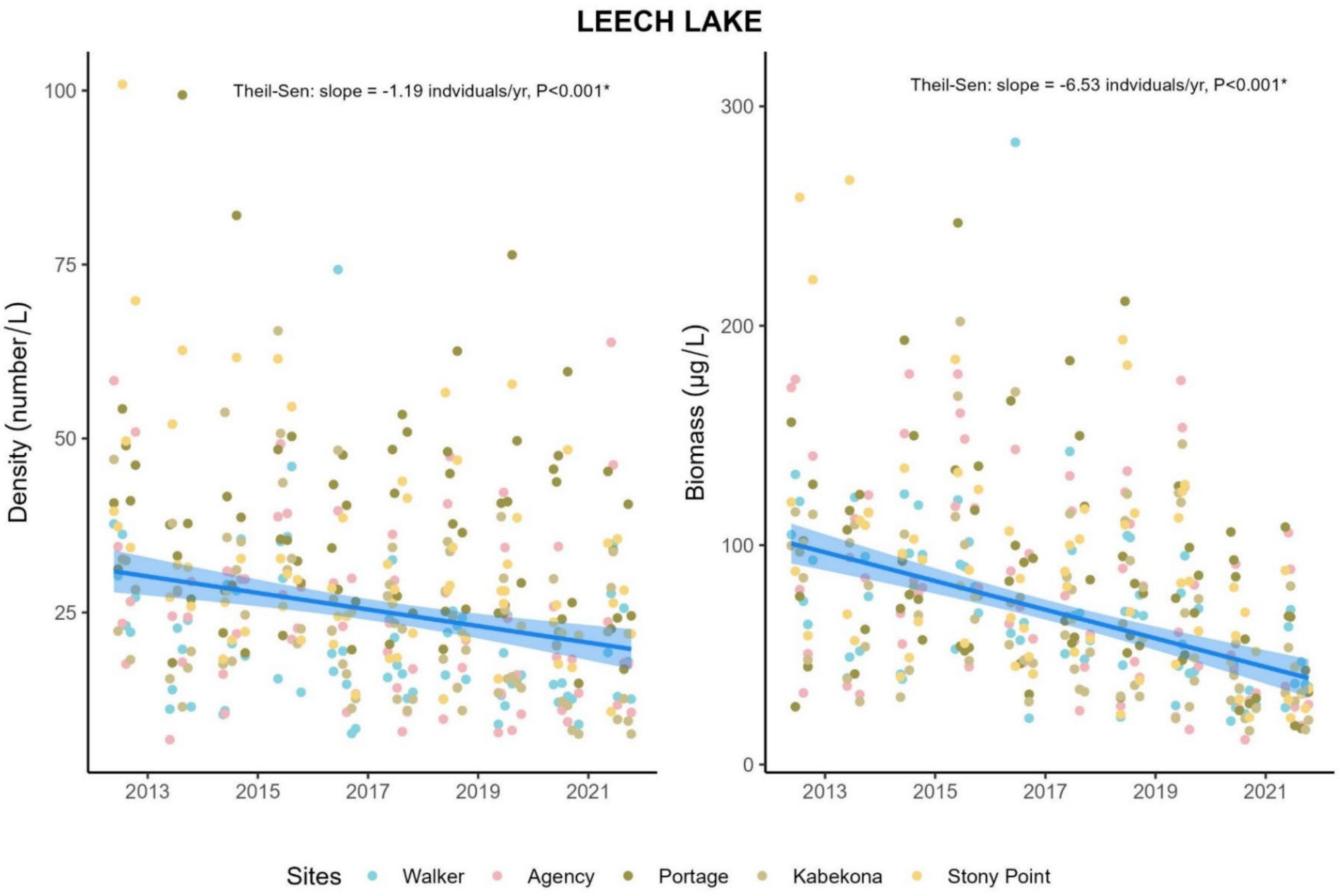

LEECH LAKE

FIGURE 24.—Trend analyses show the total density (number/L) and biomass (µg/L) of zooplankton in Leech Lake. Colored dots represent one sampling event and correspond to five collection sites. The Theil-Sen slope shows a significant decrease in density and biomass from 2012–2021.

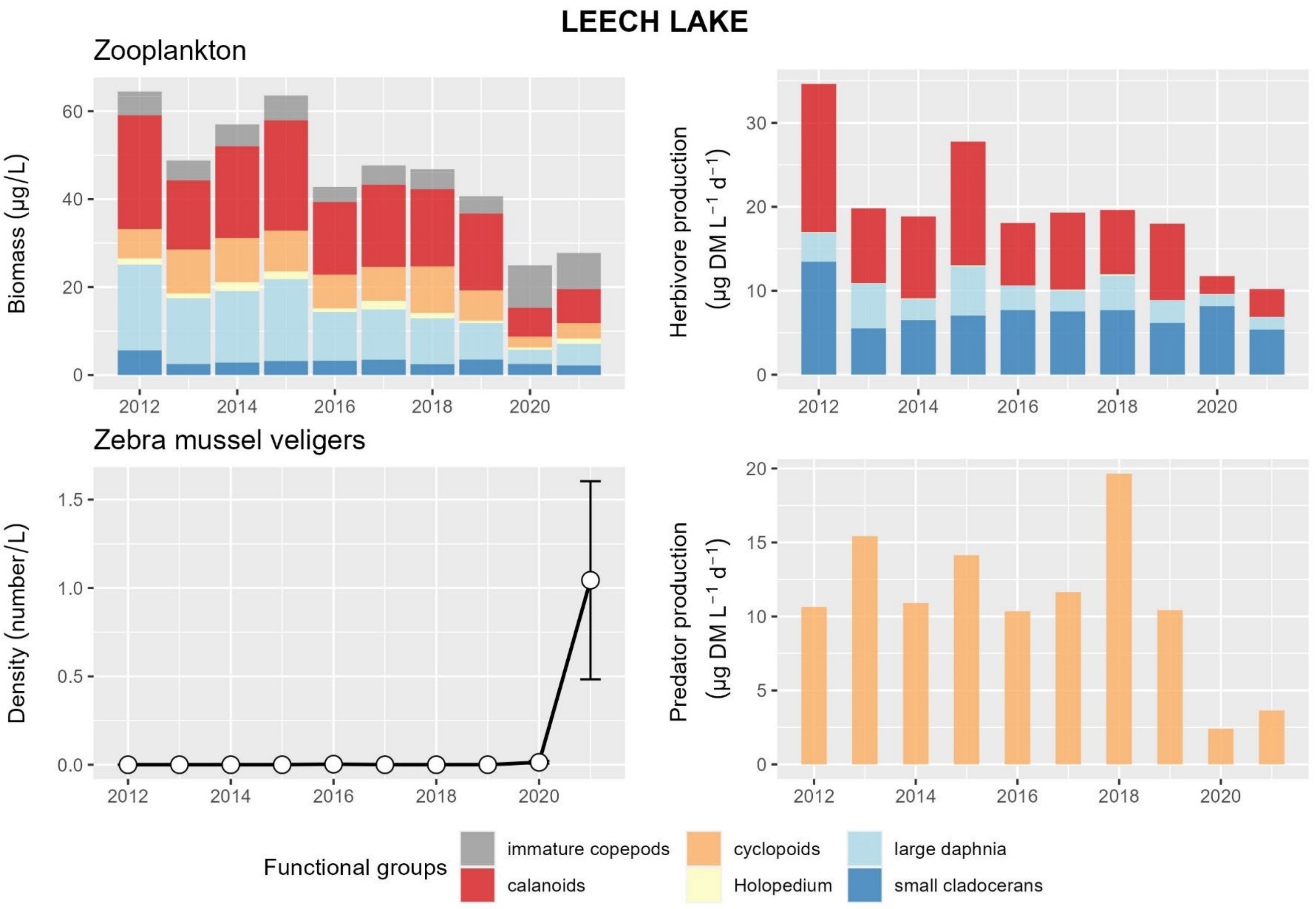


FIGURE 25.—Bar graphs show the annual mean biomass (µg/L) and secondary production (µg DM L^{-1} d^{-1}) of zooplankton in Leech Lake. Color bars represent different functional groups. Annual mean density (number/L) of zebra mussel veligers are from zooplankton samples. Error bars represent one standard error.

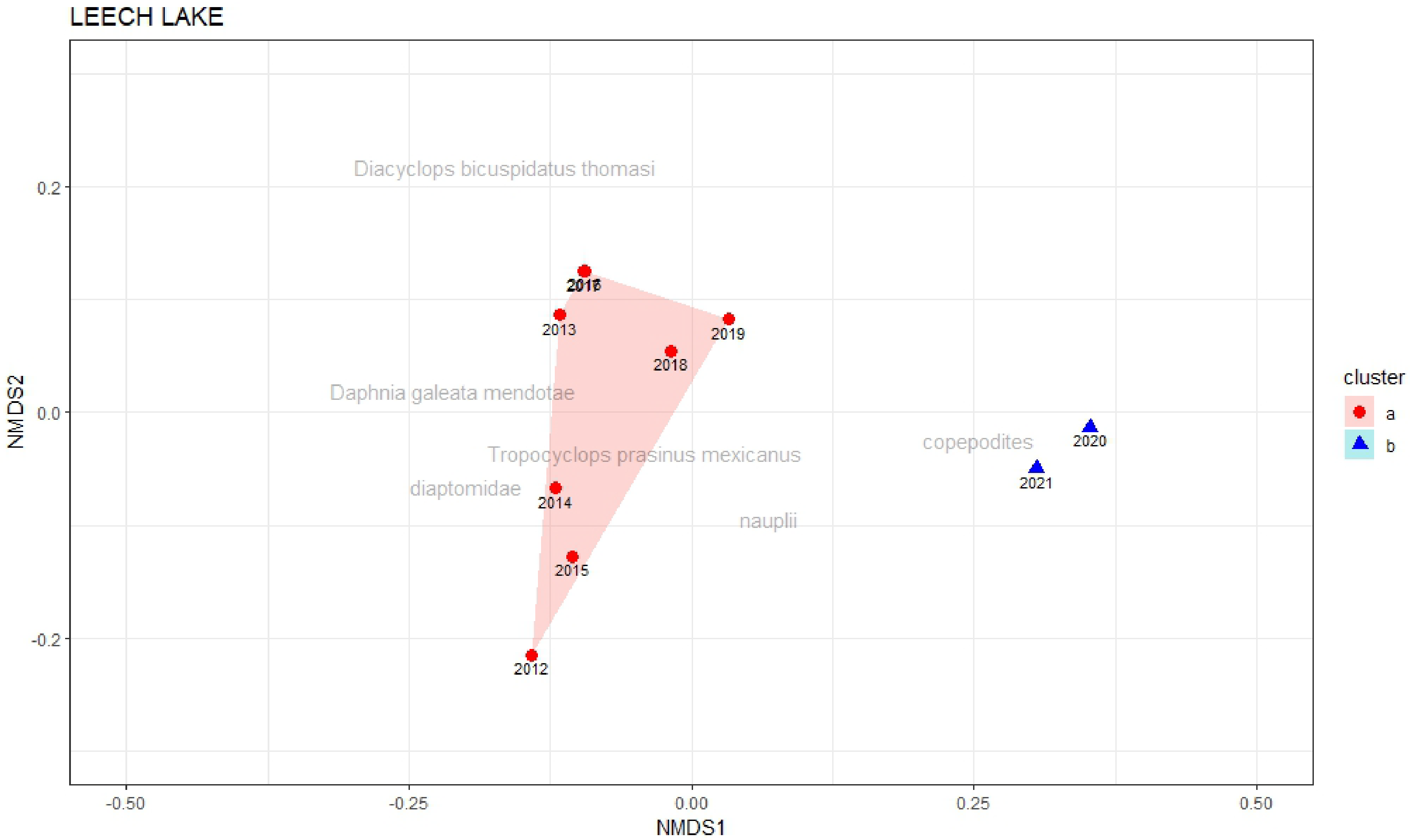


FIGURE 26.—A non-metric multidimensional scaling (NMDS) plot showing differences in zooplankton community structure in Leech Lake (2-D stress = 0.06). Symbols correspond to the zooplankton community in different years. Similar communities based on taxa and abundance are positioned closer together with the main taxa contributing to this pattern labeled in the direction of increasing abundance of those taxa.

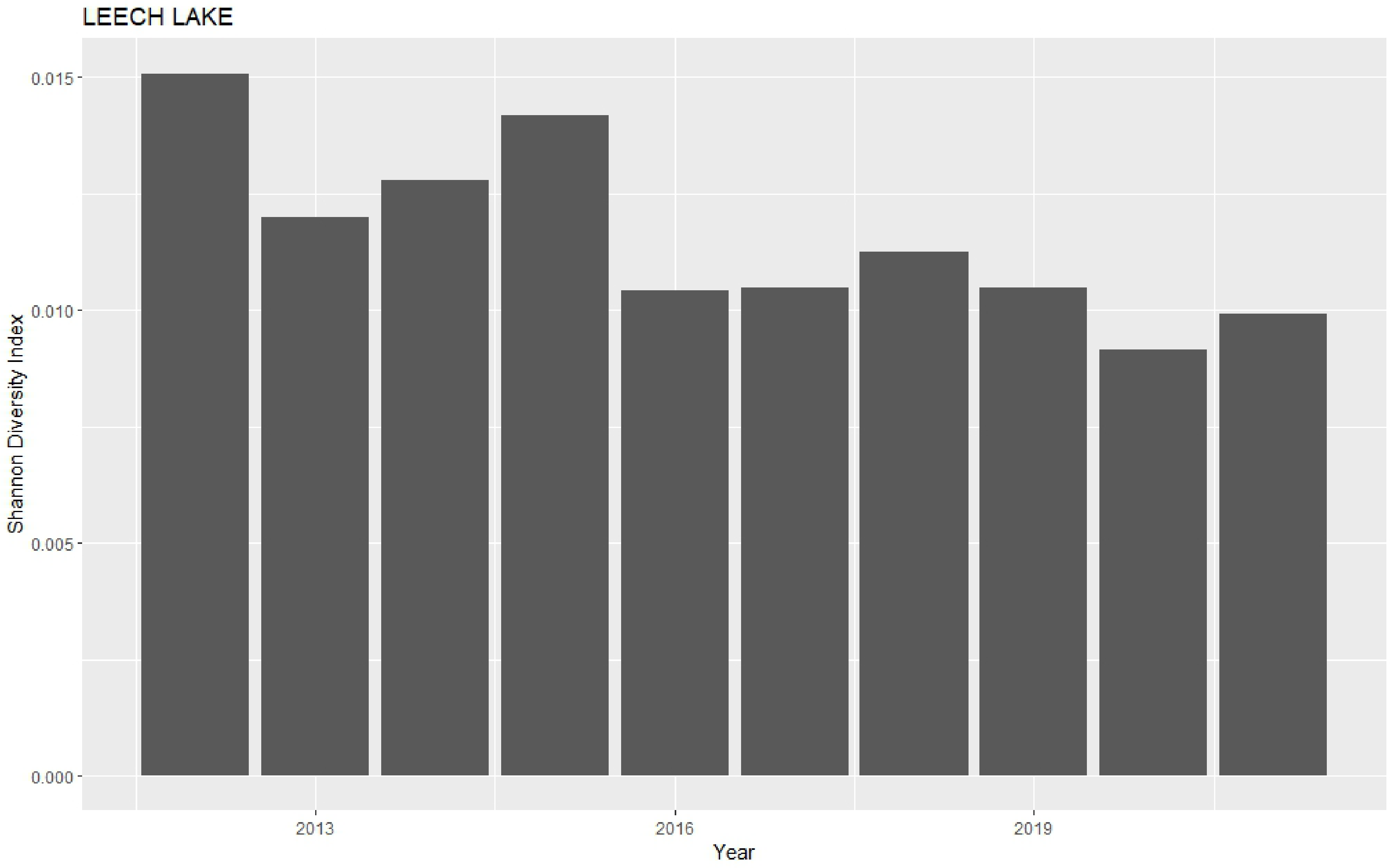


FIGURE 27.—Barplot summarizing the Shannon diversity index for the zooplankton community from 2012–2021 in Leech Lake.

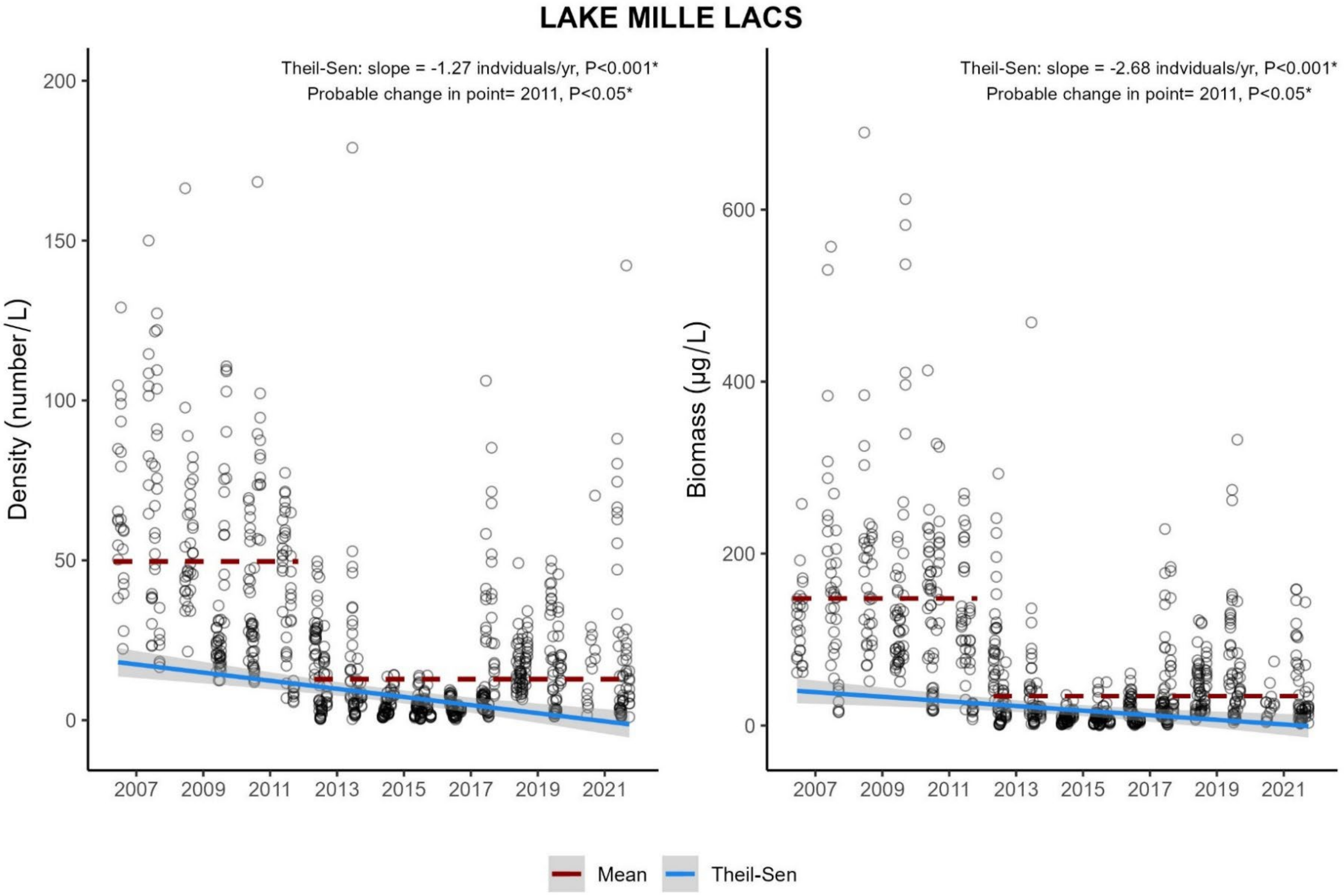


FIGURE 28.—Trend analyses show the total density (number/L) and biomass (µg/L) of zooplankton in Lake Mille Lacs. Hollow circles represent one sampling event. The Theil-Sen slope shows a significant decrease in density and biomass from 2006–2021. The Pettitt's test for single change-point detection was significant for density or biomass in 2011 (p-values< 0.05*).

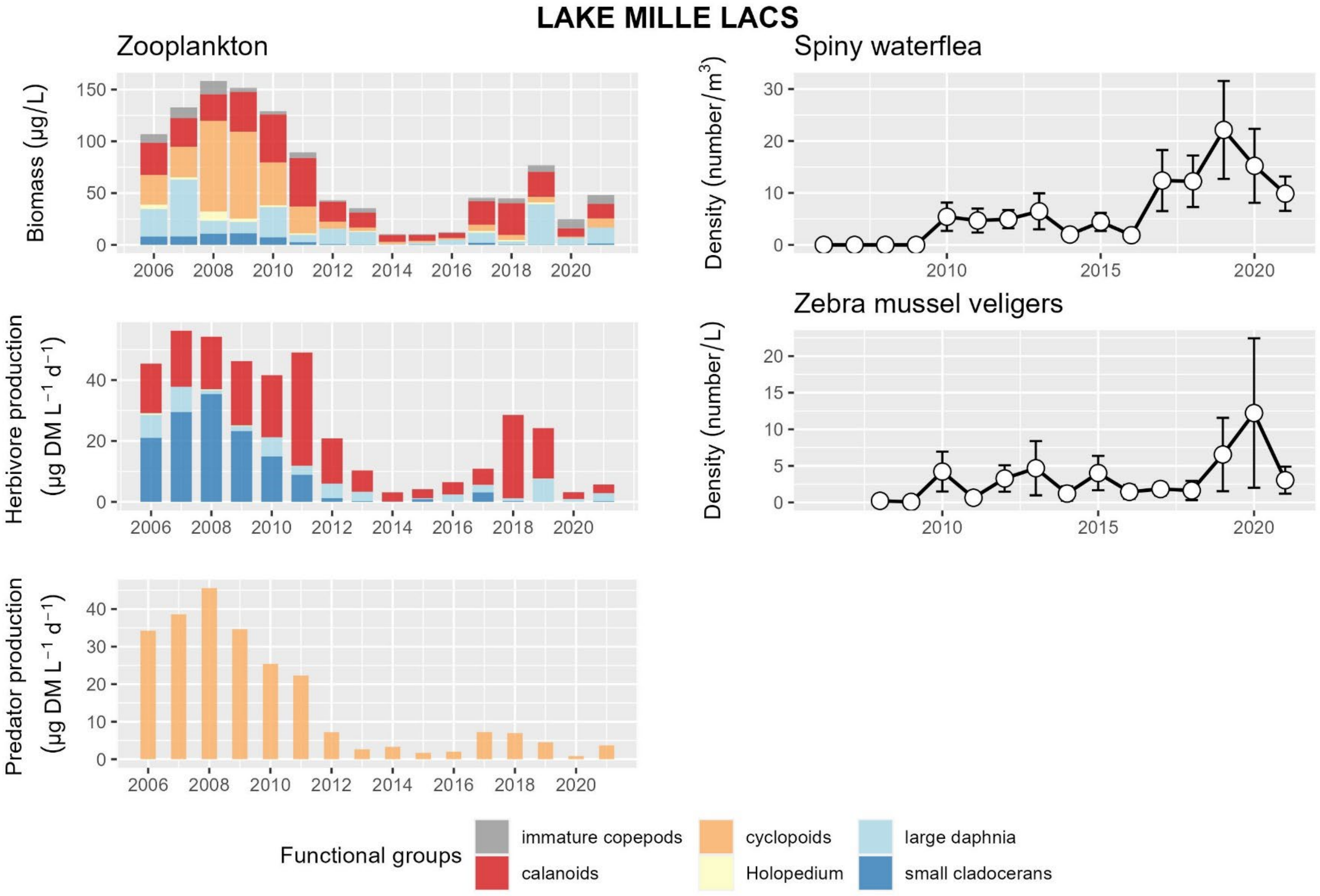


FIGURE 29.—Bar graphs show the annual mean biomass (μg/L) and secondary production (μg DM L^{-1} d^{-1}) of zooplankton in Lake Mille Lacs. Color bars represent different functional groups. Annual mean density of spiny waterflea (number/m^3) and zebra mussel veligers (number/L) are from zooplankton samples. Error bars represent one standard error.

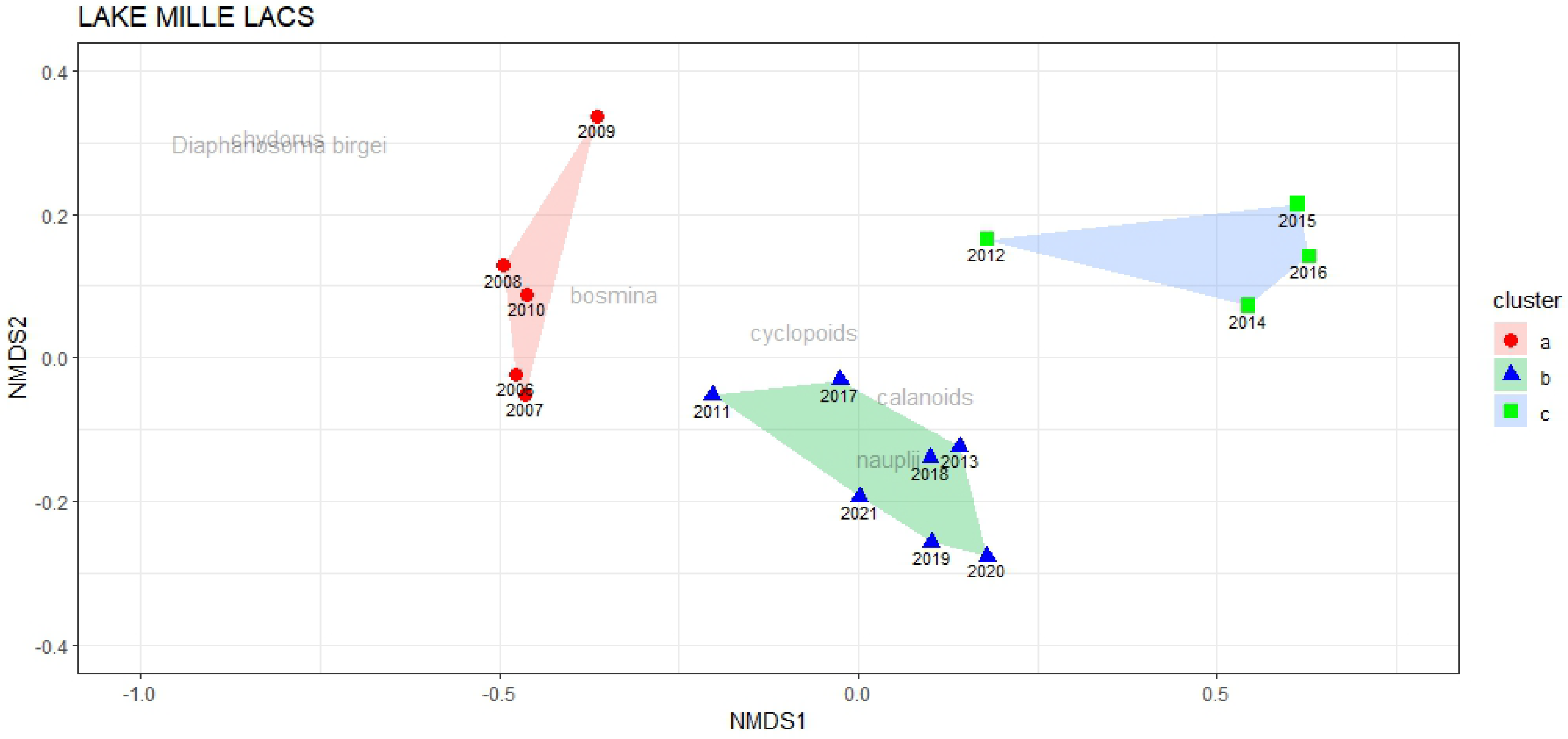


FIGURE 30.—A non-metric multidimensional scaling (NMDS) plot showing differences in zooplankton community structure in Lake Mille Lacs (2-D stress = 0.04). Symbols correspond to the zooplankton community in different years. Similar communities based on taxa and abundance are positioned closer together with the main taxa contributing to this pattern labeled in the direction of increasing abundance of those taxa.

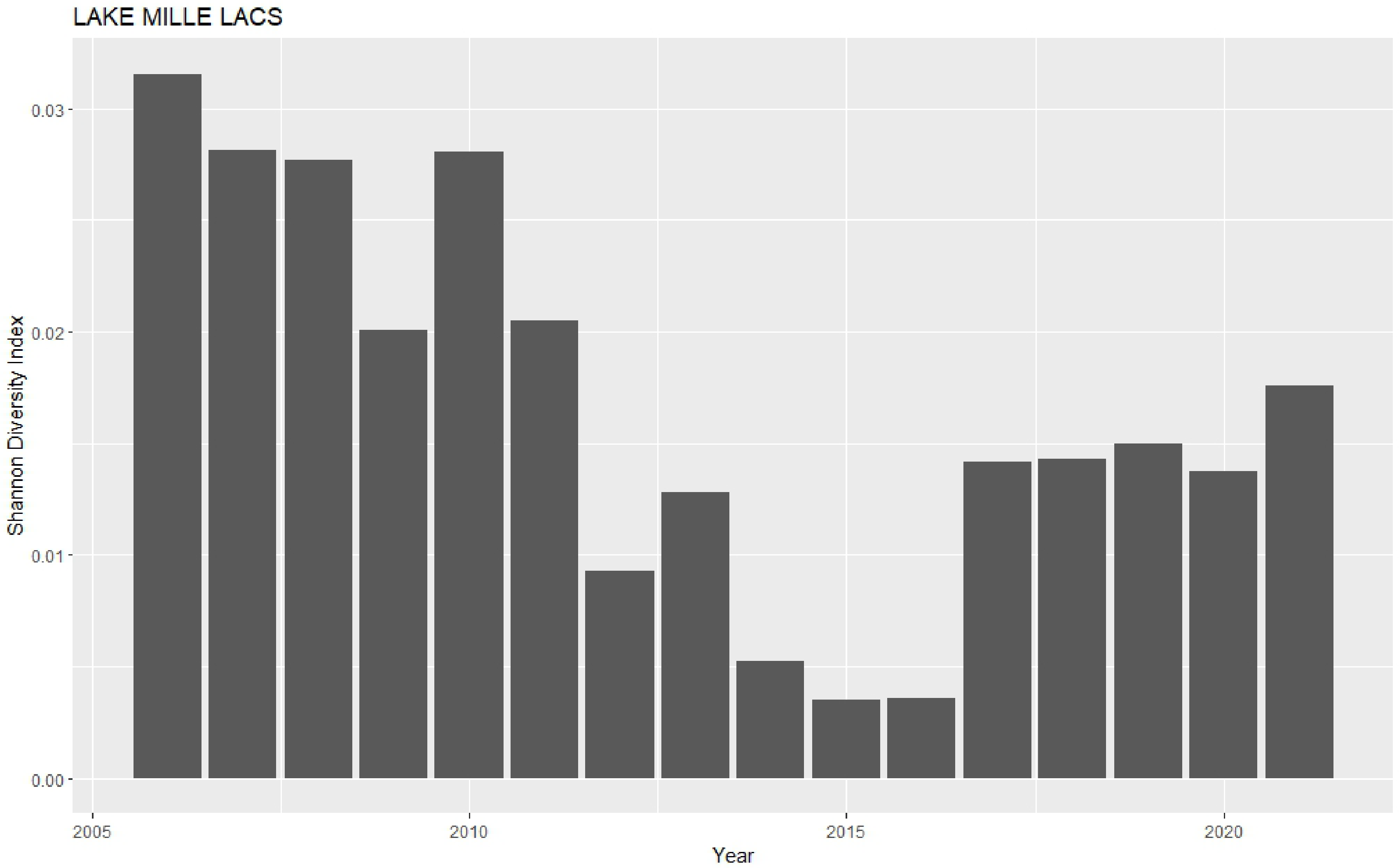


FIGURE 31.—Barplot summarizing the Shannon diversity index for the zooplankton community from 2006–2021 in Lake Mille Lacs.

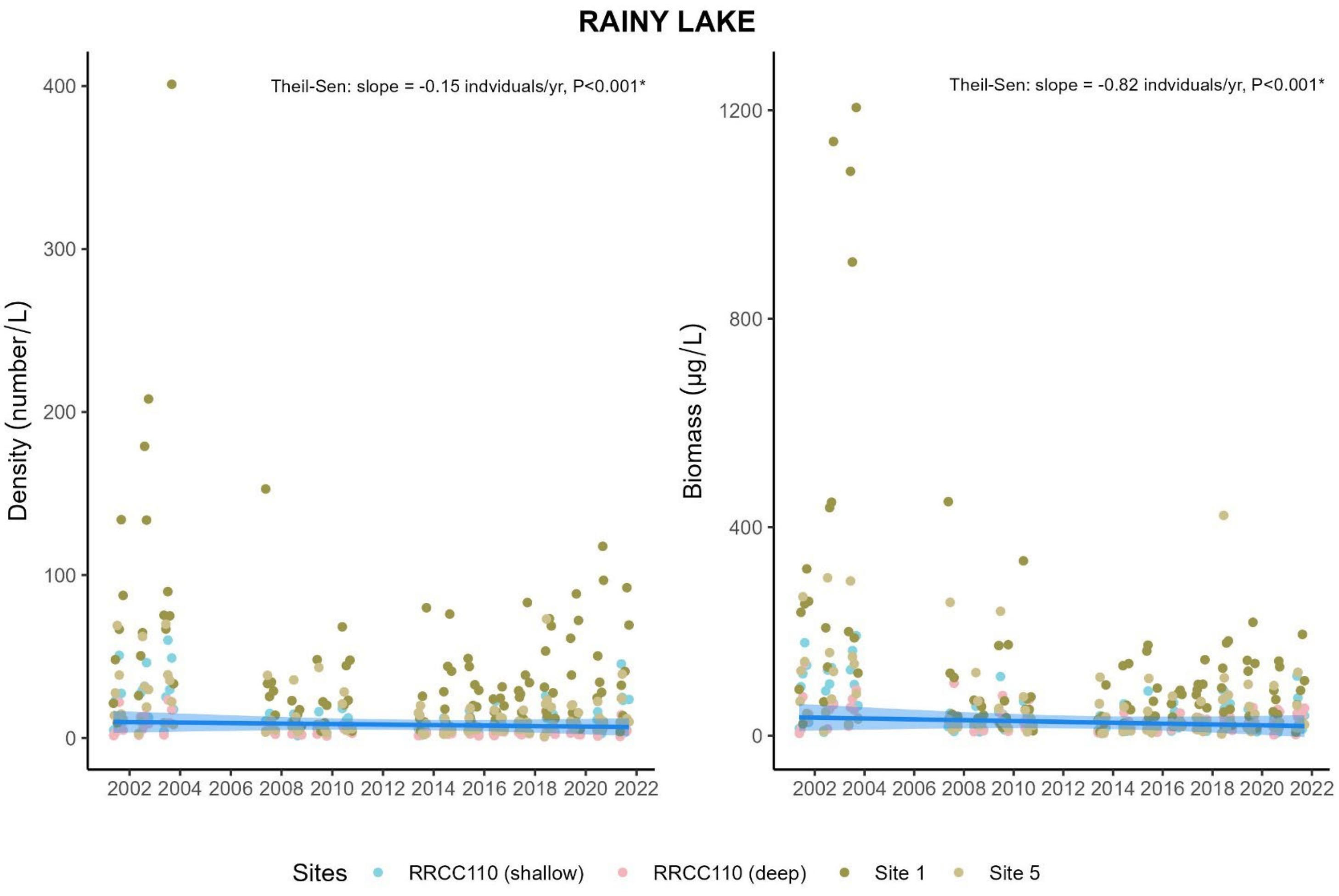


FIGURE 32.—Trend analyses show the total density (number/L) and biomass (µg/L) of zooplankton in Rainy Lake. Colored dots represent one sampling event and correspond to four collection sites. The Theil-Sen slope shows a significant decrease in density and biomass from 2001–2021.

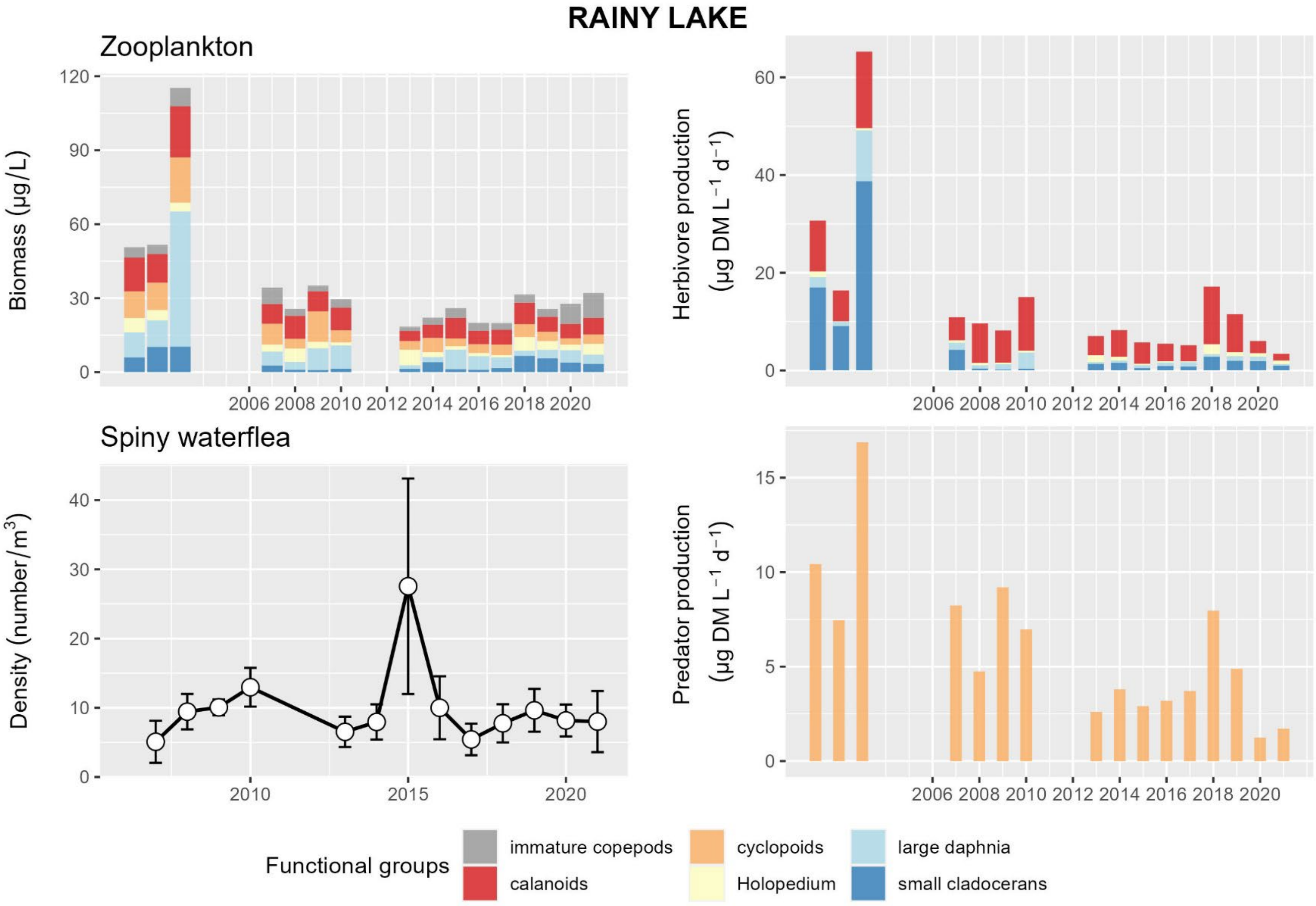


FIGURE 33.—Bar graphs show the annual mean biomass (μg/L) and secondary production (μg DM L^{-1} d^{-1}) of zooplankton in Rainy Lake. Color bars represent different functional groups. Annual mean density of spiny waterflea (number/m^3) are from zooplankton samples. Error bars represent one standard error.

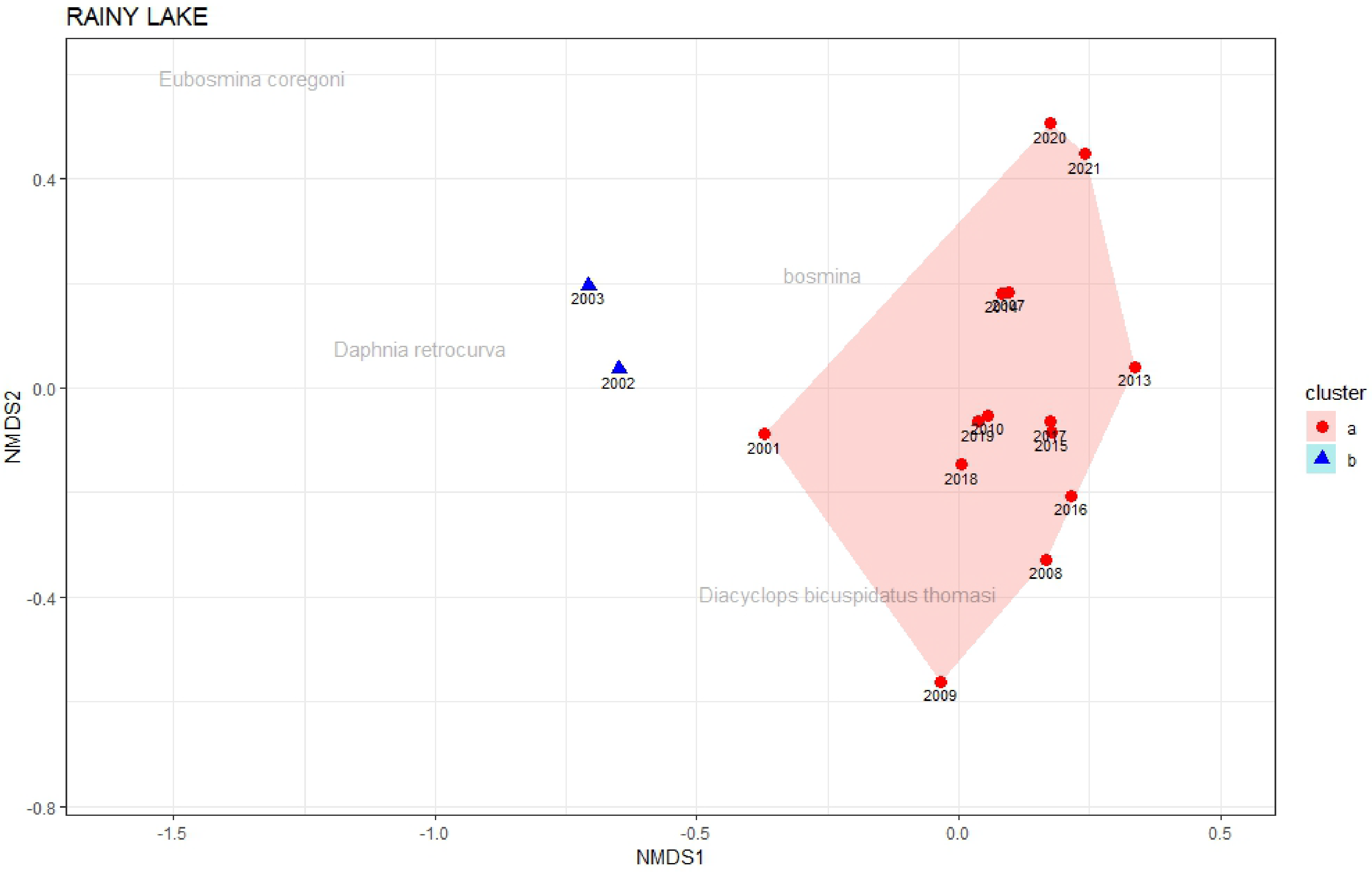


FIGURE 34.—A non-metric multidimensional scaling (NMDS) plot showing differences in zooplankton community structure in Rainy Lake (2-D stress = 0.09). Symbols correspond to the zooplankton community in different years. Similar communities based on taxa and abundance are positioned closer together with the main taxa contributing to this pattern labeled in the direction of increasing abundance of those taxa.

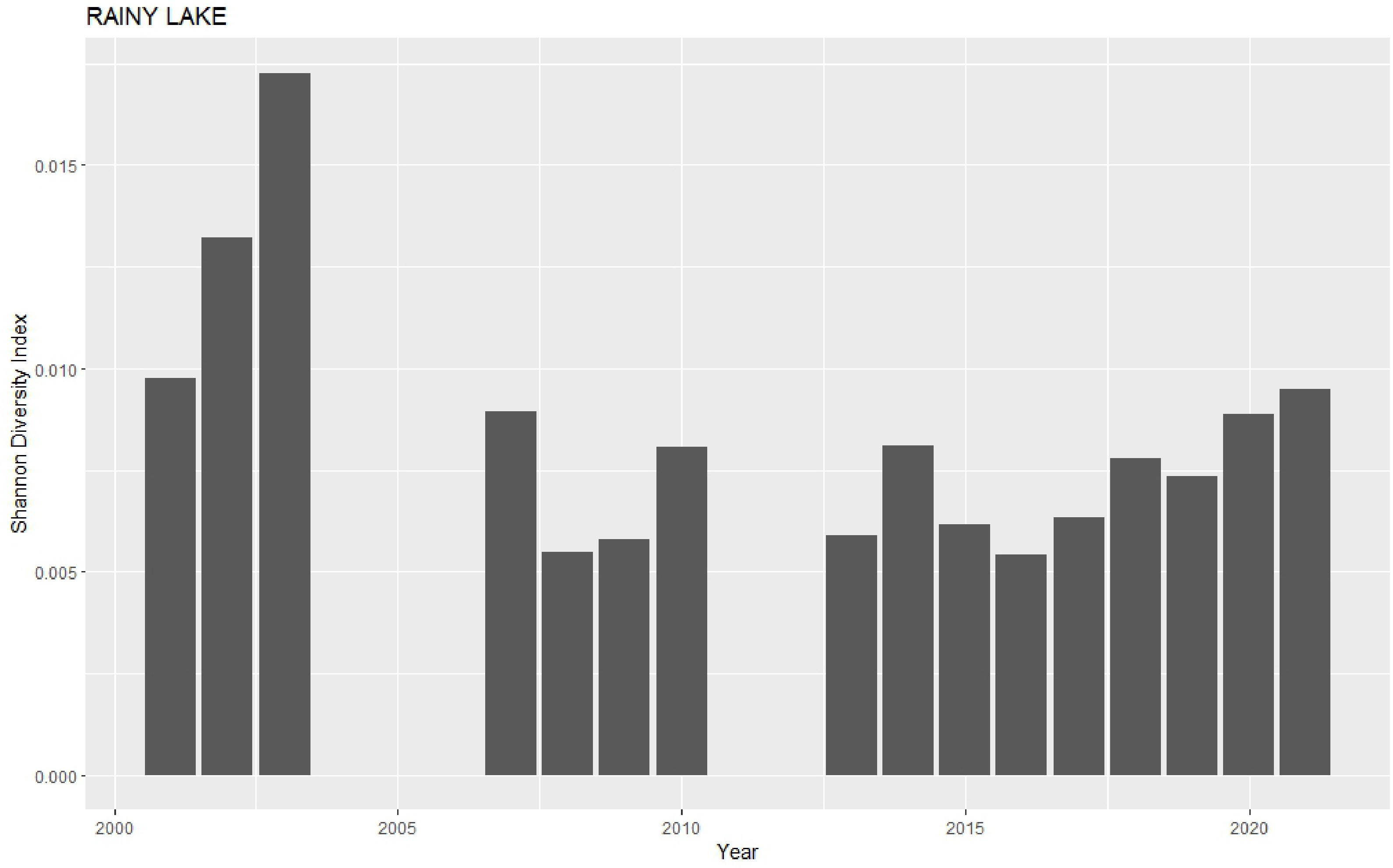


FIGURE 35.—Barplot summarizing the Shannon diversity index for the zooplankton community from 2001–2021 in Rainy Lake.

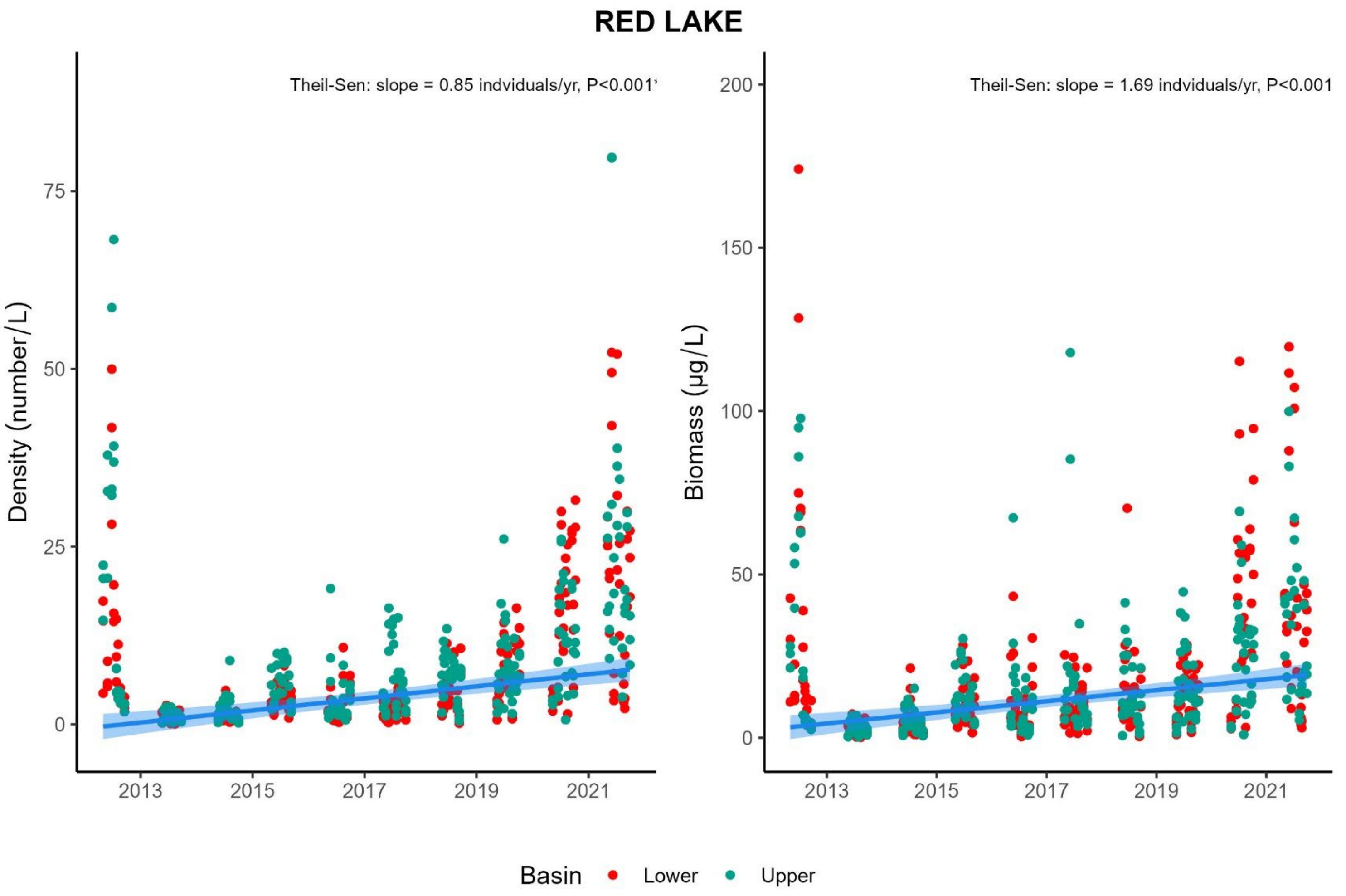


FIGURE 36.—Trend analyses show the total density (number/L) and biomass (µg/L) of zooplankton in Red Lake. Colored dots represent one sampling event and correspond to the different basins (lower and upper). The Theil-Sen slope shows a significant increase in density and biomass from 2012–2021.

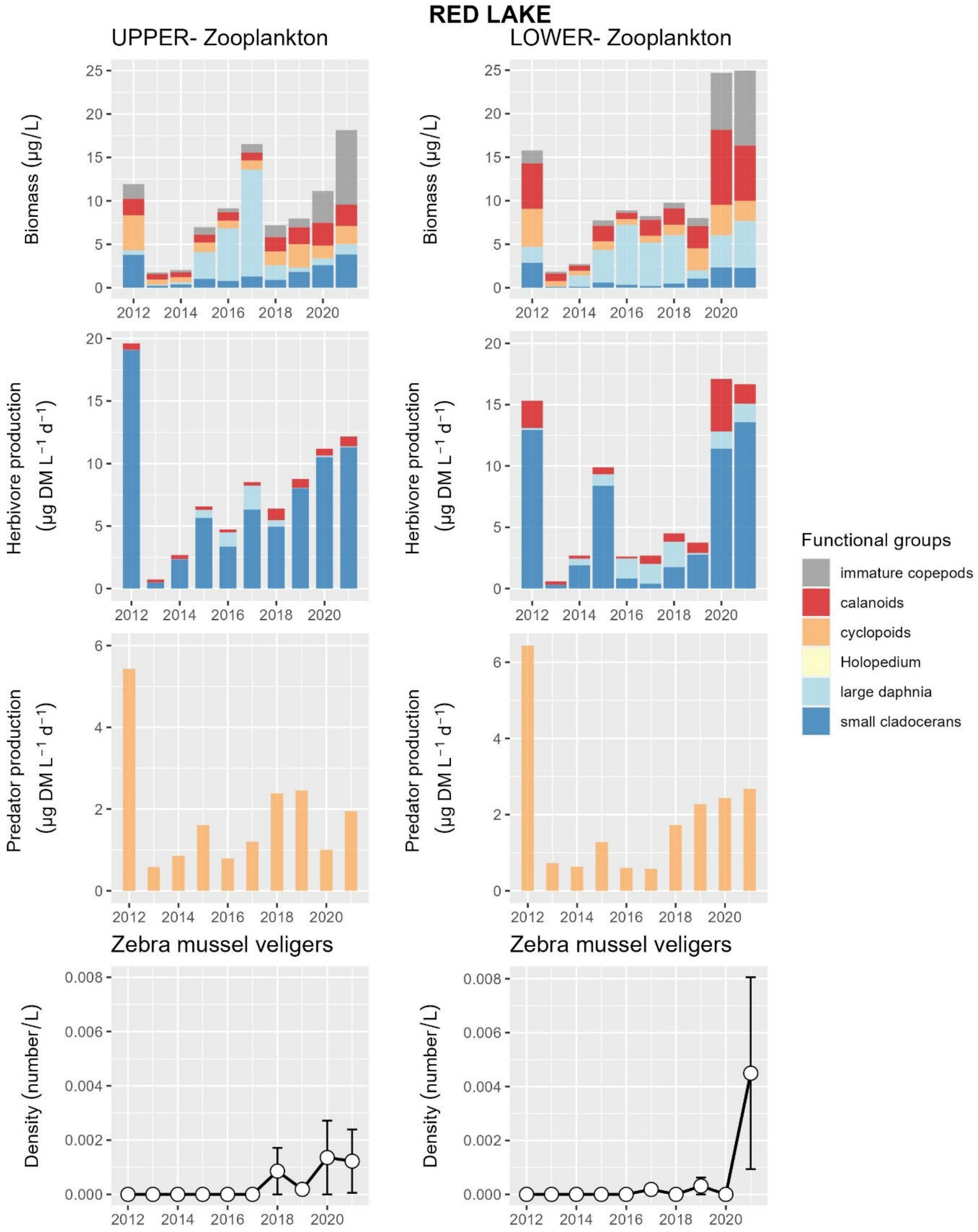


FIGURE 37.—Bar graphs show the annual mean biomass (µg/L) and secondary production (µg DM L^{-1} d^{-1}) of zooplankton in Red Lake (upper and lower basins). Color bars represent different functional groups. Annual mean density of zebra mussel veligers (number/L) are from zooplankton samples. Error bars represent one standard error.

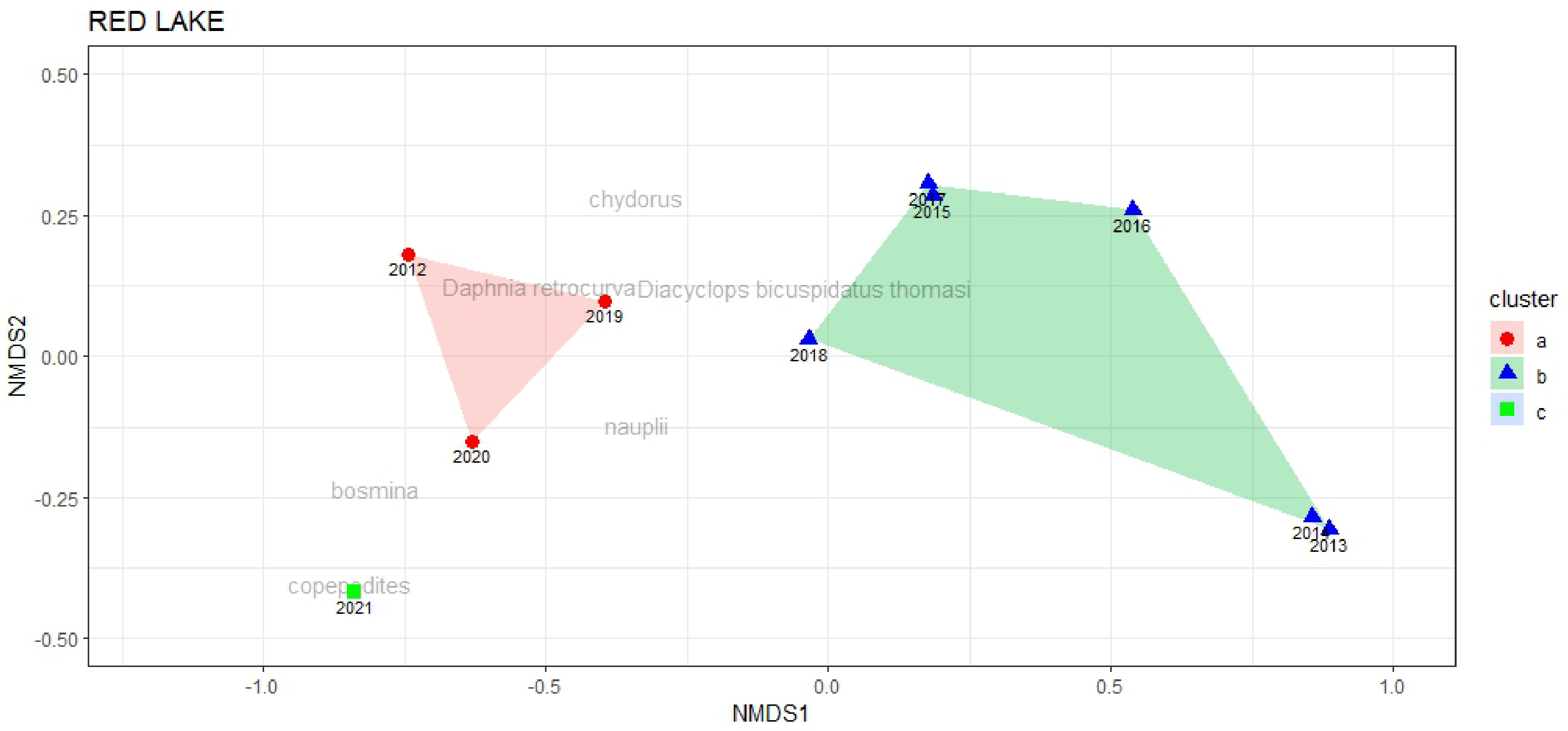


FIGURE 38.—A non-metric multidimensional scaling (NMDS) plot showing differences in zooplankton community structure in Red Lake (2-D stress = 0.006). Symbols correspond to the zooplankton community in different years. Similar communities based on taxa and abundance are positioned closer together with the main taxa contributing to this pattern labeled in the direction of increasing abundance of those taxa.

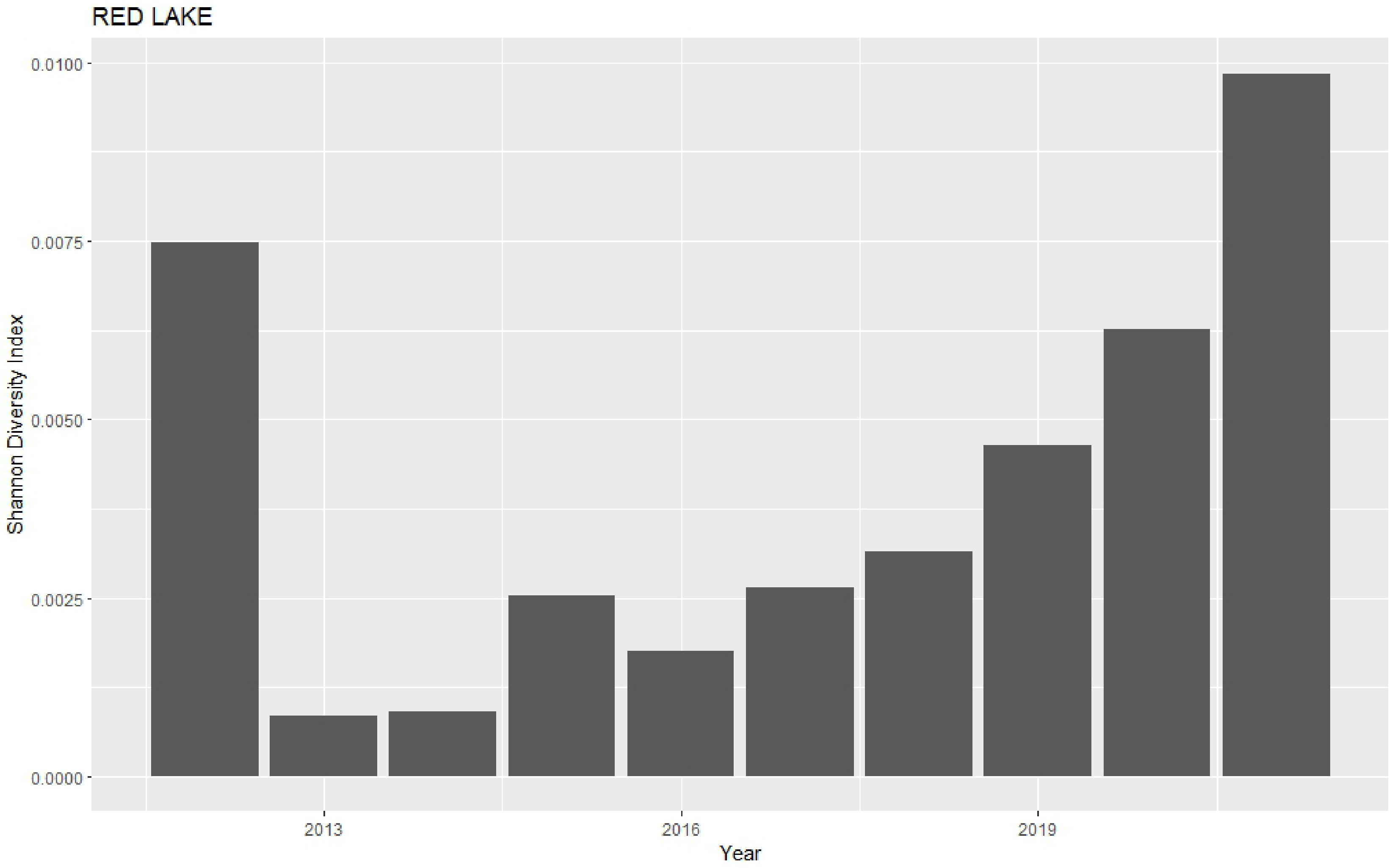


FIGURE 39.—Barplot summarizing the Shannon diversity index for the zooplankton community from 2012–2021 in Red Lake.

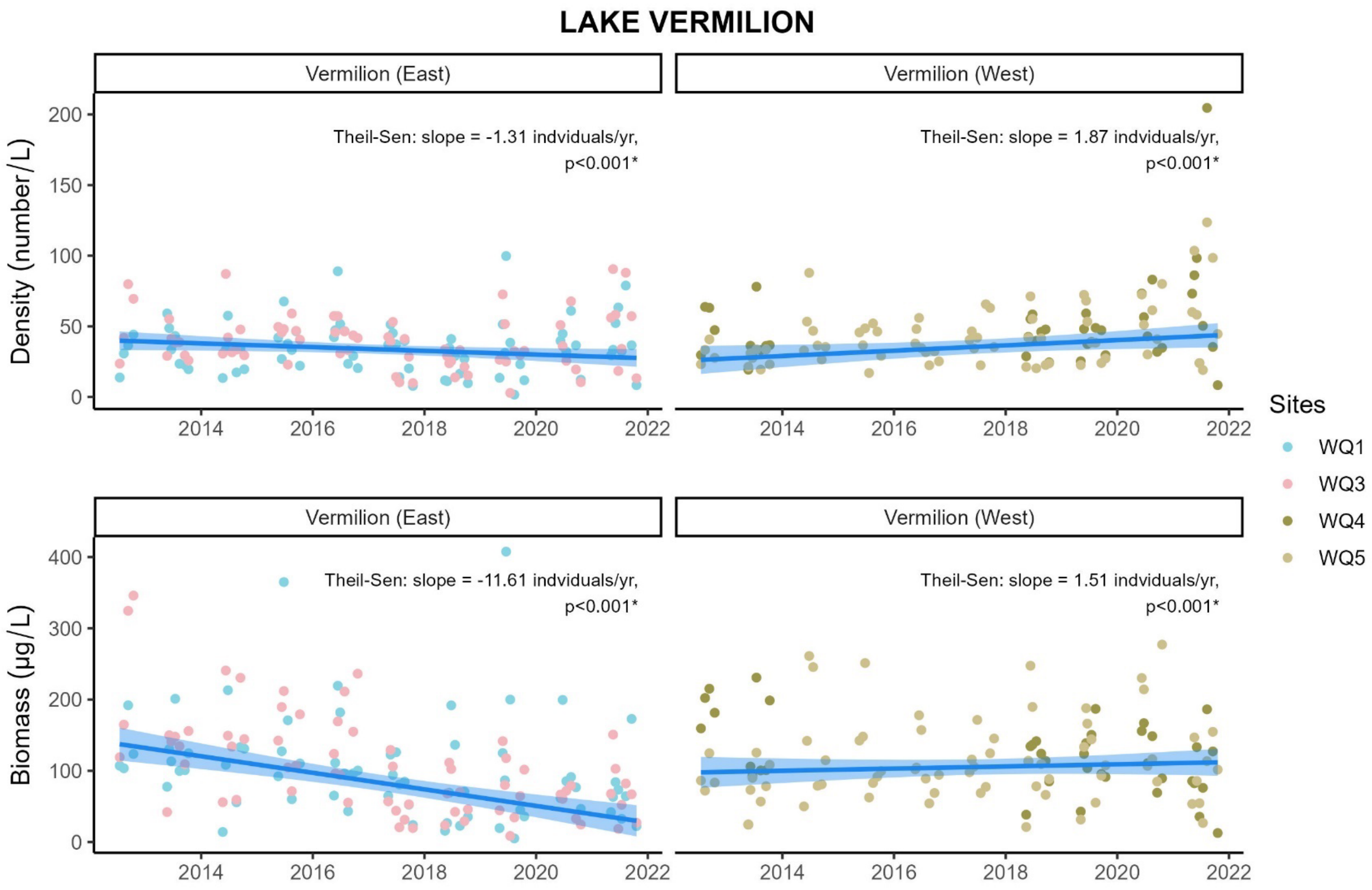


FIGURE 40.—Trend analyses show the total density (number/L) and biomass (µg/L) of zooplankton in Lake Vermilion (East and West basin). Colored dots represent one sampling event and correspond to four collection sites. In the East basin, the Theil-Sen slope shows a significant decrease in density and biomass from 2012–2021. In the est basin, the Theil-Sen slope shows a significant increase in density and biomass from 2012–2021.

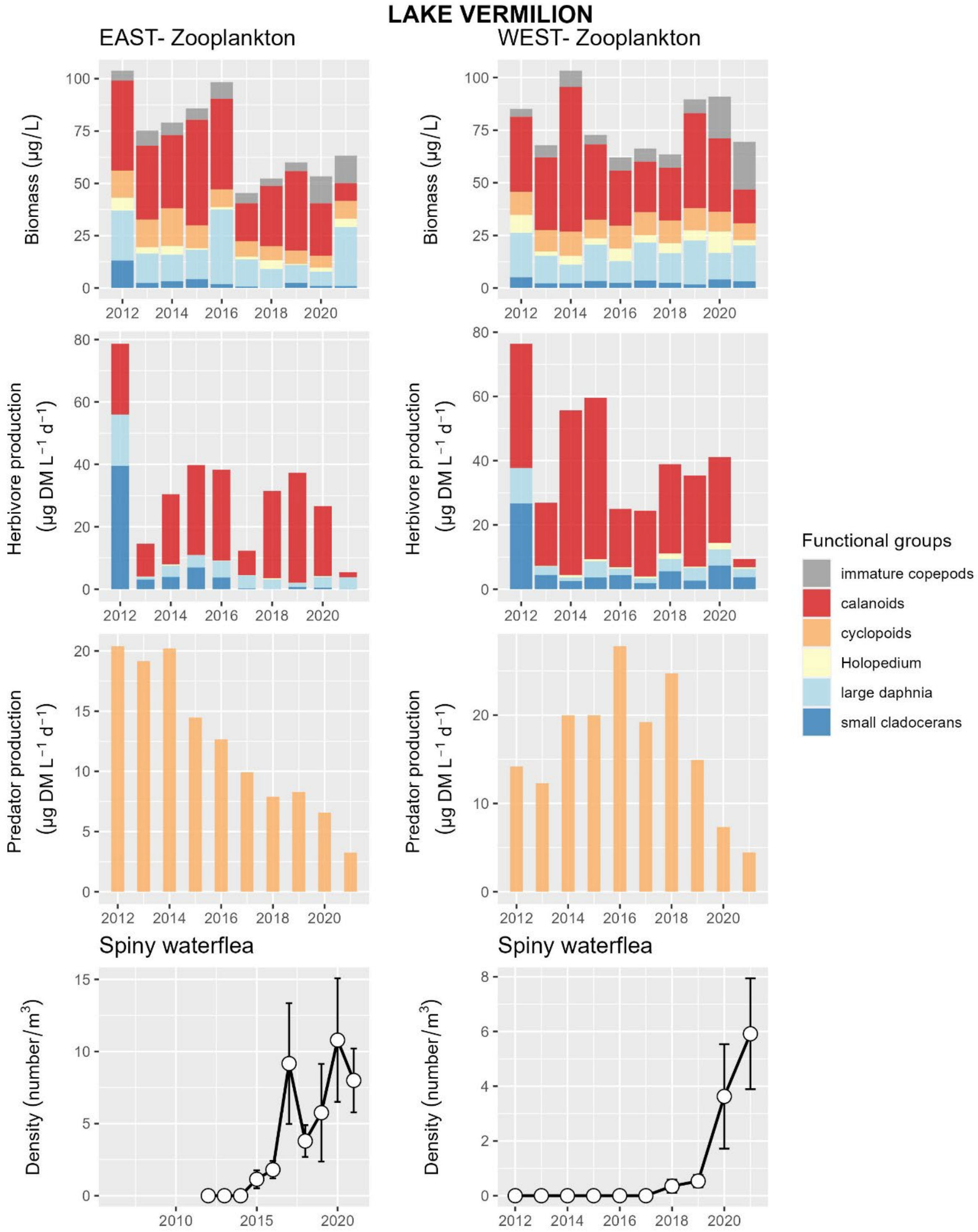


FIGURE 41.—Bar graphs show the annual mean biomass (µg/L) and secondary production (µg DM L^{-1} d^{-1}) of zooplankton in Lake Vermilion (East and West basins). Color bars represent different functional groups. Annual mean density of spiny waterflea (number/m^3) are from zooplankton samples. Error bars represent one standard error.

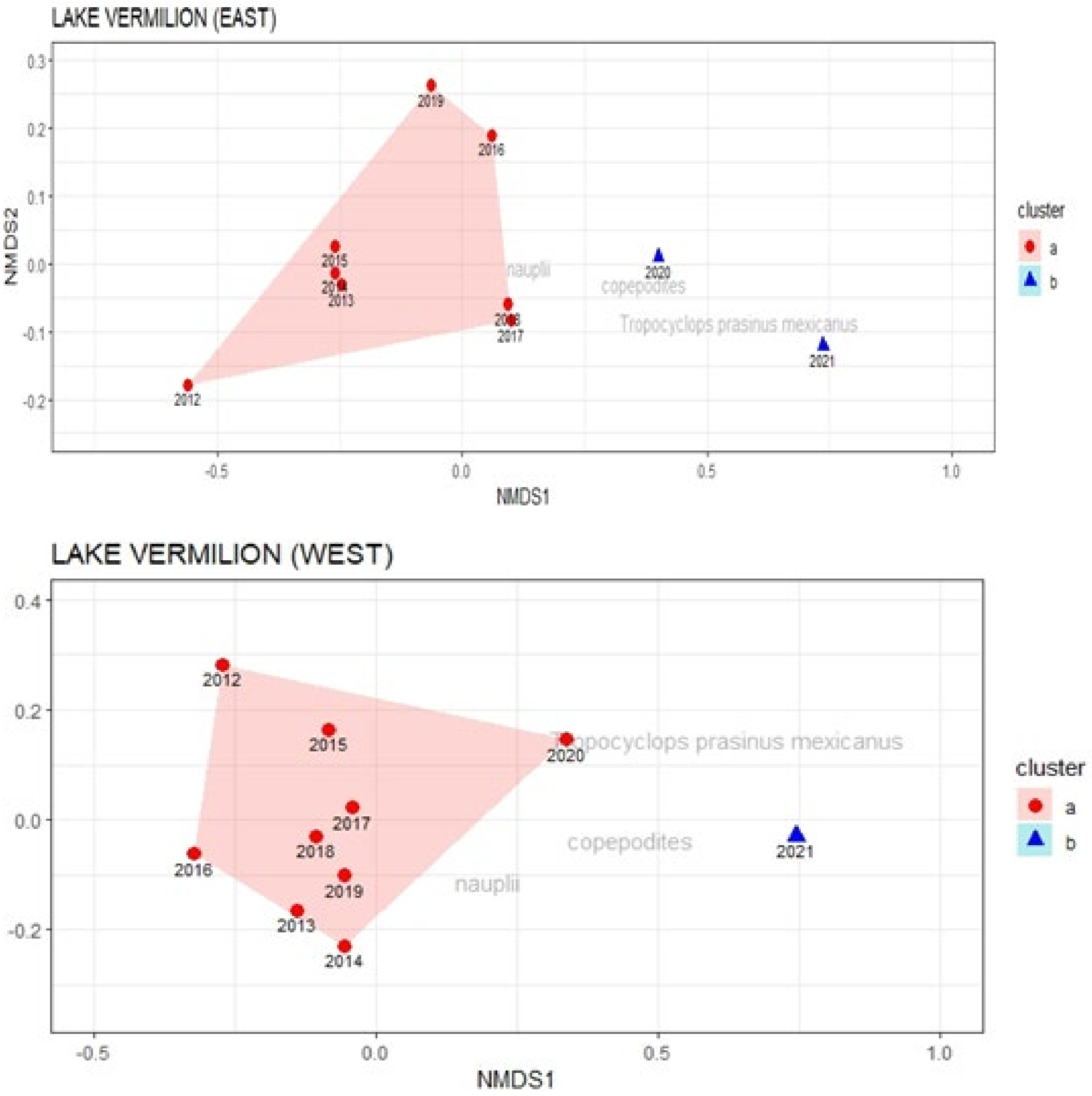


FIGURE 42.—A non-metric multidimensional scaling (NMDS) plot showing differences in zooplankton community structure in Lake Vermilion (East and West basins; 2-D stress = 0.04 and 0.06). Symbols correspond to the zooplankton community in different years. Similar communities based on taxa and abundance are positioned closer together with the main taxa contributing to this pattern labeled in the direction of increasing abundance of those taxa.

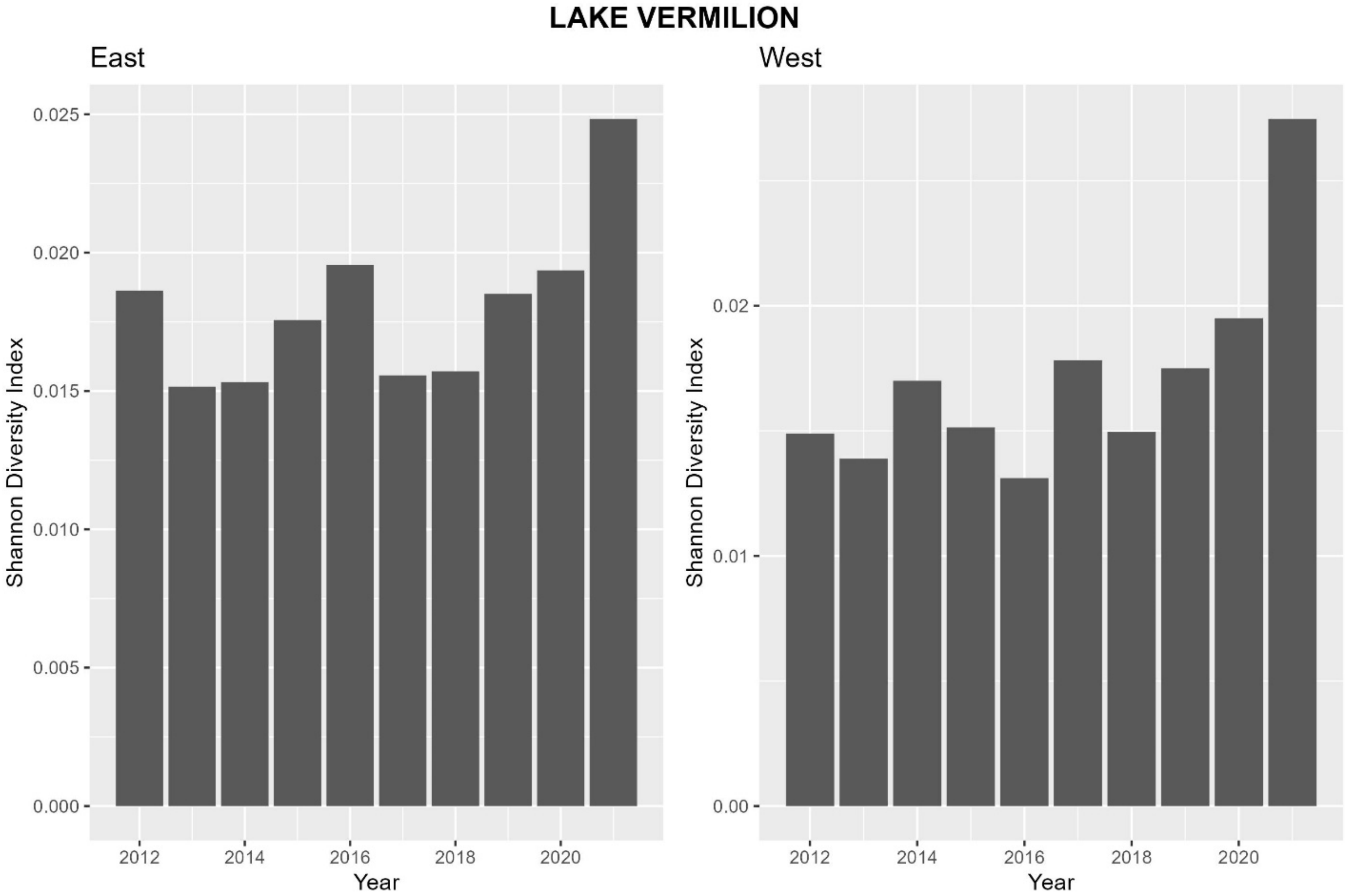


FIGURE 43.—Barplot summarizing the Shannon diversity index for the zooplankton community from 2012–2021 in Lake Vermilion (East and West basin).

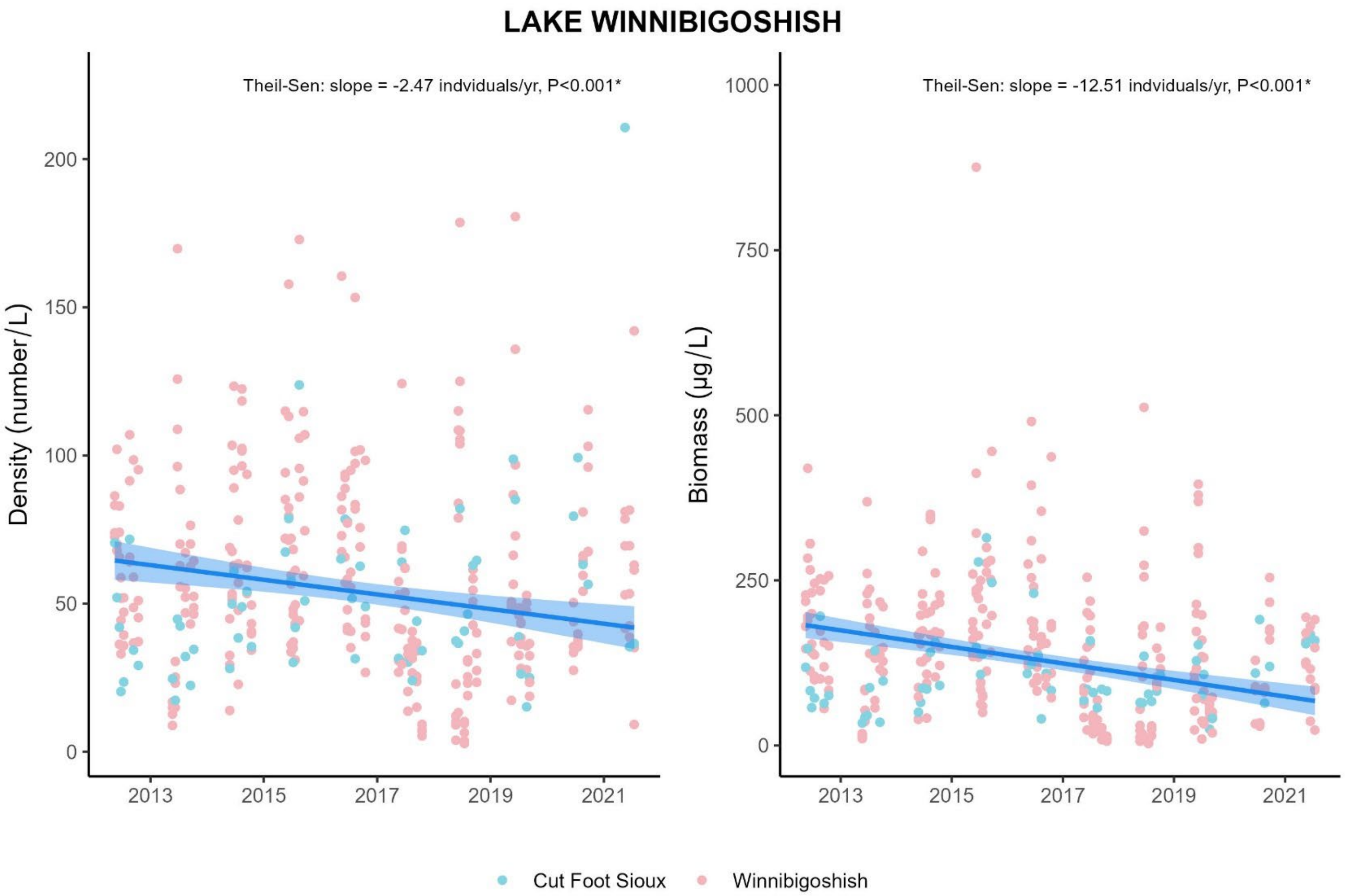


FIGURE 44.—Trend analyses show the total density (number/L) and biomass (µg/L) of zooplankton in Lake Winnibigoshish and Cut Foot Sioux. Colored dots represent one sampling event and correspond the two different waterbodies. Tthe Theil-Sen slope shows a significant decrease in density and biomass from 2012–2021.

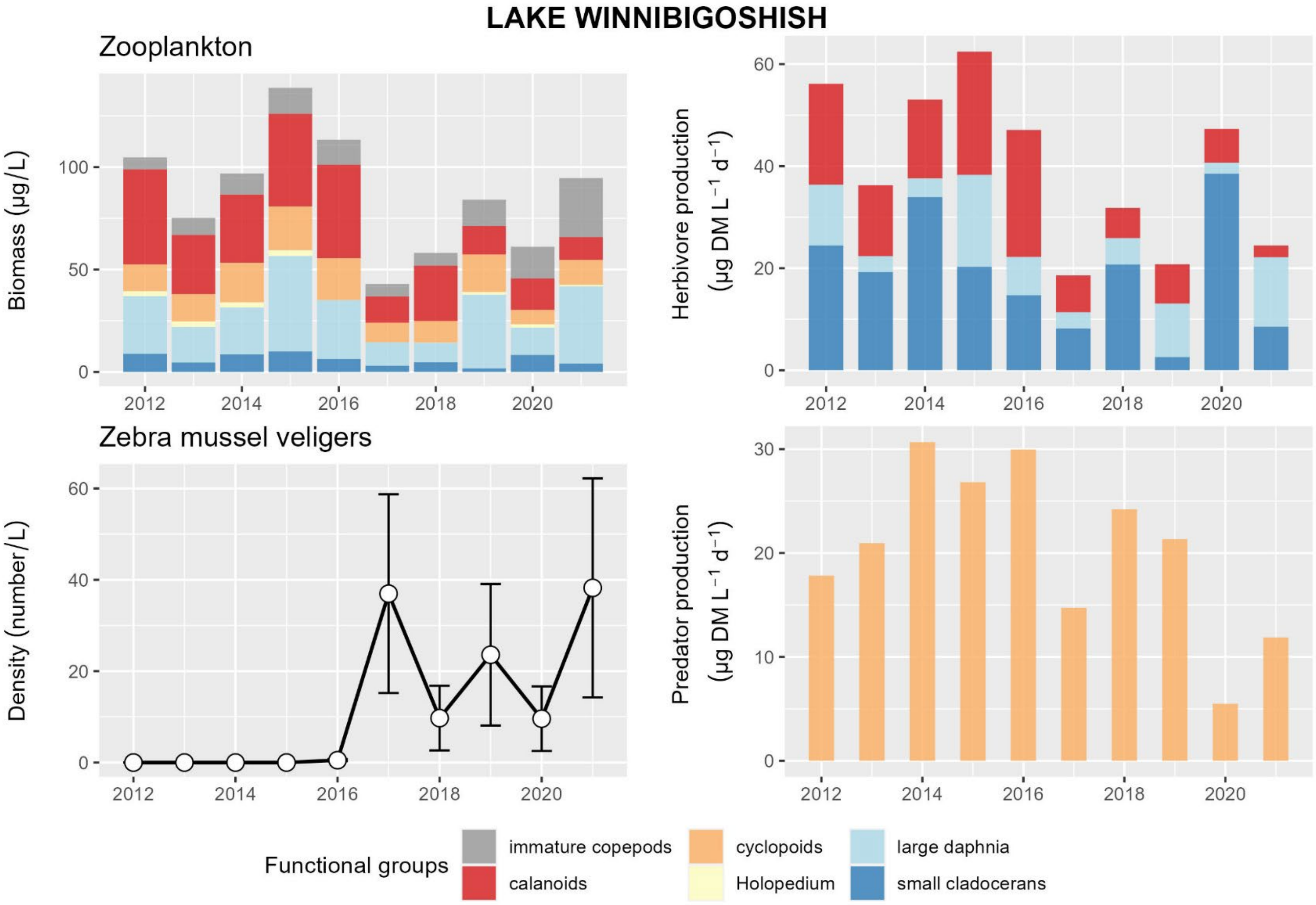


FIGURE 45.—Bar graphs show the annual mean biomass (µg/L) and secondary production (µg DM L^{-1} d^{-1}) of zooplankton in Lake Winnibigoshish and Cut Foot Sioux. Color bars represent different functional groups. Annual mean density of zebra mussel veligers (number/L) are from zooplankton samples. Error bars represent one standard error.

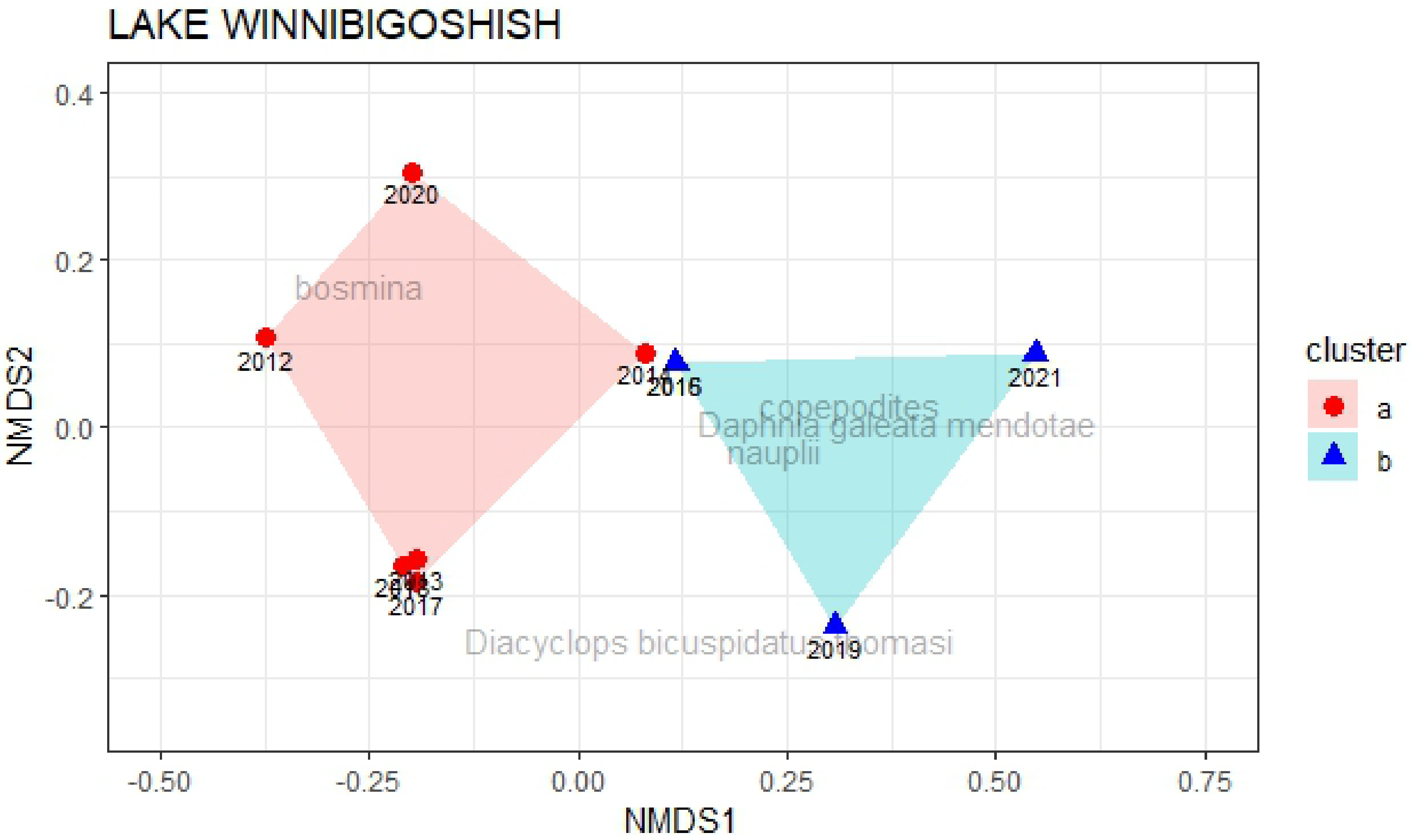


FIGURE 46.—A non-metric multidimensional scaling (NMDS) plot showing differences in zooplankton community structure in Lake Winnibigoshish and Lake Cut Foot Sioux (2-D stress = 0.08). Symbols correspond to the zooplankton community in different years. Similar communities based on taxa and abundance are positioned closer together with the main taxa contributing to this pattern labeled in the direction of increasing abundance of those taxa.

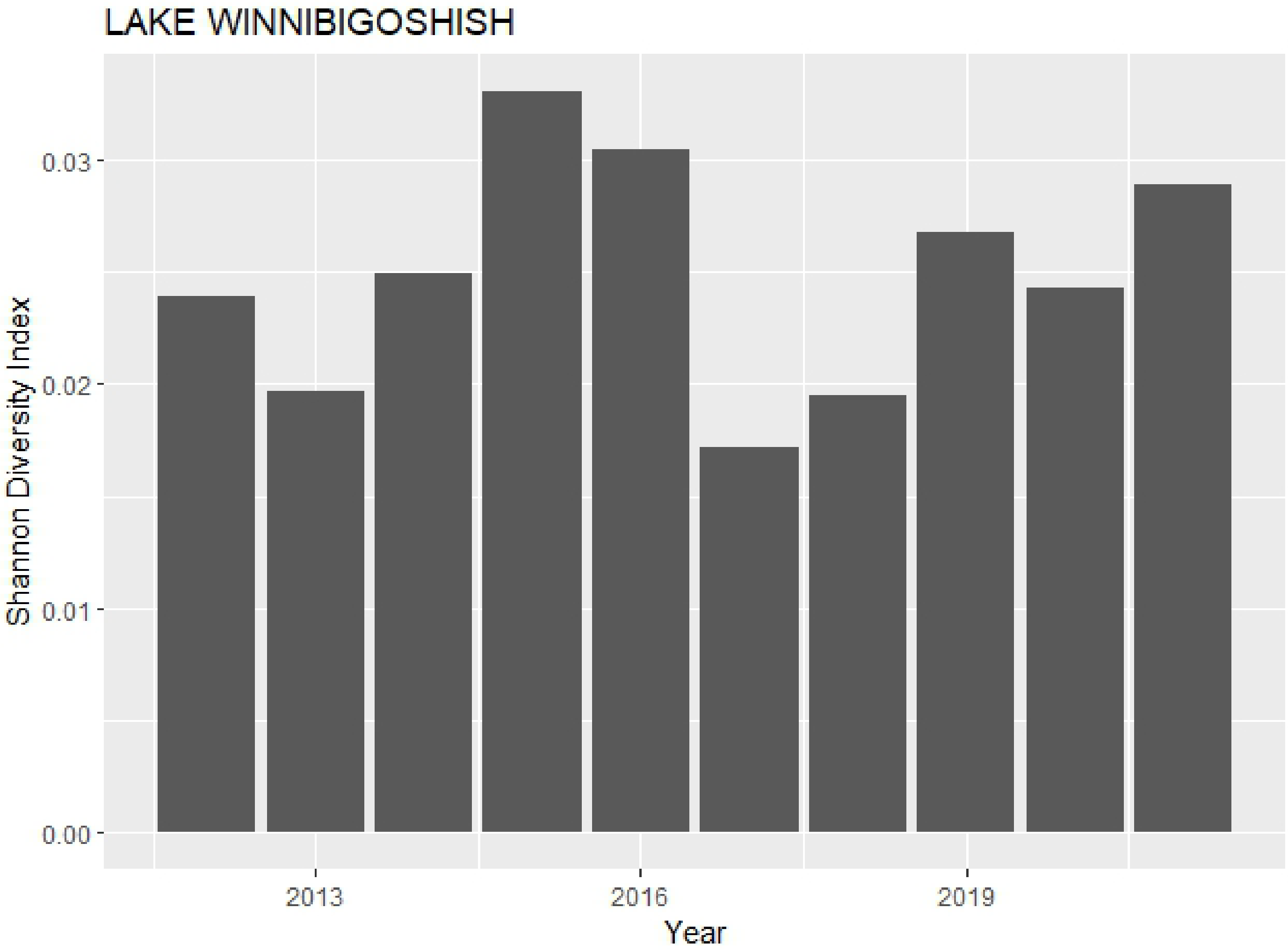
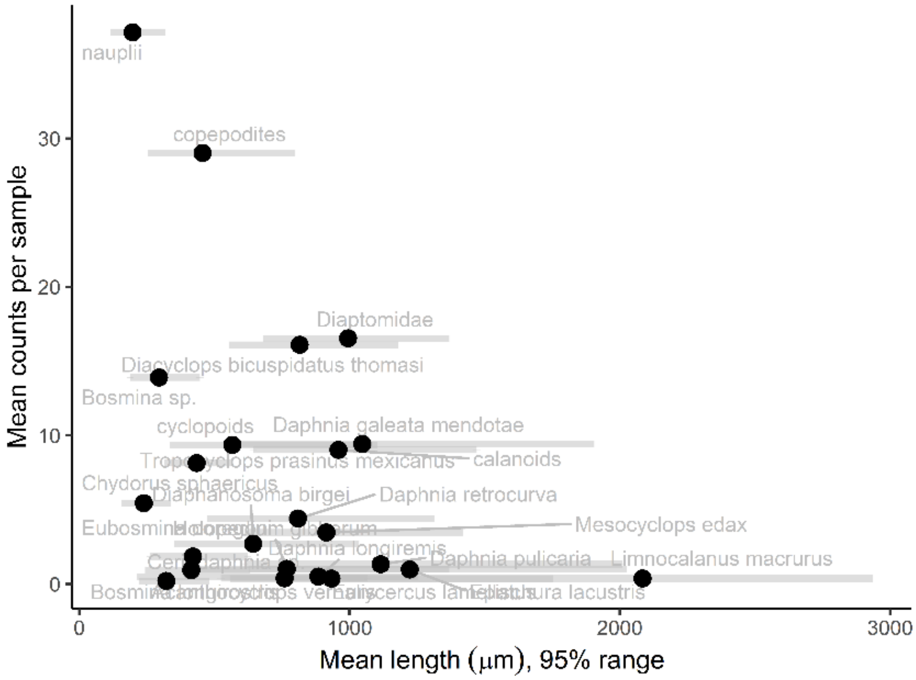


FIGURE 47.—Barplot summarizing the Shannon diversity index for the zooplankton community from 2012–2021 in Lake Winnibigoshish and Lake Cut Foot Sioux.

a) Length measurements



b) Dry weight

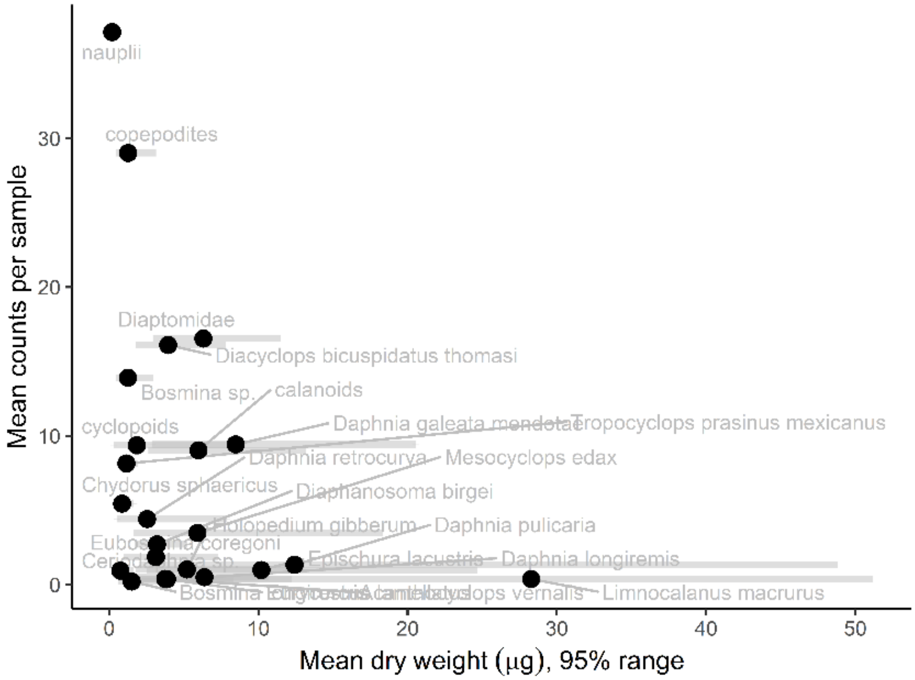


FIGURE 48.—Average number of animals counted for individual taxa in samples (gray points) and range of 95% of measurements of **(a)** individual length (μm) and **(b)** dry weight (μg). Spiny waterflea (Bythotrephes cederströemi, synonym Bythotrephes longimanus) was removed from the plot because it is exceptionally large, variable, and infrequently counted. The number of samples varies by taxon.

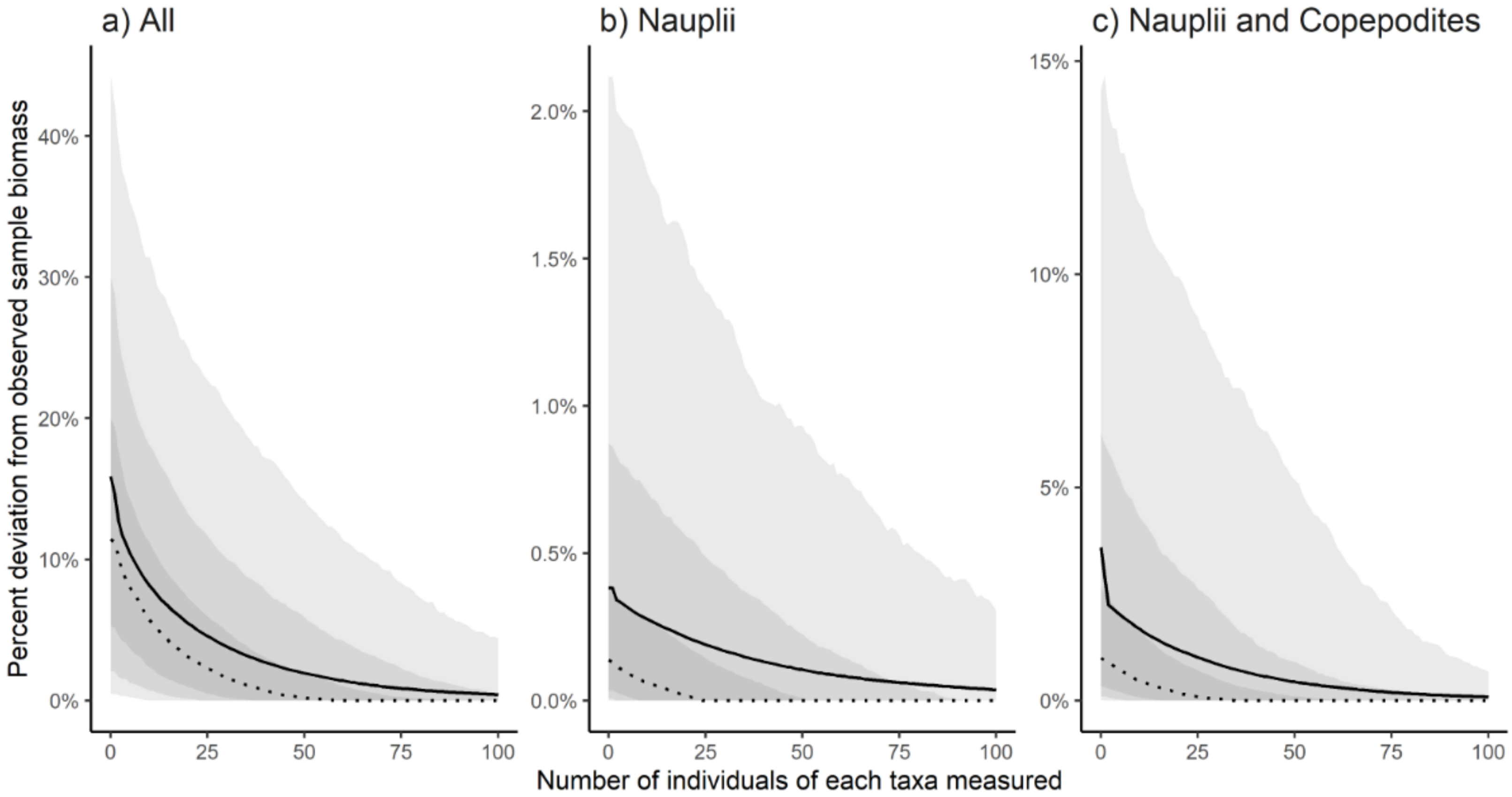


FIGURE 49.—Percent deviation of total biomass estimates from observed sample biomass under three different measurement effort scenarios. Results are plotted as the number of each taxon measured in each sample on the x-axis and the resulting sample biomass estimate as the percent deviation from the observed sample biomass on the y-axis (mean is plotted with a solid line, median is plotted as a dotted line, and shaded polygons represent the 50%, 80%, and the 95% ranges of all samples). Note the differing y-axis ranges. The three scenarios included measuring **(a)** some number of individuals for all taxa, **(b)** specified number of nauplii, plus all individuals of other taxa, and **(c)** a specified number of nauplii and copepodites, plus all individuals of other taxa.

APPENDICES

TABLE A.1.— Summary of sample recommendations for zooplankton monitoring in the Large Lakes. Current sample schedule shows the typical number of samples collected by month for each lake. Proposed sample schedule is a suggestion for the next 10 years based on the power analysis but is subject to change.

Lake name	Current sample schedule							Proposed sample schedule						
	MAY	JUN	JUL	AUG	SEPT	OCT	TOTAL	MAY	JUN	JUL	AUG	SEPT	OCT	TOTAL
Cass & Andrusia	9	8	4	4	4	4	33	5	4	2	2	2	0	15
Kabetogama	1	2	1	1	1	0	6	no change						6
Lake of the Woods	3	6	6	6	3	0	24	6	6	3	3	3	0	21
Leech	10	10	5	5	5	5	40	no change						40
Mille Lacs	18	18	9	9	9	0	63	10	10	5	5	5	0	35
Rainy	5	9	5	5	5	0	29	no change						29
Red (Upper and Lower)	12	12	12	12	12	6	66	12	12	6	6	6	0	42
Vermilion (East and West)	8	8	4	4	4	4	32	10	10	5	5	5	0	35
Winnibigoshish	10	10	5	5	5	0	35	8	8	4	4	4	0	28
						Total samples	328						Total samples	251

TABLE A.2.—Summary of zooplankton sampling effort in Minnesota's Large Lakes from 1994-2021. Additional data collected for special projects are not shown here. Standard collection methods include sampling from May through October with a 30 cm diameter, 80 µm mesh plankton net unless otherwise noted. The number of sites that were sampled varied depending on the sample event. [a] 153µm mesh net, [b] 150µm mesh net, [c] 13cm diameter net

Year sampled	Total sites	Total sample events (May – Oct)	Total samples
CASS			
2012	4	8	32
2013	4	6	23
2014	4	7	28
2015	4	8	32
2016	4	7	28
2017	4	8	32
2018	4	5	20
2019	4	7	28
2020	4	7	28
2021	4	5	20
KABETOGAMA			
2001[a]	3	7	15
2002[a]	3	7	17
2003[a]	3	6	18
2007[a]	1	6	6
2008[a]	1	6	6
2009[a]	1	6	6
2010[a]	1	5	5
2013[a]	1	6	6
2014[a]	1	6	6
2015[a]	1	8	8
2016[a]	1	7	7
2017[a]	1	7	7
2018[a]	1	8	8
2019[a]	1	7	7
2020[a]	1	6	6
2021[a]	1	6	6
LAKE OF THE WOODS			
1994	2	3	6
1995	2	1	2
1996	2	3	6

Year sampled	Total sites	Total sample events (May – Oct)	Total samples
1997	2	3	6
1998	2	3	6
1999	2	2	4
2007	2	8	15
2008 [b]	2	6	12
2009 [b]	2	5	10
2010 [b]	2	1	4
2011 [b]	2	6	12
2012 [b]	2	1	2
2013	2	7	11
2014	2	8	14
2015	2	8	14
2016	2	7	14
2017	2	5	10
2018	3	6	17
2019	3	8	24
2020	3	9	24
2021	3	8	24
LEECH			
2012	5	6	30
2013	5	6	29
2014	5	7	34
2015	5	7	35
2016	5	8	35
2017	5	9	40
2018	5	8	40
2019	5	8	37
2020	5	7	35
2021	5	7	35
MILLE LACS			
2006 [c]	8	4	24
2007 [c]	9	5	47
2008 [c]	9	4	34
2009	9	6	49
2010	9	7	54
2011	9	6	46
2012	9	8	75
2013	9	8	58

Year sampled	Total sites	Total sample events (May – Oct)	Total samples
2014	9	7	65
2015	9	9	81
2016	9	9	72
2017	9	8	72
2018	9	7	61
2019	9	7	54
2020	3	5	14
2021	9	7	56
RAINY			
2001 [a]	6	14	35
2002 [a]	6	14	36
2003 [a]	4	7	24
2007 [a]	7	9	40
2008 [a]	5	6	28
2009 [a]	5	6	29
2010 [a]	5	7	30
2013 [a]	5	10	29
2014 [a]	5	8	29
2015 [a]	5	10	38
2016 [a]	5	7	33
2017 [a]	5	9	38
2018 [a]	5	8	36
2019 [a]	5	8	32
2020 [a]	5	6	29
2021 [a]	5	7	33
RED			
2012	6	8	46
2013	6	9	55
2014	6	10	54
2015	6	9	53
2016	6	11	58
2017	6	11	57
2018	6	10	57
2019	6	11	64
2020	6	10	60
2021	6	11	59
VERMILION			
2011	5	2	5

Year sampled	Total sites	Total sample events (May – Oct)	Total samples
2012	5	4	18
2013	4	7	30
2014	3	8	24
2015	3	7	22
2016	3	9	24
2017	3	8	24
2018	4	8	32
2019	4	8	32
2020	4	6	24
2021	4	8	32
WINNIBIGOSHISH (includes Cut Foot Sioux)			
2012	5	8	41
2013	5	7	35
2014	6	7	42
2015	6	8	42
2016	6	9	40
2017	6	7	42
2018	6	7	41
2019	6	8	41
2020	6	4	24
2021	6	3	18

TABLE A.3—Zooplankton taxa list from the nine largest walleye lakes in Minnesota (1994–2021). Specimen were identified to the lowest taxonomic level at the time they were analyzed and marked accordingly with an X. Grp shows the 5 major functional groups (calanoids [cal], cyclopoids [cyc], holopedium [holo], large daphnia [ldaph], small cladocerans [sclad]). Red indicates invasive zooplankton.

		Cass	Kabetogama	Lake of the Woods	Leech	Mille Lacs[a]	Rainy	Red	Vermilion	Winnibigoshish
Cladocerans	**Grp**									
Acroperus harpae	sclad	X			X	X				X
Alona sp.	sclad	X	X		X	X	X	X	X	X
Bosmina sp.	sclad	X	X	X	X	X	X	X	X	X
Bythotrephes cederströmii			X	X		X	X		X	
Camptocercus sp.	sclad						X			
Ceriodaphnia sp.	sclad	X	X	X	X	X	X	X	X	X
Chydorus sphaericus	sclad	X	X	X	X	X	X	X	X	X
Daphnia galeata mendotae	ldaph	X	X	X	X	X	X	X	X	X
Daphnia longiremis	sclad	X			X				X	
Daphnia parvula	sclad	X			X	X	X		X	X
Daphnia pulicaria	ldaph	X	X		X	X	X	X	X	X
Daphnia retrocurva	sclad	X	X	X	X	X	X	X	X	X
Diaphanosoma birgei	sclad	X	X	X	X	X	X	X	X	X
Eubosmina coregoni	sclad	X	X	X	X	X	X	X	X	X
Eurycercus lamellatus	sclad		X		X	X	X	X	X	X
Holopedium gibberum	holo	X	X	X	X	X	X		X	X

		Cass	Kabetogama	Lake of the Woods	Leech	Mille Lacs[a]	Rainy	Red	Vermilion	Winnibigoshish
Ilyocryptus sp.	sclad						X			
Latona setifera	sclad				X	X				
Leptodora kindti		X	X	X	X	X	X	X	X	X
Macrothricidae	sclad						X			
Polyphemus pediculus	sclad		X				X			
Sida crystallina	sclad		X	X		X	X			
Simocephalus sp.	sclad					X				
Copepods										
Acanthocyclops vernalis	cyc		X	X	X		X	X	X	
Diacyclops bicuspidatus thomasi	cyc	X	X	X	X		X	X	X	X
Diaptomidae	cal	X	X	X	X	X	X	X	X	X
Epischura lacustris	cal	X	X	X	X		X	X	X	X
Ergasilus sp.	cyc	X	X		X			X	X	X
Limnocalanus macrurus	cal		X	X	X		X			
Macrocyclops sp.	cyc				X			X		X
Mesocyclops edax	cyc	X	X	X	X		X	X	X	X
Senecella calanoides	cal		X				X			
Tropocyclops prasinus mexicanus	cyc	X	X	X	X		X	X	X	X
Richness (taxa per lake)		20	24	18	25	19	27	19	22	21

[a] identified as cyclopoid only

TABLE A.4.—Output of linear mixed effects models testing for effects of different analysts (2 taxonomists over the span of the project) on the relative proportions of density (d) and biomass (b) estimates for the different copepod groups (nauplii, copepodites, adult calanoids, and adult cyclopoids) using 2018-2021 data. Lakes were included in the models as a random effect. ** = *P* <0.01, *** = *P* <0.001.

Taxa			Estimate	Std. error	df	t	Pr(>|t|)	
nauplii	^{d}	(Intercept)	0.255	0.030	15	8.49	4.64E-07	***
		Analyst 2	0.081	0.023	33	3.60	0.00104	**
	^{b}	(Intercept)	0.020	0.004	19	4.61	0.00019	***
		Analyst 2	0.031	0.004	33	7.25	2.57E-08	***
copepodites	^{d}	(Intercept)	0.195	0.019	18	10.21	7.18E-09	***
		Analyst 2	0.220	0.018	33	12.17	9.63E-14	***
	^{b}	(Intercept)	0.084	0.017	25	4.95	4.42E-05	***
		Analyst 2	0.314	0.020	33	15.32	< 2e-16	***
calanoids	^{d}	(Intercept)	0.223	0.018	19	12.32	1.54E-10	***
		Analyst 2	-0.144	0.018	33	-7.85	4.75E-09	***
	^{b}	(Intercept)	0.519	0.040	15	12.83	1.65E-09	***
		Analyst 2	-0.206	0.031	33	-6.56	1.86E-07	***
cyclopoids	^{d}	(Intercept)	0.328	0.022	16	15.00	7.49E-11	***
		Analyst 2	-0.157	0.018	33	-8.53	7.45E-10	***
	^{b}	(Intercept)	0.377	0.035	16	10.78	9.30E-09	***
		Analyst 2	-0.139	0.030	33	-4.72	4.22E-05	***

^{d} density

^{b} biomass

TABLE A.5.—Output from Wilcoxon Rank Sum tests for changes in median zooplankton density and biomass from study lakes for zooplankton taxa groups in the presence of spiny waterflea (top) and zebra mussel (bottom). Lake Mille Lacs data were included in the Spiny Waterflea category. Invasion = status of lakes when sample was collected, pre = prior to invasion, post = after invasion, n = sample size, W = Wilcoxon statistic, * = P <0.05, ** = P <0.01, *** = P <0.001.

SPINY WATERFLEA

			Density (number/L)				Biomass (µg/L)			
Taxa	Invasion	n	Median	W	p-value		Median	W	p-value	
All	pre	104	3.16	16413	<0.001	***	9.51	14499	<0.001	***
	post	425	1.80				5.00			
Calanoid	pre	104	2.82	22332	0.003	**	16.10	21003	<0.001	***
	post	528	1.12				7.88			
Cyclopoid	pre	156	2.27	27573	<0.001	***	7.91	24794	<0.001	***
	post	544	0.99				1.68			
Cladoceran	pre	333	1.76	70757	<0.001	***	4.76	83423	<0.001	***
	post	743	0.64				1.18			

ZEBRA MUSSEL

			Density (number/L)				Biomass (µg/L)			
Taxa	Invasion	n	Median	W	p-value		Median	W	p-value	
All	pre	270	1.81	41598	0.023	*	4.89	39955	0.166	
	post	277	2.09				5.06			
Calanoid	pre	184	1.27	20211	0.005	**	9.11	19923	0.011	**
	post	188	1.57				11.2			
Cyclopoid	pre	225	0.92	24458	0.100		2.88	22634	0.845	
	post	199	1.10				3.28			
Cladoceran	pre	390	0.94	53942	0.092		2.68	55888	0.351	
	post	299	0.79				2.44			

TABLE A.6.—Output from generalized additive models (GAMs) to test for effects of zebra mussels (ZM), spiny waterflea (SWF), or both (SWF & ZM) on biomass of different zooplankton functional groups from May-September in the Large Lakes. Lake Vermilion was separated into East and West basins for this analysis. AIS3 = ZM were modeled with a three-year lag time; AIS2 = ZM were modeled with a two-year lag time; dd = time was expressed as cumulative degree-days (base 0°C); doy = time was expressed as day-of-year; lake = lake sample was collected from; year = year sample was collected in; s= smoothed variable; 'fs' = variable was a factor smooth; 're' = random variable; AIC = Akaike information criterion score; df = degrees of freedom; ML = maximum likelihood score; n = sample size.

Model	AIC	Deviance explained %	Residual deviance	Residual df	-ML
Large daphnia (*D. pulicaria* & *D. galeata mendotae*) (n=778)					
AIS3 + s(dd, AIS3, 'fs') + s(dd) + s(lake, 're') + s(year, 're')	2677.44	48.5	1278.80	737.53	1369.6
AIS2 + s(dd, AIS2, 'fs') + s(dd) + s(lake, 're') + s(year, 're')	2683.69	48.1	1288.29	737.28	1372.7
AIS3 + s(DOY, AIS3, 'fs') + s(DOY) + s(lake, 're') + s(year, 're')	2724.41	45.2	1359.93	737.97	1394.3
Small cladocerans (n=844)					
AIS3 + s(dd, AIS3, 'fs') + s(dd) + s(lake, 're') + s(year, 're')	2931.85	57.3	1431.80	799.70	1505.6
AIS2 + s(dd, AIS2, 'fs') + s(dd) + s(lake, 're') + s(year, 're')	2938.67	57.0	1441.45	799.13	1509.5
AIS3 + s(DOY, AIS3, 'fs') + s(DOY) + s(lake, 're') + s(year, 're')	2947.88	56.5	1460.81	800.15	1513.1
Calanoids (n=877)					
AIS3 + s(DOY, AIS3, 'fs') + s(DOY) + s(lake, 're') + s(year, 're')	2276.82	68.7	620.49	832.28	1178.7
AIS3 + s(dd, AIS3, 'fs') + s(dd) + s(lake, 're') + s(year, 're')	2298.01	67.9	637.74	833.71	1189.2
AIS2 + s(dd, AIS2, 'fs') + s(dd) + s(lake, 're') + s(year, 're')	2339.82	66.3	669.65	834.21	1208.4
Cyclopoids (n=887)					
AIS3 + s(dd, AIS3, 'fs') + s(dd) + s(lake, 're') + s(year, 're')	2322.45	65.5	637.39	838.81	1212.7
AIS2 + s(dd, AIS2, 'fs') + s(dd) + s(lake, 're') + s(year, 're')	2350.68	64.3	658.02	838.83	1226.2
AIS3 + s(DOY, AIS3, 'fs') + s(DOY) + s(lake, 're') + s(year, 're')	2352.59	64.3	659.55	838.90	1225.3

TABLE A.7.—Output from generalized additive models selected to predict the impacts of zebra mussels, spiny waterflea, and both species together on season biomass of native zooplankton groups (Large *Daphnia* spp., small cladocerans, calanoid copepods, and cyclopoid copepods). Std Error = standard error. Definitions of other abbreviations can be found in Table A.6.

Model					
Large daphnia (*D. pulicaria* & *D. galeata mendotae*) (n=778)					
Component	Term	Estimate	Std Error	t-value	p-value
A. parametric coefficients	Intercept	1.98	0.42	4.77	<0.001
	ZM	-0.83	0.31	-2.65	0.008
	SWF	-0.72	0.31	-2.34	0.020
	SWF & ZM	-1.40	0.51	-2.75	0.005
Component	Term	edf	Ref. df	F-value	p-value
B. smooth terms	s(dd, AIS3)	10.17	35.00	1.91	<0.001
	s(dd)	6.66	7.64	6.30	<0.001
	s(lake)	8.66	9.00	46.82	<0.001
	s(year)	11.43	18.00	3.33	<0.001
Small cladocerans (n=844)					
Component	Term	Estimate	Std Error	t-value	p-value
A. parametric coefficients	Intercept	2.35	0.45	5.21	<0.001
	ZM	-0.76	0.40	-1.89	0.117
	SWF	-0.62	0.42	-3.88	<0.001
	SWF & ZM	-3.81	0.57	-6.67	<0.001
Component	Term	edf	Ref. df	F-value	p-value
B. smooth terms	s(dd, AIS3)	10.77	35.00	3.00	<0.001
	s(dd)	5.69	6.72	2.82	0.009
	s(lake)	8.82	9.00	50.61	<0.001
	s(year)	15.82	18.00	21.83	<0.001
Calanoids (n=877)					
Component	Term	Estimate	Std Error	t-value	p-value
A. parametric coefficients	Intercept	2.77	0.46	6.05	<0.001

Model					
	ZM	-0.90	0.26	-3.53	<0.001
	SWF	-0.48	0.26	-1.87	0.062
	SWF & ZM	-0.37	0.36	-1.01	0.314
Component	Term	edf	Ref. df	F-value	p-value
B. smooth terms	s(dd, AIS3)	12.12	35.00	3.59	<0.001
	s(dd)	4.89	5.80	1.96	0.072
	s(lake)	8.89	9.00	185.74	<0.001
	s(year)	13.81	18.00	9.41	<0.001
Cyclopoids (n=877)					
Component	Term	Estimate	Std Error	t-value	p-value
A. parametric coefficients	Intercept	3.03	0.36	8.33	<0.001
	ZM	-0.70	0.16	-4.44	<0.001
	SWF	-0.70	0.18	-4.00	<0.001
	SWF & ZM	-2.07	0.32	-6.48	<0.001
Component	Term	edf	Ref. df	F-value	p-value
B. smooth terms	s(dd, AIS3)	12.44	35.00	4.19	<0.001
	s(dd)	7.10	7.88	6.17	<0.001
	s(lake)	8.80	9.00	126.30	<0.001
	s(year)	16.13	18.00	34.14	<0.001

TABLE A.8.—Species-level summaries of measured length (µm) and estimated dry weight (µg) data from the DNR Large Lakes Program is sorted by prevalence in the 2,786 samples. S.D. = standard deviation; C.V. = coefficient of variation (ratio of the standard deviation to the mean); n = number of measured individuals needed to have 95% confidence that the sampled mean is within 10% of the actual mean.

			Length (µm)				**Weight (µg)**		
Species	Count	Count/ Sample	**Mean**	S.D.	C.V.	n	**Mean**	S.D.	C.V.
Nauplii	103477	37.14	**198**	53	0.27	29	**0.20**	0.11	0.55
Copepodites	80852	29.02	**456**	135	0.29	34	**1.27**	0.69	0.54
Diaptomidae	46095	16.55	**994**	177	0.18	13	**6.31**	2.24	0.35
Diacyclops bicuspidatus thomasi	44865	16.10	**816**	156	0.19	15	**3.95**	1.51	0.38
Bosmina sp.	38722	13.90	**295**	67	0.23	20	**1.25**	0.78	0.62
Daphnia galeata mendotae	26285	9.43	**1047**	366	0.35	48	**8.48**	4.78	0.56
Cyclopoids	26088	9.36	**567**	209	0.37	53	**1.85**	2.61	1.41
Calanoids	25159	9.03	**959**	218	0.23	20	**5.98**	2.94	0.49
Tropocyclops prasinus mexicanus	22696	8.15	**434**	65	0.15	9	**1.15**	0.34	0.30
Chydorus sphaericus	15159	5.44	**239**	48	0.20	16	**0.86**	0.39	0.45
Daphnia retrocurva	12284	4.41	**809**	226	0.28	31	**2.54**	1.99	0.78
Mesocyclops edax	9680	3.47	**914**	208	0.23	20	**5.91**	4.47	0.76
Diaphanosoma birgei	7554	2.71	**643**	178	0.28	30	**3.20**	0.93	0.29
Eubosmina coregoni	5169	1.86	**421**	94	0.22	19	**3.12**	1.67	0.53
Daphnia pulicaria	3716	1.33	**1115**	349	0.31	38	**12.41**	11.96	0.96
Holopedium gibberum	2851	1.02	**768**	258	0.34	44	**5.20**	2.79	0.54
Epischura lacustris	2747	0.99	**1223**	418	0.34	45	**10.19**	6.68	0.66
Ceriodaphnia sp.	2620	0.94	**415**	104	0.25	25	**0.75**	0.37	0.50
Daphnia longiremis	1395	0.50	**885**	222	0.25	25	**6.38**	2.27	0.36

			Length (μm)				**Weight (μg)**		
Species	Count	Count/ Sample	**Mean**	S.D.	C.V.	n	**Mean**	S.D.	C.V.
Acanthocyclops vernalis	1069	0.38	**760**	142	0.19	14	**3.75**	1.87	0.50
Limnocalanus macrurus	1051	0.38	**2084**	617	0.30	34	**28.28**	14.15	0.50
Eurycercus lamellatus	1021	0.37	**933**	303	0.32	41	**3.86**	2.77	0.72
Bythotrephes longimanus	612	0.22	**2538**	535	0.21	18	**123.76**	53.10	0.43
Bosmina longirostris	588	0.21	**322**	68	0.21	18	**1.51**	0.74	0.49
Senecella calanoides	66	0.02	**2005**	542	0.27	29	**25.87**	11.97	0.46
Daphnia parvula	46	0.02	**840**	386	0.46	82	**6.71**	5.11	0.76
Chydorus sp.	42	0.02	**228**	41	0.18	13	**0.78**	0.27	0.35
Alona sp.	38	0.01	**545**	198	0.36	51	**0.53**	0.31	0.59
Ergasilus sp.	15	0.01	**763**	136	0.18	13	**3.28**	1.42	0.43
Daphnia sp.	12	<0.01	**623**	169	0.27	29	**2.44**	1.73	0.71
Macrocyclops sp.	8	<0.01	**1360**	439	0.32	40	**20.32**	17.15	0.84
Acroperus harpae	7	<0.01	**605**	177	0.29	33	**0.61**	0.27	0.44
Sida crystallina	7	<0.01	**994**	350	0.35	48	**5.05**	1.86	0.37
Latona setifera	6	<0.01	**795**	214	0.27	28	**3.99**	1.12	0.28
Alona setulosa	4	<0.01	**606**	197	0.32	41	**0.62**	0.34	0.54
Camptocercus sp.	2	<0.01	**571**	288	0.51	99	**1.49**	1.32	0.89
Chydoridae	2	<0.01	**362**	79	0.22	19	**1.92**	0.81	0.42
Leptodora kindti	2	<0.01	**2121**	427	0.20	16	**6.48**	2.40	0.37
Polyphemus pediculus	2	<0.01	**638**	125	0.20	15	**0.96**	0.39	0.41
Ilyocryptus sp.	1	<0.01	**556**	---	---	---	**1.26**	---	---
Simocephalus sp.	1	<0.01	**591**	---	---	---	**0.54**	---	---

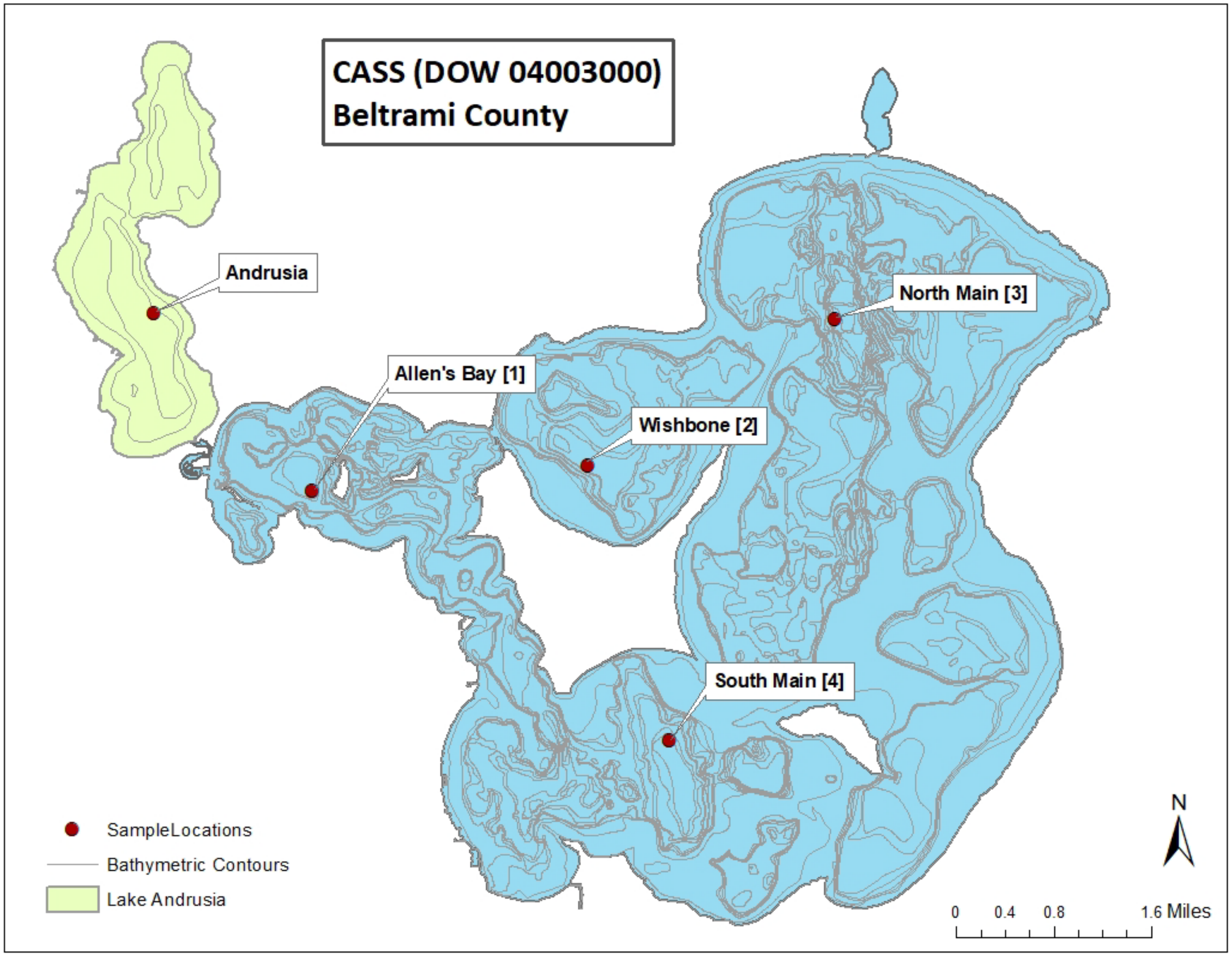


FIGURE A.1.—Map showing zooplankton sample locations in Cass Lake and Lake Andrusia, Beltrami County.

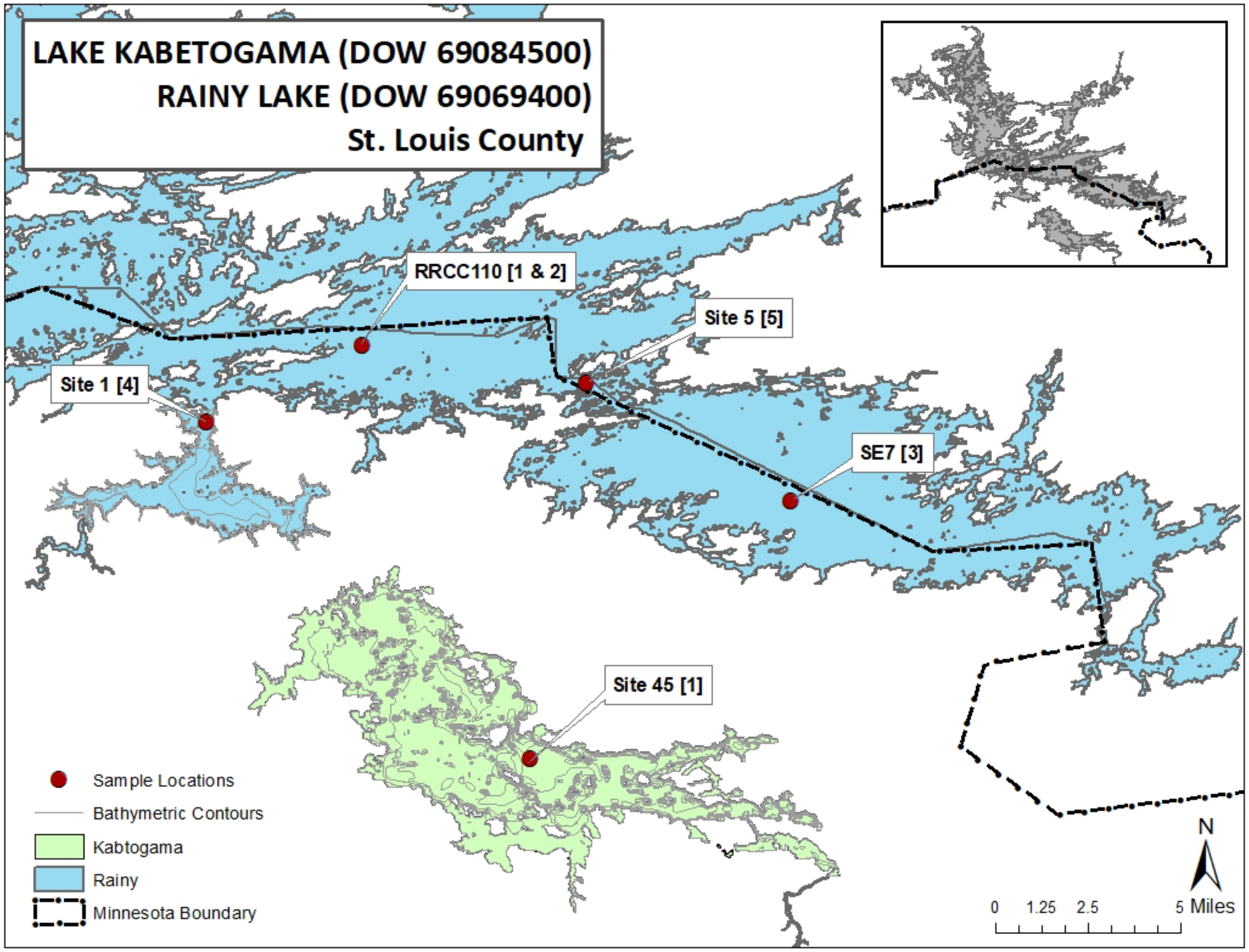


FIGURE A.2.—Map showing zooplankton sample locations in Lake Kabetogama and Rainy Lake, St. Louis County.

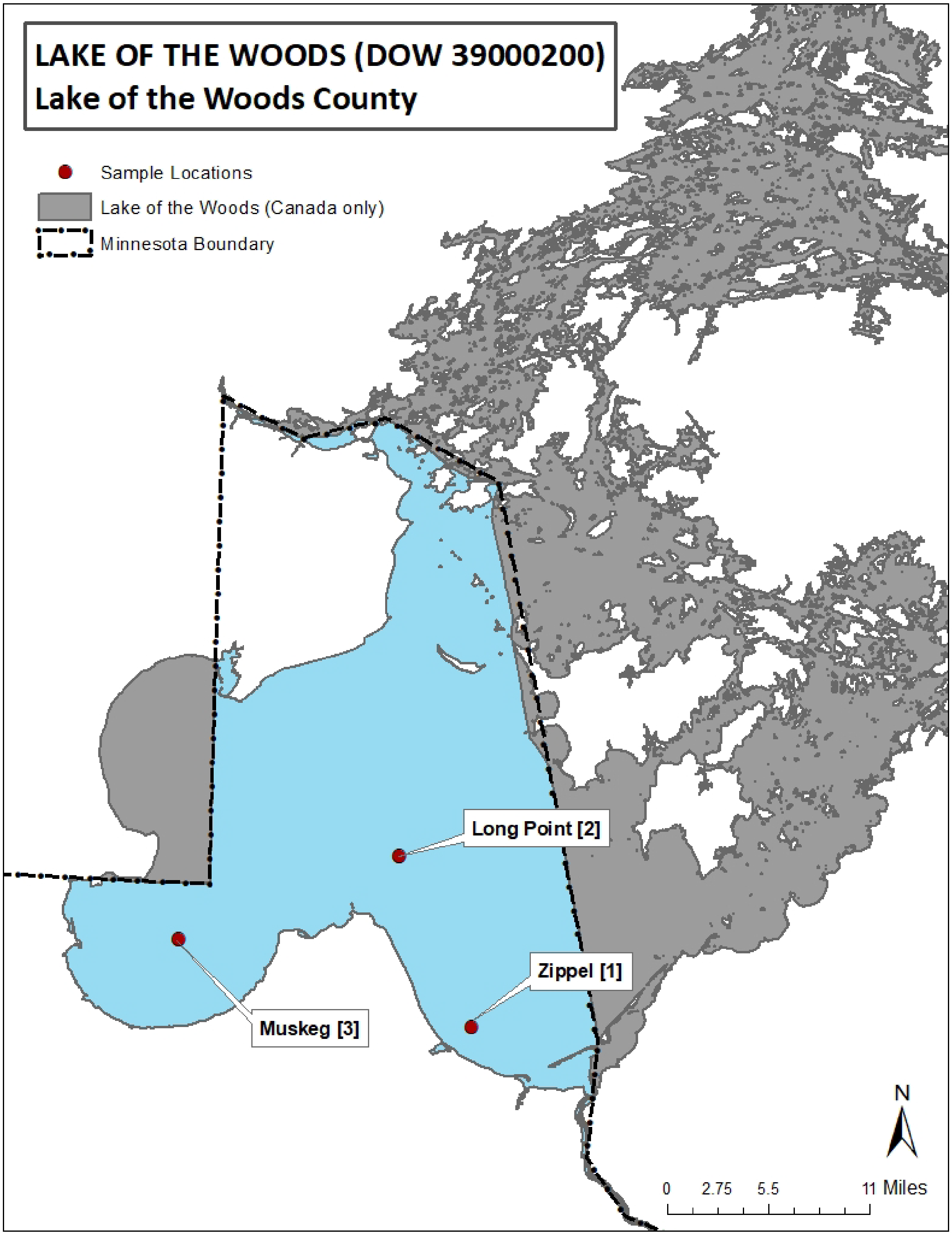


FIGURE A.3.—Map showing zooplankton sample locations in Lake of the Woods, Lake of the Woods County.

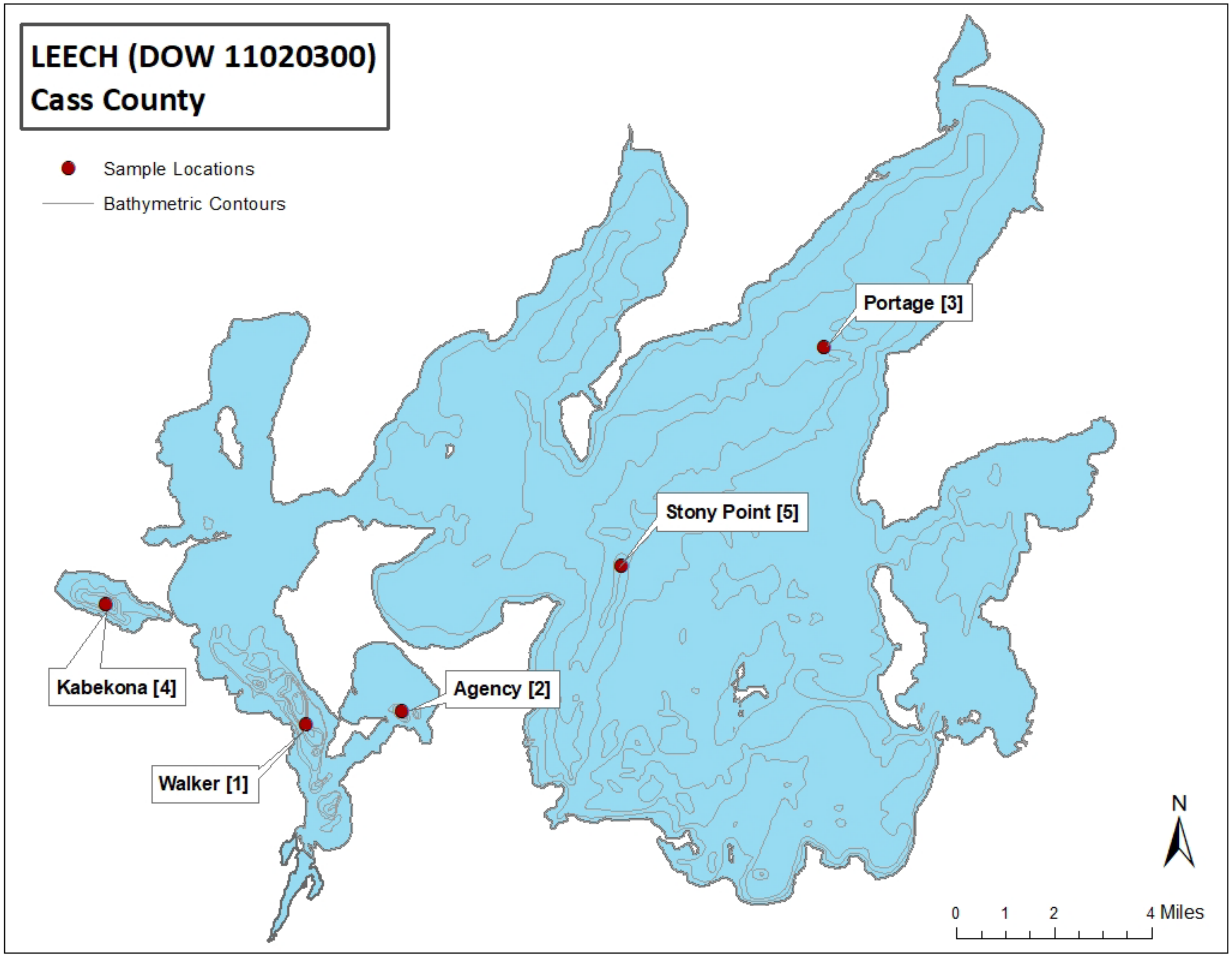


FIGURE A.4.—Map showing zooplankton sample locations in Leech Lake, Cass County.

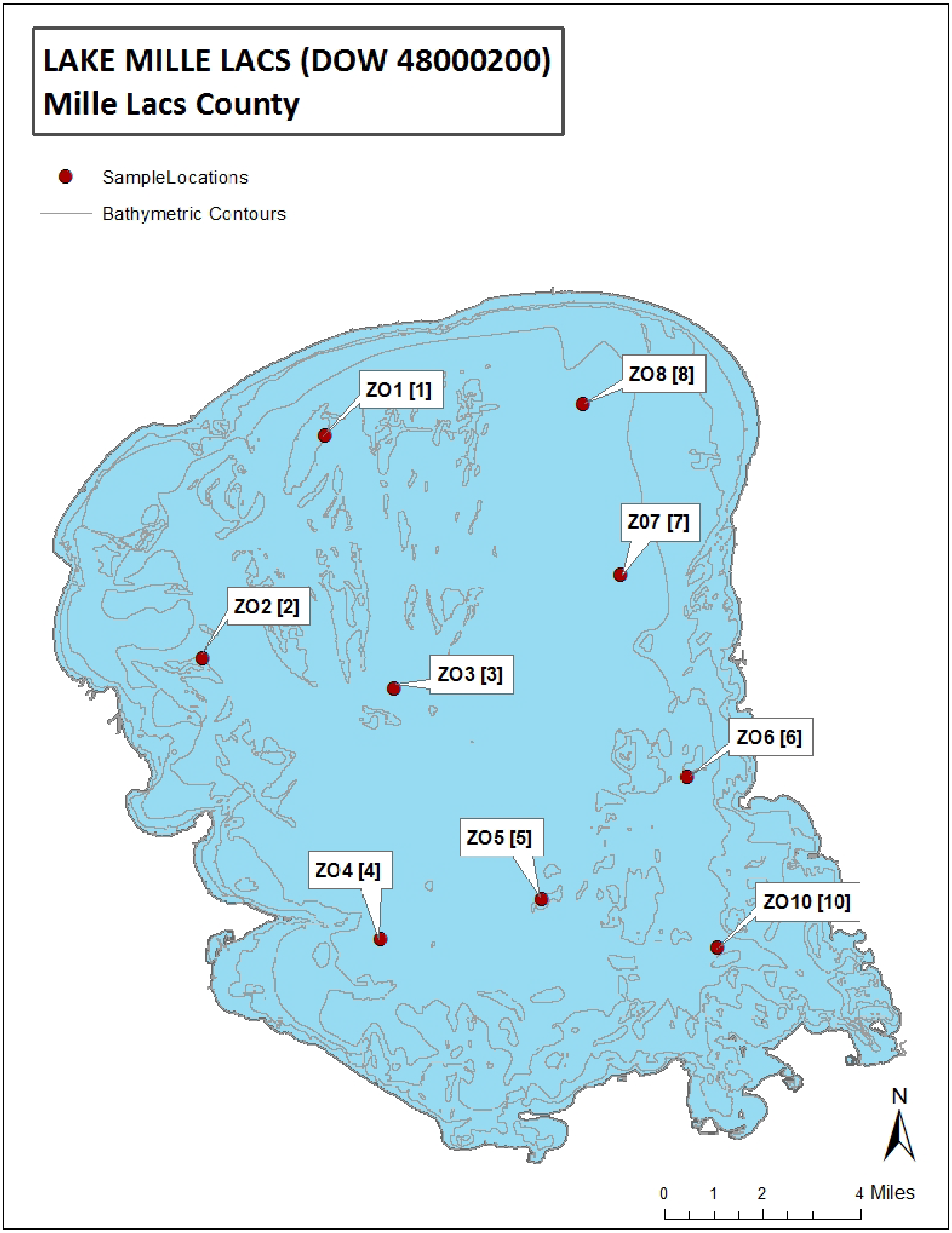


FIGURE A.5.—Map showing zooplankton sample locations in Lake Mille Lacs, Mille Lacs County.

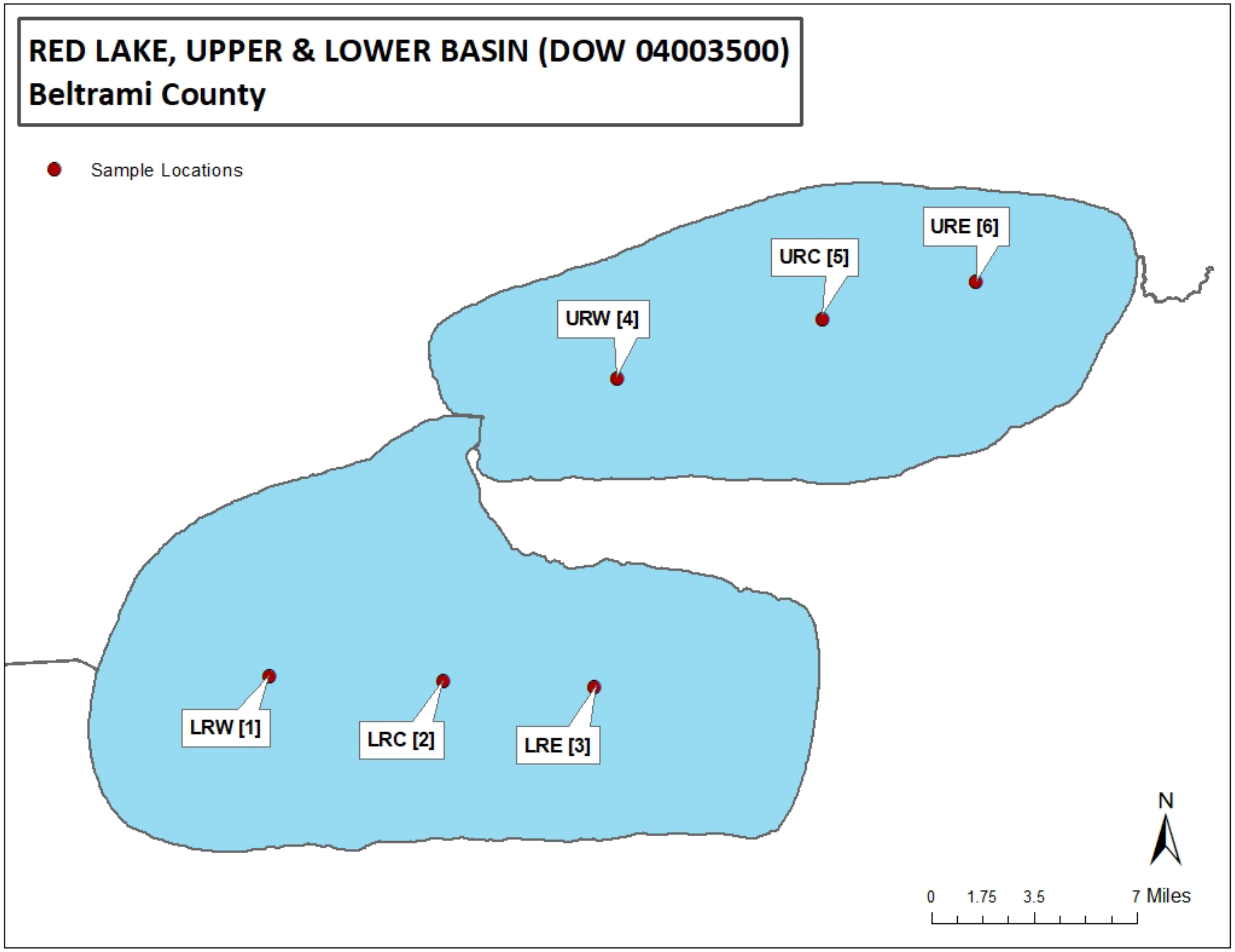


FIGURE A.6.—Map showing zooplankton sample locations in Red Lake, Beltrami County.

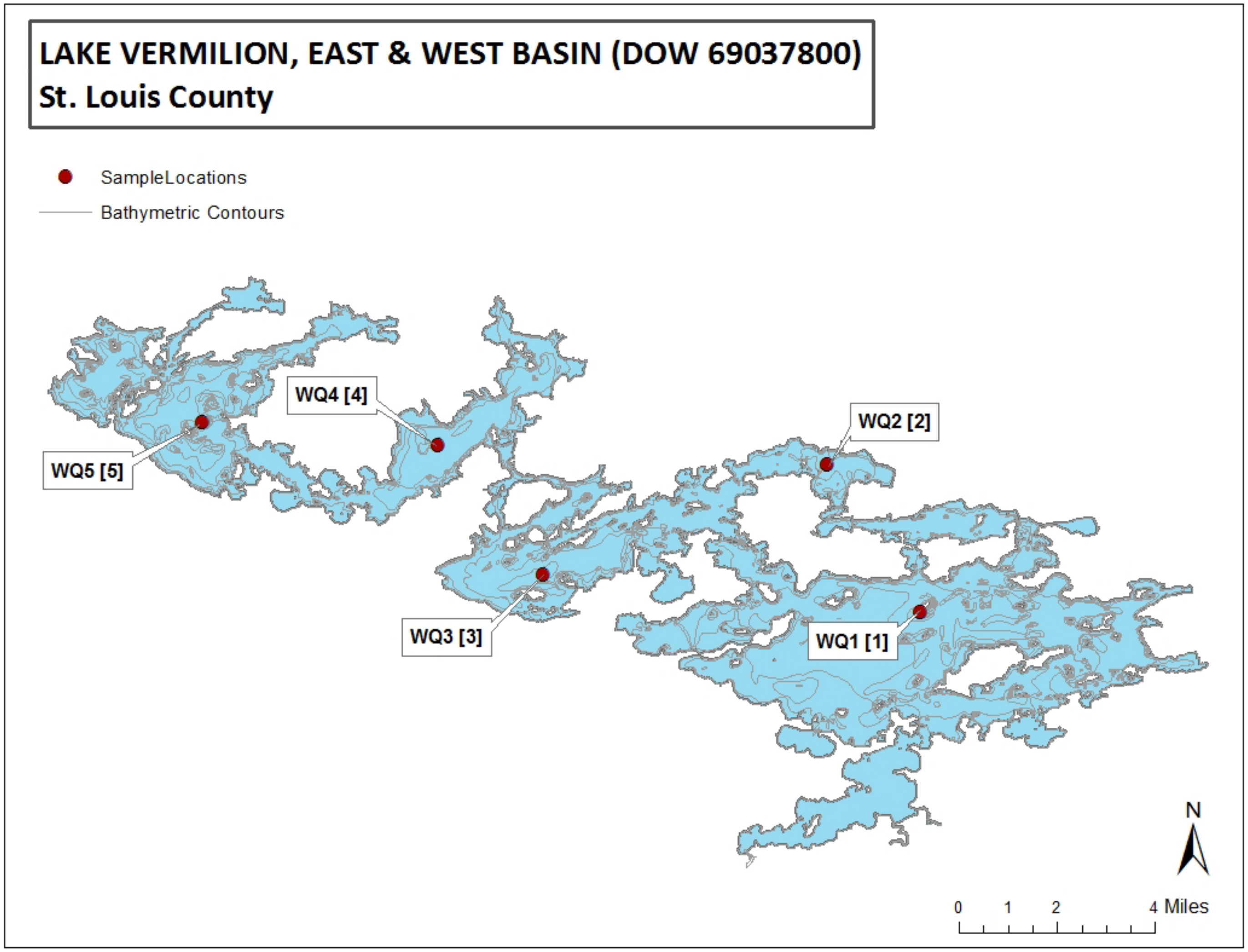


FIGURE A.7.—Map showing zooplankton sample locations in Lake Vermilion, St. Louis County.

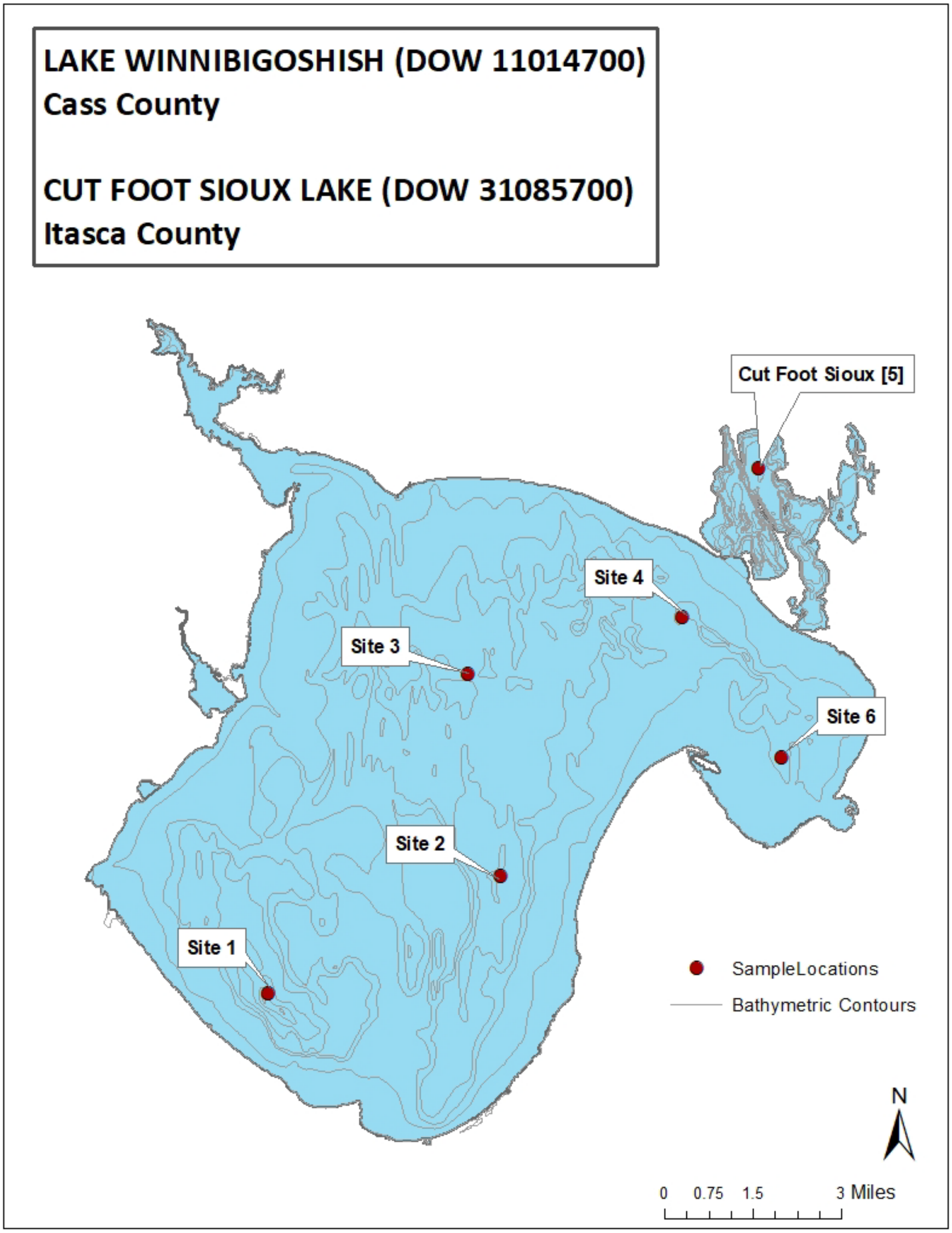


FIGURE A.8. —Map showing zooplankton sample locations in Lake Winnibigoshish and Cut Foot Sioux Lake, Cass and Itasca County.

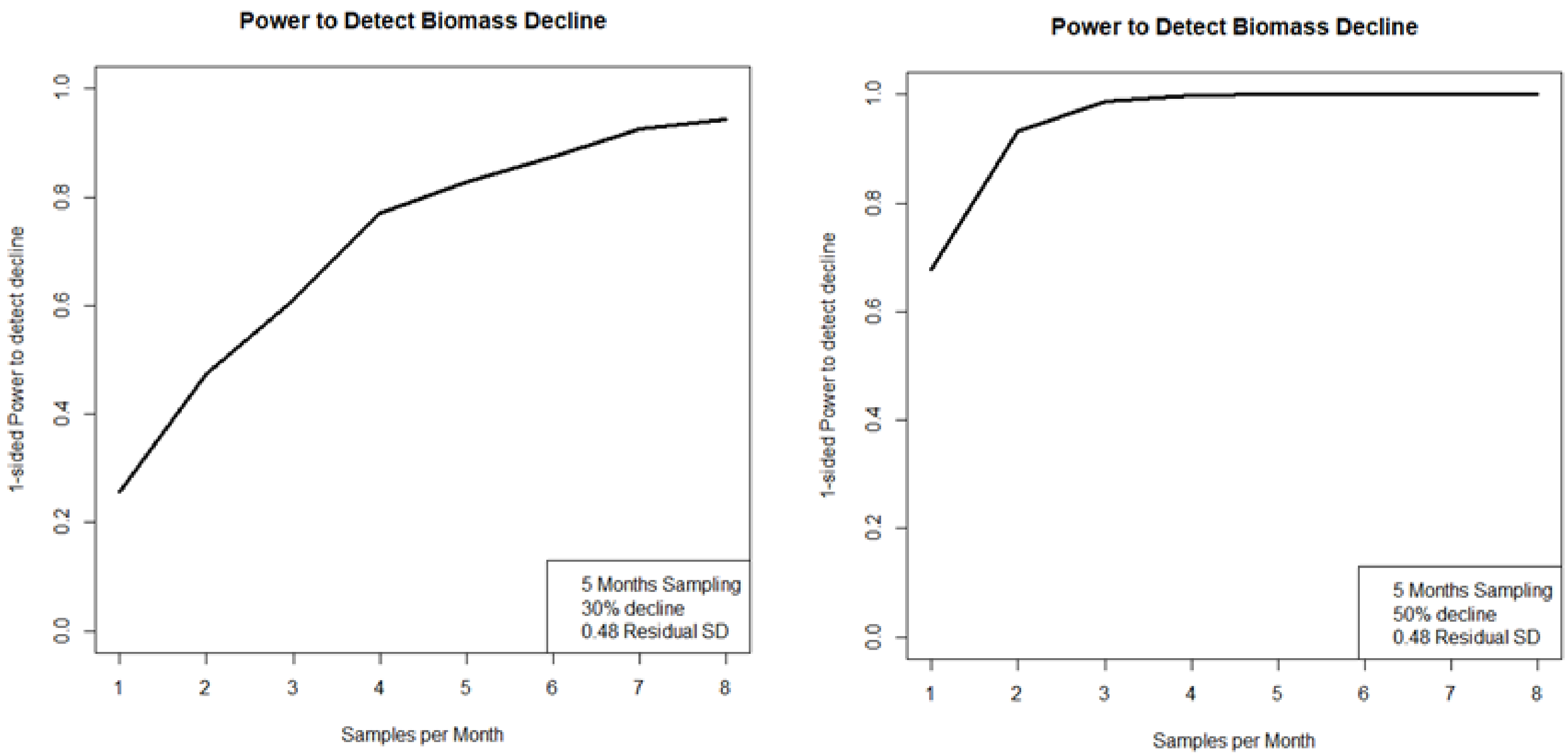


FIGURE A.9.—Results from power analysis to determine the number of samples per month needed in Cass Lake to detect a 30% or 50% decrease in zooplankton biomass between years.

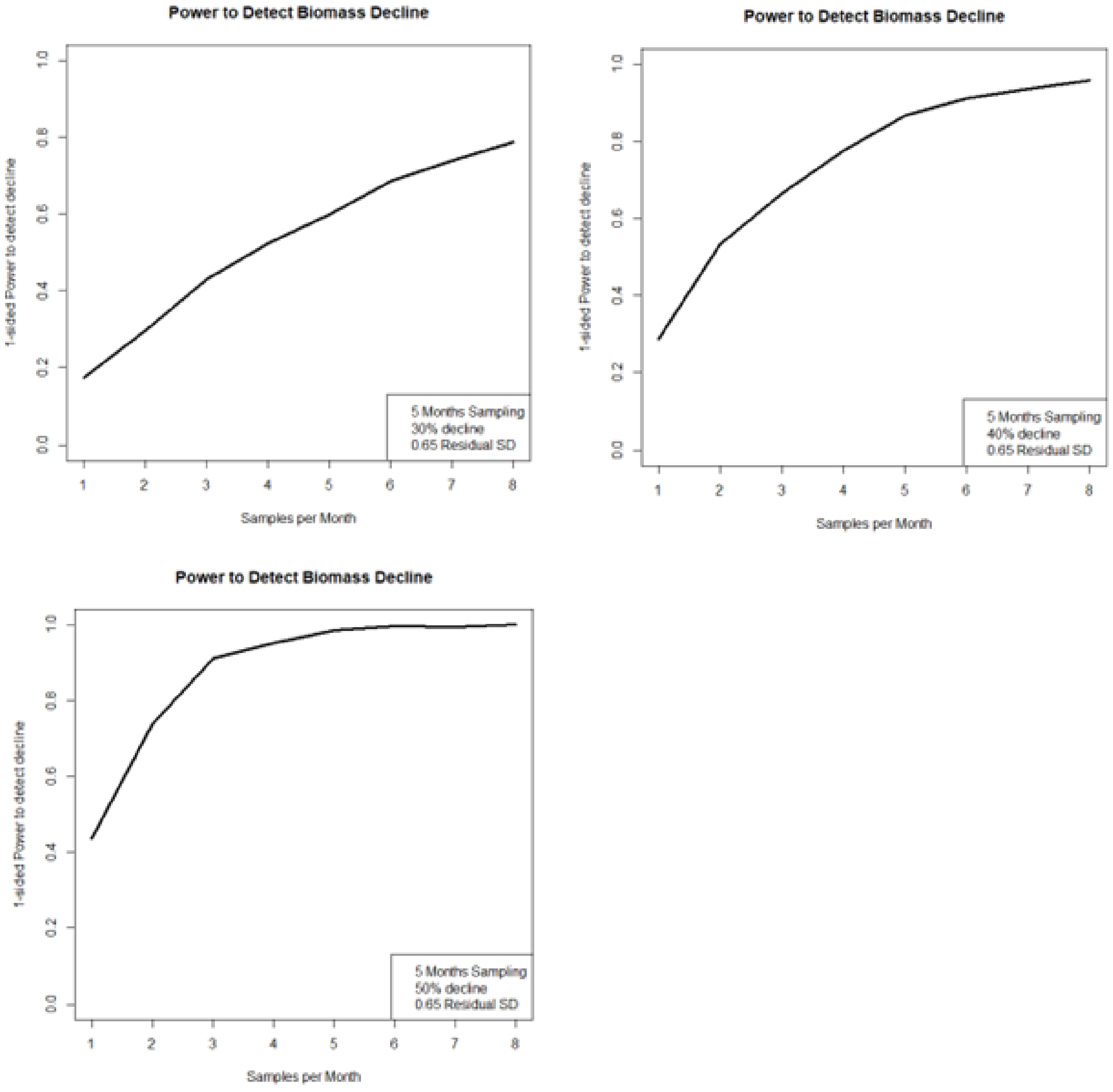


FIGURE A.10.—Results from power analysis to determine the number of samples per month needed in Lake of the Woods to detect a 30%, 40% or 50% decrease in zooplankton biomass between years.

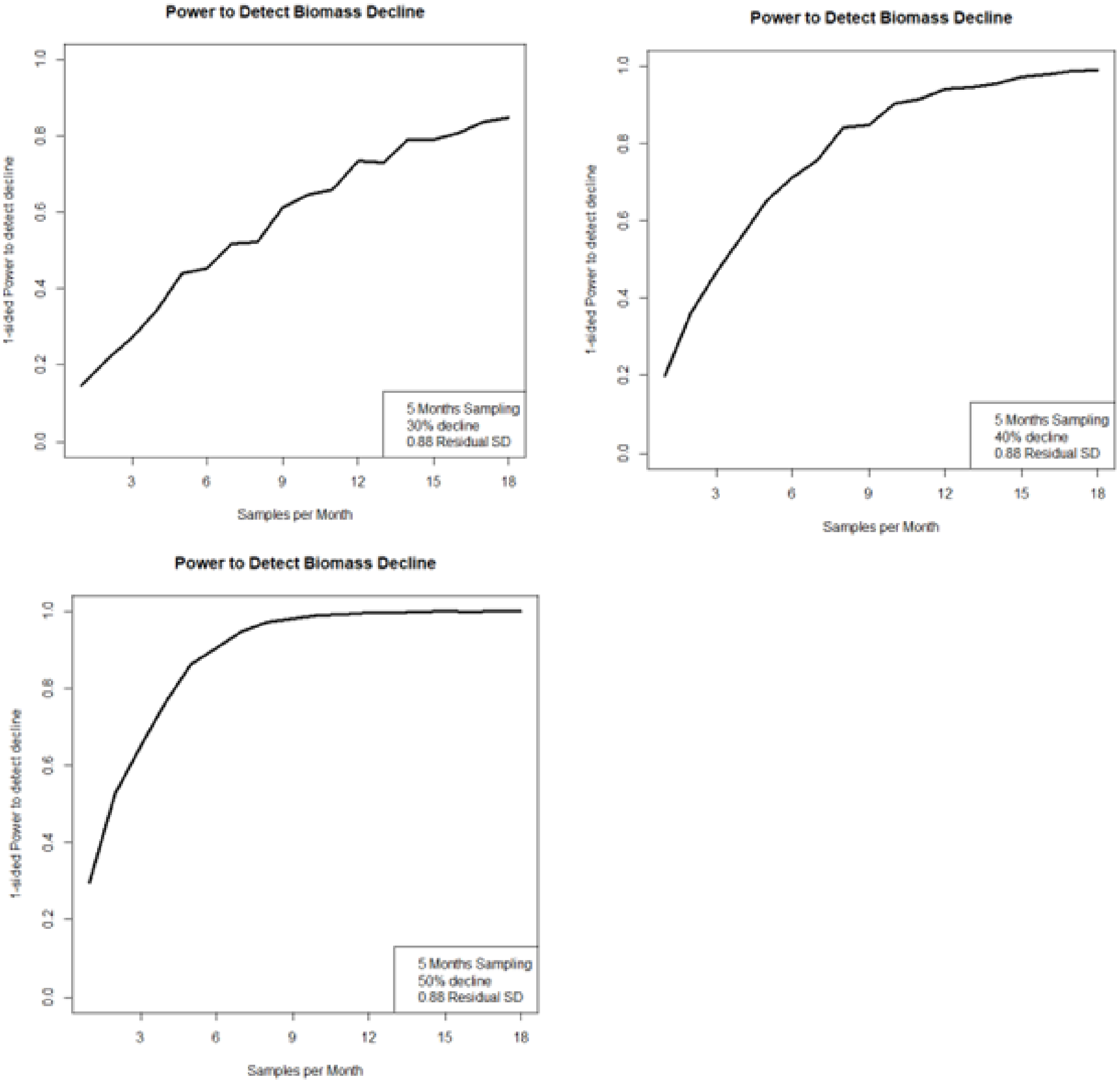


FIGURE A.11.—Results from power analysis to determine the number of samples per month needed in Lake Mille Lacs to detect a 30%, 40% or 50% decrease in zooplankton biomass between years.

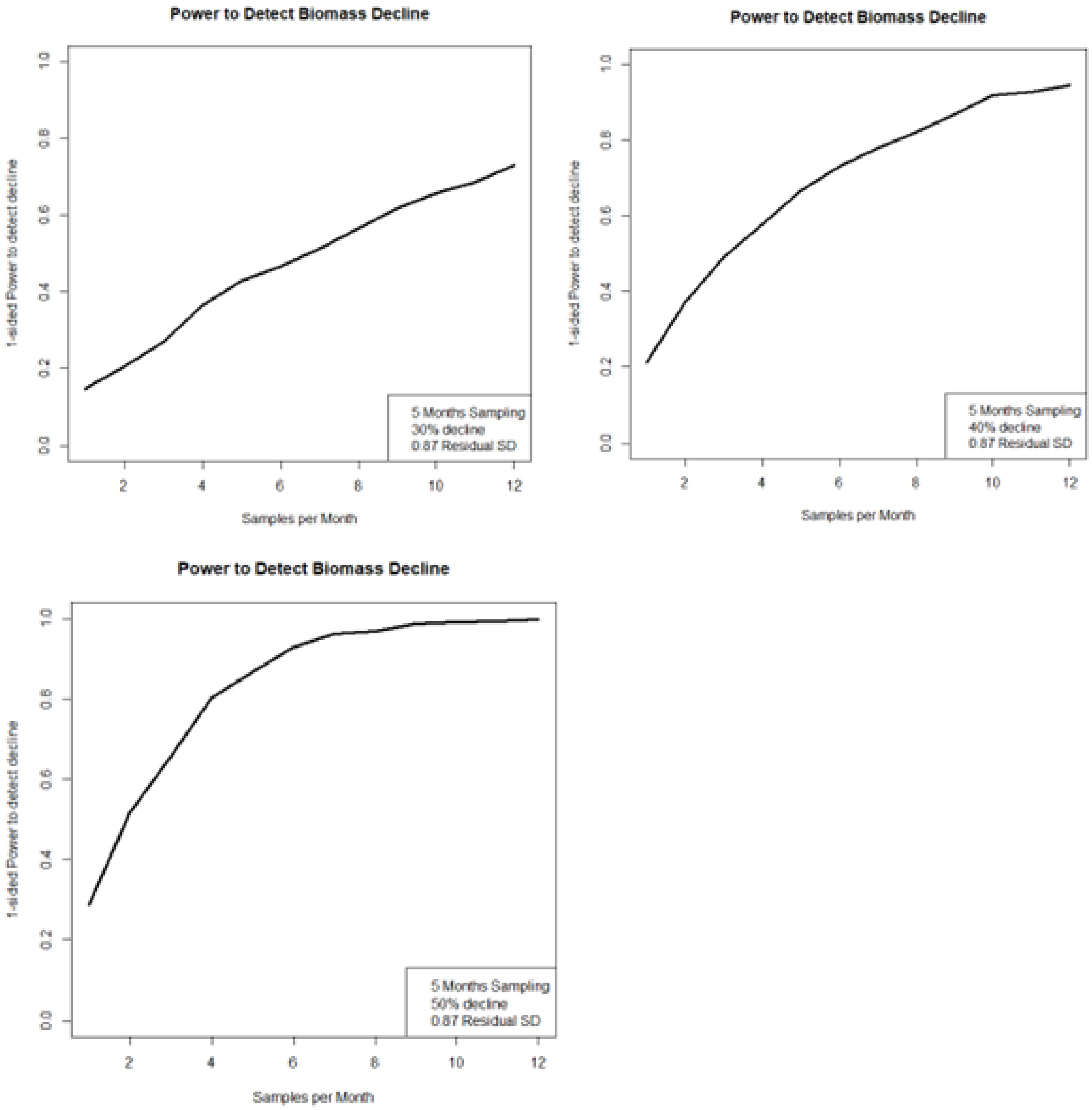


FIGURE A.12.—Results from power analysis to determine the number of samples per month needed in Red Lake to detect a 30%, 40% or 50% decrease in zooplankton biomass between years.

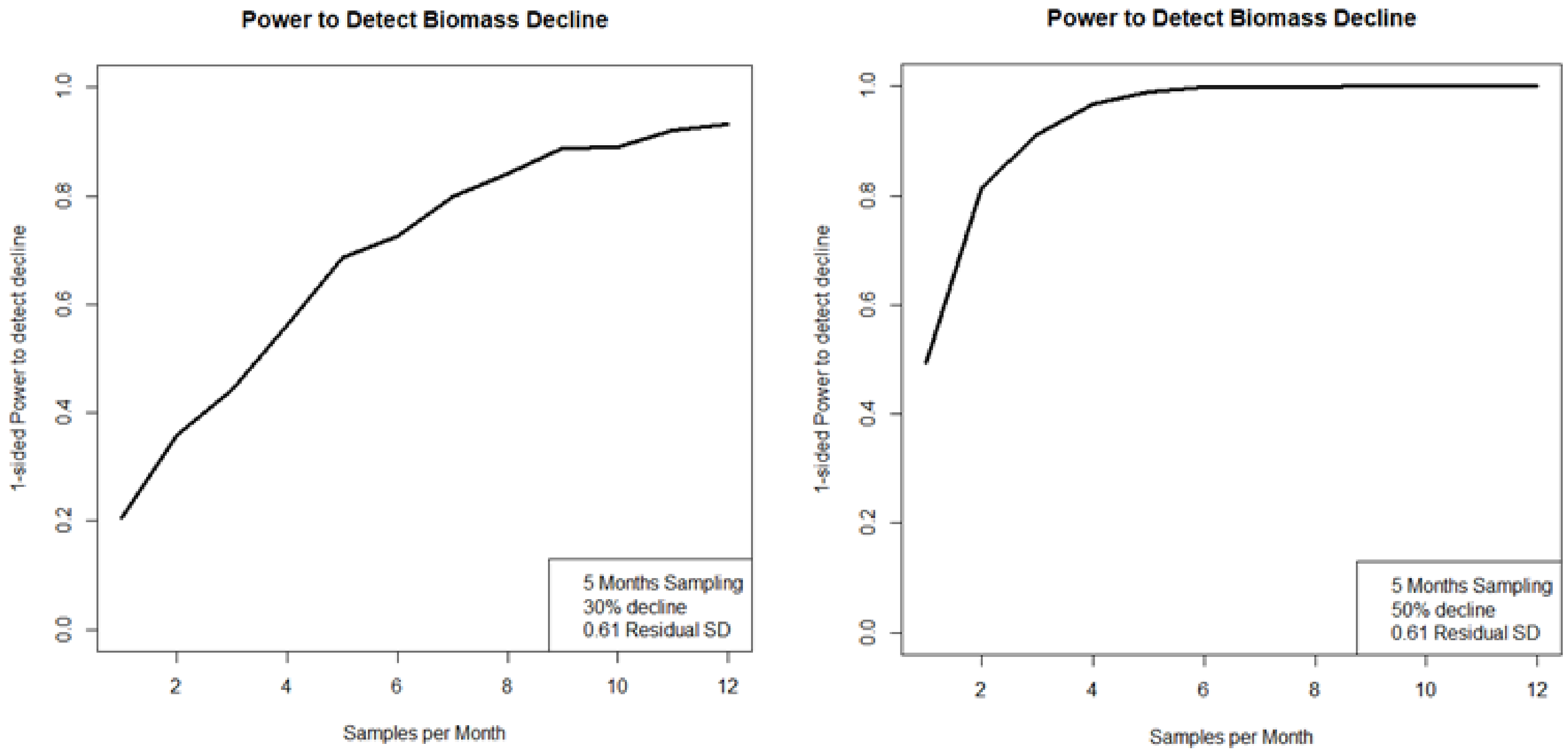
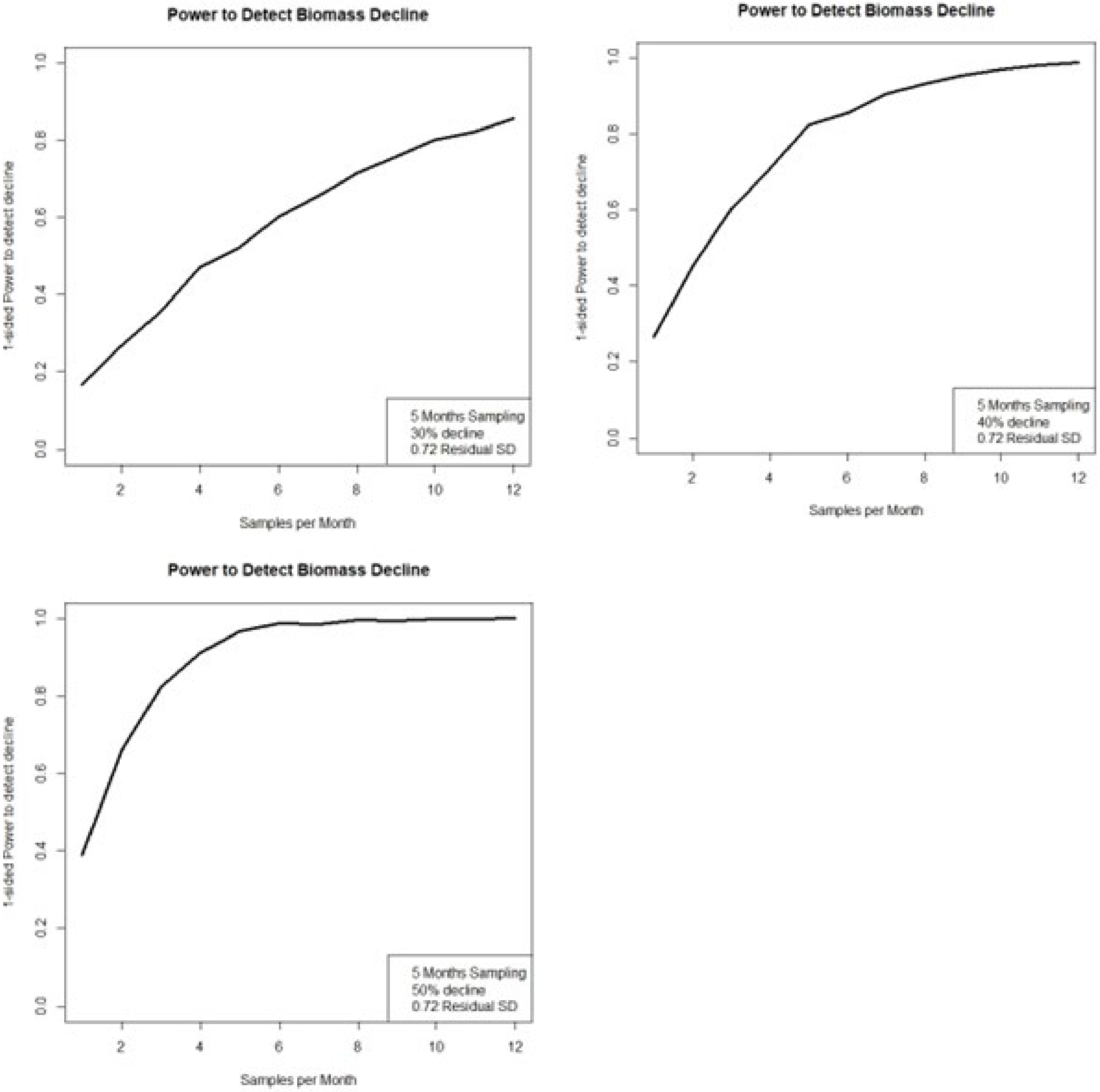


FIGURE A.13.—Results from power analysis to determine the number of samples per month needed in Lake Vermilion to detect a 30% or 50% decrease in zooplankton biomass between years.


Power to Detect Biomass Decline

Power to Detect Biomass Decline

Power to Detect Biomass Decline

FIGURE A.14.—Results from power analysis to determine the number of samples per month needed in Lake Winnibigoshish to detect a 30%, 40% or 50% decrease in zooplankton biomass between years.