National Lakes Assessment 2012

Zooplankton Communities in Minnesota Lakes

This is part of a series based on Minnesota's participation in U.S. EPA's 2012 National Lakes Assessment

Minnesota Pollution Control Agency

Minnesota Department of Natural Resources

July 2014

National Lakes Assessment 2012

Zooplankton Communities in Minnesota Lakes

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This report was developed in collaboration with MPCA as a part of Minnesota's overall 2012 National Lakes Assessment Project. This work was supported in part by a USEPA National Lake Assessment Grant (#100E00998)

ACKNOWLEDGEMENTS

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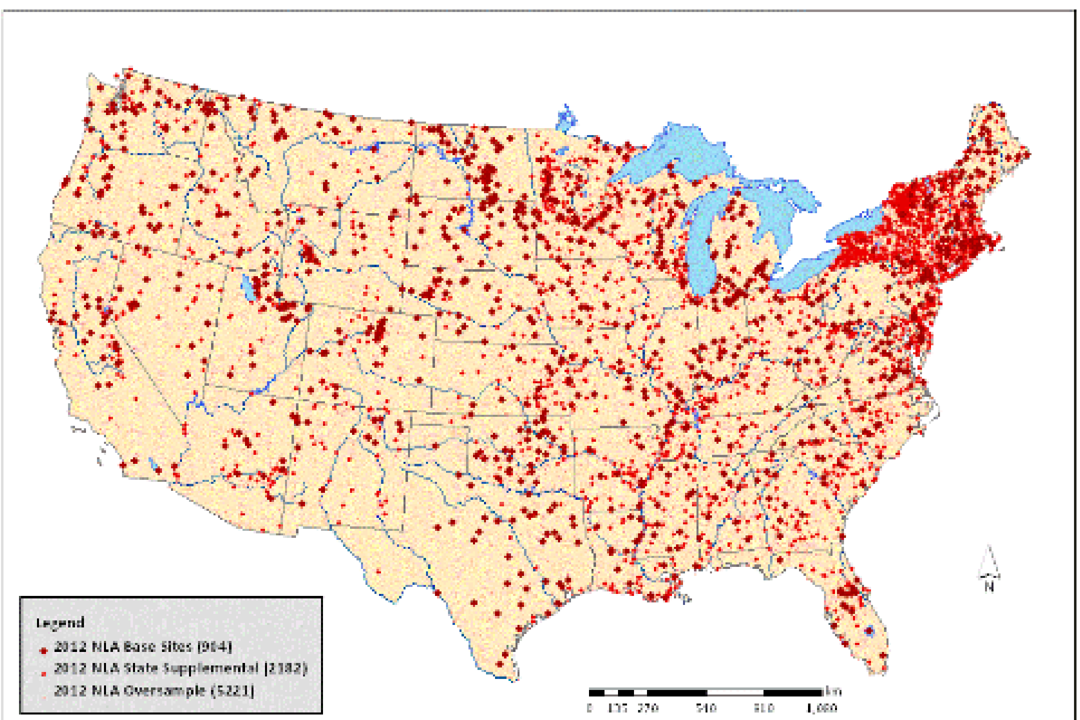
Introduction

National Lakes Assessment Project (NLAP) Overview

The United States Environmental Protection Agency (EPA) has a responsibility to assess the health of the nation's water resources. One of the methods for assessment is statistically based surveys. The National Lakes Assessment Project (NLAP) is one of a series of water surveys being conducted by states, tribes, the EPA, and other partners. In addition to lakes, partners also study coastal waters, wadable streams, rivers, and wetlands in a revolving sequence. The purpose of these surveys is to generate statistically-valid and environmentally relevant reports on the condition of the nation's water resources at nationwide and regional scales.

The sampling design for this survey is a probability-based network which provides statistically-valid estimates of the condition of all lakes with known confidence. It is designed using modern survey techniques. Sample sites are selected using a stratified-random design to represent the condition of all lakes across the nation and each region. A total of 1000 lakes in the United States were included in the 2012 Lakes Survey. The sample set is comprised of freshwater lakes, ponds, and reservoirs greater than one hectare and at least one meter in depth located in the conterminous U.S.

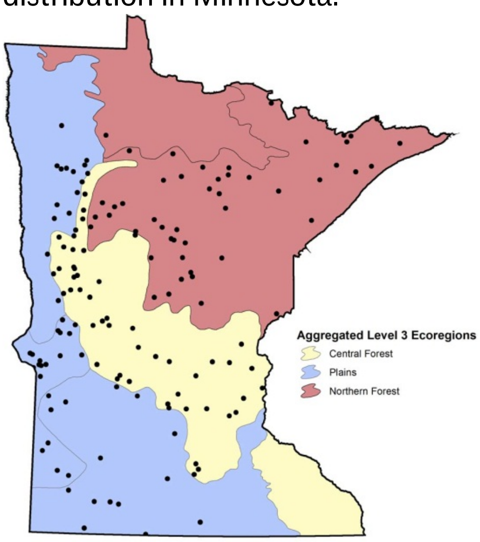
Design Sites for the
2012 National Lakes Assessment



Minnesota's NLAP Overview

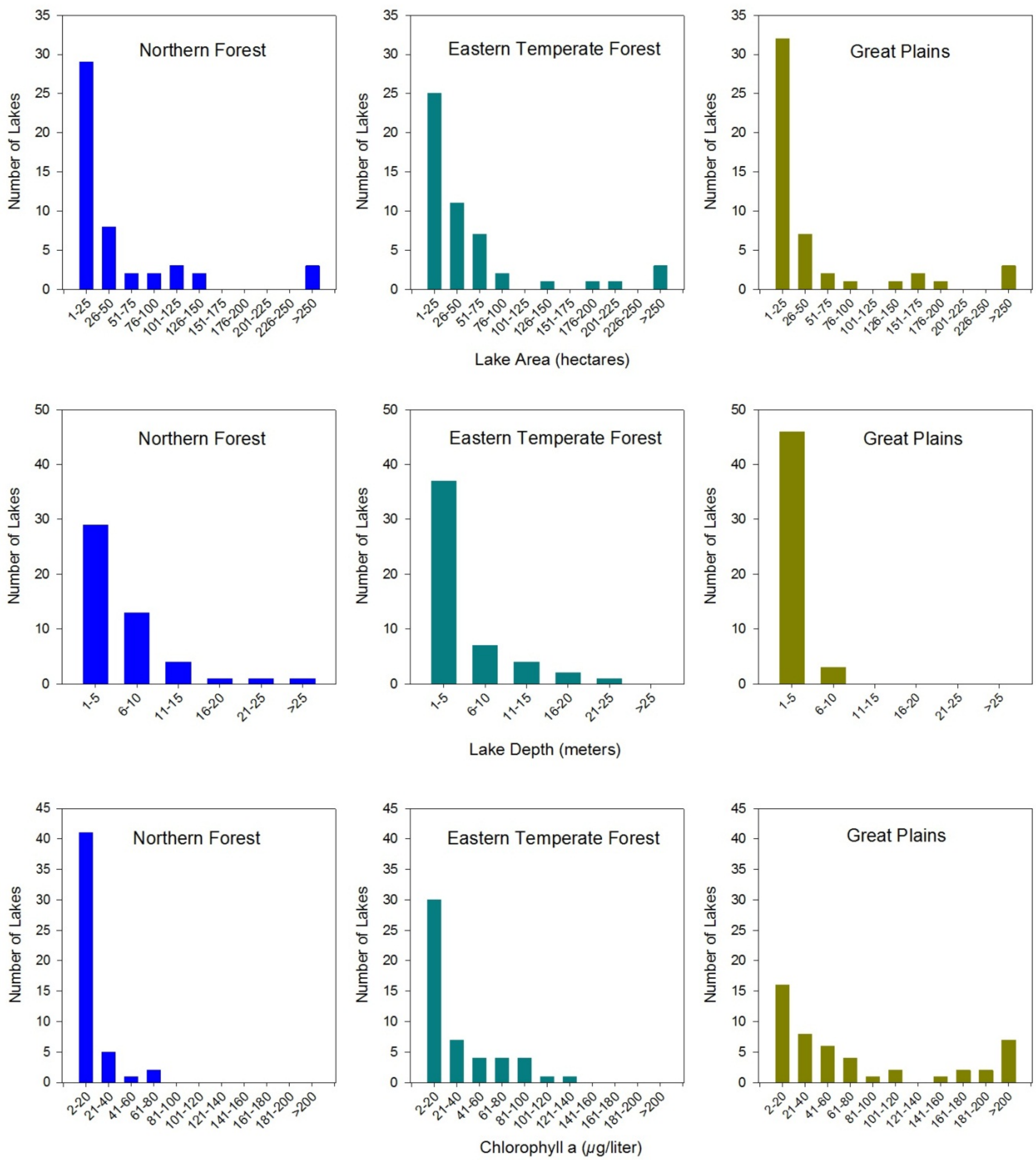
Minnesota's participation in the 2012 NLAP survey involved a collaborative approach that included EPA, U.S. Forest Service (USFS), MN Department of Natural Resources (MDNR), MN Department of Health (MDH), MN Department of Agriculture (MDA) and the Red Lake and White Earth Native American Bands. Minnesota drew 42 lakes as a part of the initial draw for this statistically based national survey effort and added 8 lakes to allow for state-based assessment. All 50 lakes received the national level of assessment and contributed to both the state-frame and national-frame assessments. In addition, 100 lakes were added from EPA's randomized list of lakes to allow for ecoregion-based assessments (50 per major ecoregion) in Minnesota. The 2012 NLAP survey also involved resampling some lakes from the 2007 NLAP survey and, where applicable, comparisons between 2007 and 2012 results are included in this report.

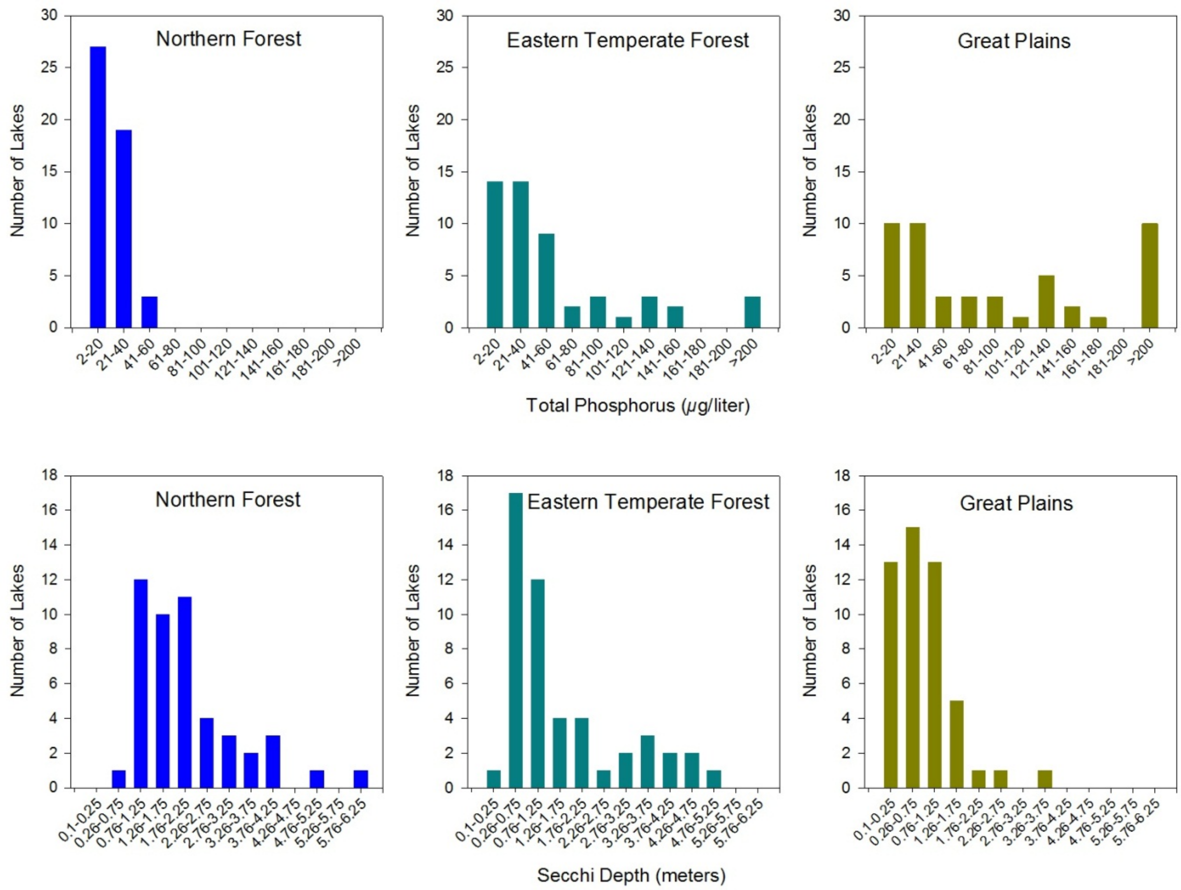
This report will focus on the crustacean zooplankton communities sampled from both state-based and ecoregion-based Minnesota NLAP lakes. The results should only be viewed as reflective of the zooplankton communities sampled in these lakes based on the method used in this survey, which was limited to a single vertical tow from most lakes, collected once between June 6 and September 6, 2012. Although zooplankton variability among lakes is generally greater than variability within lakes (Rusack et al. 2002; Olden et al. 2006), zooplankton tend to have both spatial and temporal variation within lakes due to patchy distribution and short generation times. In addition, over half the lakes chosen for this survey were small (under 25 hectares) and shallow (less than 5 meters maximum depth), atypical of what may be considered a classic recreational Minnesota lake. Therefore, this survey is not intended to provide a comprehensive assessment for the individual lakes nor a statistically-valid sample of typical recreational lakes in Minnesota. Nevertheless, this survey did provide the opportunity to develop a large and comprehensive zooplankton community dataset from a wide variety of lakes across the state of Minnesota. This will have value in examining the potential use of zooplankton as indices of change as well as documenting zooplankton species presence and distribution in Minnesota.



Distribution of MN State and National-Based NLAP Lakes

As background for this report, the following histograms display morphometric parameters (lake area and maximum lake depth) and productivity parameters (chlorophyll a, total phosphorus, and Secchi depth) for all state and national-frame NLAP lakes within each of the three major ecoregions in Minnesota.




Northern Forest
Eastern Temperate Forest
Great Plains
Number of Lakes
2-20 21-40 41-60 61-80 81-100 101-120 121-140 141-160 161-180 181-200 >200
Total Phosphorus (μg/liter)
Northern Forest
Eastern Temperate Forest
Great Plains
Number of Lakes
0.1-0.25 0.26-0.75 0.76-1.25 1.26-1.75 1.76-2.25 2.26-2.75 2.76-3.25 3.26-3.75 3.76-4.25 4.26-4.75 4.76-5.25 5.26-5.75 5.76-6.25
Secchi Depth (meters)

Report focus: Zooplankton Communities in Minnesota Lakes

Background

The crustacean zooplankton community is an important component of the aquatic food web in lakes. Zooplankton are known to be efficient phytoplankton grazers as well as primary food sources for larger invertebrates and young-of-the-year-fish. Many adult fish species also rely on zooplankton for prey. Because of their intermediate trophic position and interactions with nutrient cycling, zooplankton play key roles in the functioning of lake ecosystems (Dodson and Frey 2001).

Many studies have shown promising results using crustacean zooplankton as indicators of lake productivity and changing environmental conditions in north-temperate lakes (Rusack et al. 2002; Beisner et al. 2003; Olden et al. 2006). Furthermore, zooplankton indices to assess lake productivity were examined from 24 sentinel lakes in Minnesota (Hirsch 2013). Because of their importance in the aquatic food web and potential use as indicators of lake productivity, coupled with the opportunity to document the distribution of species from a randomly selected cross section of Minnesota lakes, the collection of zooplankton was included as part of the Minnesota NLAP study.

Methods

Field Collection

One vertical zooplankton tow was collected from each of 149 lakes using a 13cm mouth, 80μm mesh Wisconsin style zooplankton net. An additional tow was collected from two of the lakes on repeat visits. Samples were collected between June 6, 2012 and September 6, 2012. Zooplankton tows were taken from an anchored boat where a net was lowered to within 0.5 m of the bottom and hauled up at a rate of approximately 0.5 m/sec. Contents were rinsed into sample bottles labeled with lake name, inventory number, date and tow depth. Samples were preserved with 100% reagent alcohol and delivered to the Minnesota Department of Natural Resources (MDNR) for analysis.

Note: In addition to the zooplankton tows collected from the 149 state and national-frame lakes using methods described above, sets of additional tows were collected from the 50 national-frame lakes using 2012 EPA methods, as well as tows collected from 20 revisit lakes using 2007 EPA methods for comparison. These methods are described in the EPA's NLAP Field Operations Manual: http://water.epa.gov/type/lakes/assessmonitor/lakessurvey/upload/NLA2012_FieldOperationsManual_120517_FINAL_CombinedQRG.pdf.

Laboratory Analysis

Each sample was adjusted to a known volume by filtering the entire sample volume through 80μm mesh netting and rinsing specimens into a graduated beaker. Water was added to the beaker to a volume that provided at least 150 organisms per 5 ml aliquot. After mixing, a 5ml aliquot was withdrawn from each sample using a bulb pipette and transferred to a counting chamber. Specimens from each aliquot were counted and measured to the nearest 0.01 mm using a dissecting microscope and a computerized zooplankton counting system. All cladocerans in aliquot were identified to lowest taxonomic level possible (most to species); whereas copepods were identified to four major groups (cyclopoids, calanoids, copepodites and nauplii) for quantitative purposes. Adult copepods from each sample were further identified under a compound microscope down to species level, for a qualitative lake taxa list.

Data generated included density (number/liter), biomass (μg/liter), percent composition by number and weight, mean length (mm), mean weight (μg) and total count of each taxon identified. Mean weight and biomass estimates were calculated using length-weight regression coefficients from Culver et al. (1985) and Dumont et al. (1975). These data were automatically recorded from the counting system into the MDNR zooplankton database. Summary reports of these data for each lake sampled are not included in this report, but are available from the MDNR-Ecological and Water Resources Division. An example of an individual lake report is illustrated in Appendix A.

Results and Discussion

General Overview

A total of 51 crustacean zooplankton species were identified among the 149 lakes sampled. Of those 51 species identified, 33 were cladocerans, 9 were cyclopoid copepods, and 9 were calanoid copepods. All species identified and the percentage of lakes where that species occurred in each ecoregion are listed in Table 1.

<u>Number of Species</u>: The number of species identified per lake ranged from 1 to 15, with most lakes having 4 to 8 species present overall (Figure 1). The Northern Forest and Eastern Temperate Forest Ecoregions both followed the statewide NLAP pattern with most lakes having 4 to 8 species present, whereas in the Great Plains Ecoregion, most lakes had fewer species (3-7). Because only one sample was collected from the majority of the lakes, and zooplankton tend to have short generation times, the actual number of species per lake may be higher in some lakes than reported from this survey. Nevertheless, these values can be used as relative indices of species richness when comparing lakes.

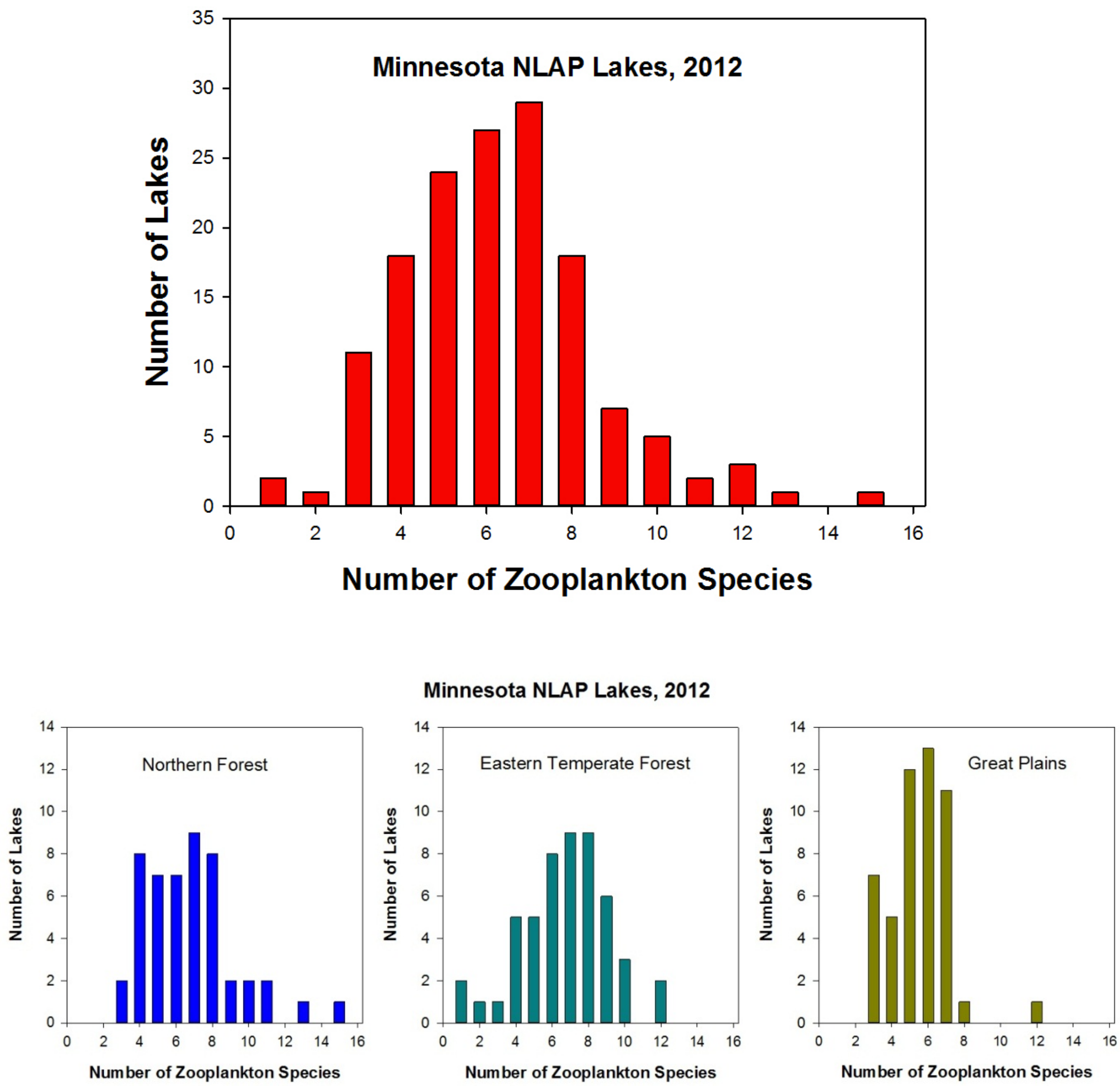


Figure 1. Distribution of the number of zooplankton species per lake collected from MN NLAP Lakes, 2012. (Graphs in lower half of figure show number of taxa per lake in each of the three ecoregions).

Statewide Ubiquitous Species: Three cladocerans (*Bosmina spp*., *Ceriodaphnia lacustris*, and *Diaphanosoma birgei*) were ubiquitous among the samples, i.e., present in over 40% of the lakes in each ecoregion. *Mesocyclops edax* was the only ubiquitous cyclopoid copepod and no calanoid copepods were ubiquitous in the samples. These species also appear to be ubiquitous among the 24 Minnesota sentinel lakes (Hirsch 2013).

Ecoregion Exclusive Species: Species found exclusively from lakes in the Northern Forest Ecoregion included *Daphnia longiremis* and *Leptodiaptomus minutus*. Both these species are known to prefer oligotrophic conditions, especially *D. longiremis*, a cold water stenotherm, usually found below the thermocline in well-oxygenated lakes (Balcer et al. 1984). Species that were relatively common in lakes sampled from the Eastern Temperate Forest and Great Plains ecoregions, but absent from the Northern Forest Ecoregion, included *Eubosmina coregoni*, *Acanthocyclops vernalis*, *Aglaodiaptomus clavipes*, and *Leptodiaptomus siciloides*. *E. coregoni* is a European species that is relatively uncommon in the Great Lakes region (Balcer et al. 1984), although it has been collected from many of the sentinel lakes in Minnesota, especially those in the southern portion of the state (Hirsch 2013). Both *L. siciloides* and *A. clavipes* are calanoid copepods that prefer more eutrophic, productive waters (Torke 2001).

Uncommon Species: There were 29 uncommon species, present in less than 10% of the lakes sampled in any one ecoregion (Table 1). Many of these were littoral species collected from shallower lakes. In the past, most zooplankton surveys in Minnesota have focused on the collection of limnetic species. Therefore, many of the vegetative-dwelling species of the littoral zone have not been well documented from Minnesota lakes. Examples of a few of the more uncommon littoral species collected in this survey included *Chydorus faviformis, Pleuroxus denticulatus*, and *Pleuroxus procurvatus.* Photographs of these, along with examples of more common species, are shown in Appendix B.

Table 1. List of zooplankton species collected from Minnesota NLAP lakes, 2012. (Numbers in table are the percentage of lakes sampled from each ecoregion where species occurred).

	% Northern Forest Lakes	% Eastern Temperate Forest Lakes	% Great Plains Lakes
Cladocerans			
Acroperus harpae	2.0	2.0	2.0
Alona setulosa	10.0	12.0	4.1
Alona sp.	4.0	0.0	0.0
Alonella sp.	4.0	0.0	0.0
Bosmina spp.	92.0	72.0	79.6
Ceriodaphnia lacustris	42.0	48.0	51.0
Ceriodaphnia quadrangula	0.0	0.0	2.0
Ceriodaphnia reticulata	0.0	4.0	2.0
Chydorus faviformis	2.0	0.0	0.0
Chydorus sphaericus	36.0	44.0	26.5
Daphnia ambigua	10.0	8.0	0.0
Daphnia galeata mendotae	40.0	32.0	14.3
Daphnia laevis	0.0	2.0	2.0
Daphnia longiremis	8.0	0.0	0.0
Daphnia parvula	6.0	16.0	18.4
Daphnia pulicaria	26.0	24.0	12.2
Daphnia retrocurva	24.0	20.0	2.0
Daphnia schodleri	2.0	0.0	0.0
Diaphanosoma birgei	74.0	56.0	75.5
Diaphanosoma brachyurum	6.0	12.0	2.0
Eubosmina coregoni	0.0	10.0	4.1
Eurycercus lamellatus	0.0	2.0	0.0
Holopedium gibberum	16.0	2.0	0.0
Ilyocryptus sp.	2.0	8.0	2.0
Leptodora kindti	2.0	4.0	0.0
Pleuroxus denticulatus	2.0	0.0	2.0
Pleuroxus procurvatus	2.0	2.0	0.0
Polyphemus pediculus	0.0	2.0	0.0
Sida crystallina	2.0	6.0	2.0
Simocephalus serrulatus	0.0	4.0	2.0
Simocephalus sp.	2.0	2.0	0.0
Simocephalus vetulus	0.0	2.0	0.0
Streblocerus serricaudatus	0.0	0.0	2.0

Table 1. Continued.

	% Northern Forest Lakes	% Eastern Temperate Forest Lakes	% Great Plains Lakes
Cyclopoid Copepods			
Acanthocyclops vernalis	0.0	20.0	30.6
Diacyclops bicuspidatus thomasi	10.0	6.0	0.0
Ergasilus sp.	8.0	4.0	4.1
Eucyclops serrulatus	0.0	8.0	0.0
Eucyclops speratus	0.0	2.0	0.0
Macrocyclops albidus	0.0	2.0	0.0
Mesocyclops edax	62.0	54.0	40.8
Mesocyclops leuckarti	0.0	0.0	2.0
Tropocyclops prasinus mexicanus	46.0	24.0	10.2
Calanoid Copepods			
Aglaodiaptomus clavipes	0.0	8.0	24.5
Aglaodiaptomus leptopus	2.0	4.0	4.1
Aglaodiaptomus saskatchewanensis	0.0	6.0	0.0
Epischura lacustris	6.0	4.0	0.0
Leptodiaptomus minutus	10.0	0.0	0.0
Leptodiaptomus nudus	0.0	0.0	2.0
Leptodiaptomus siciloides	0.0	40.0	67.3
Skistodiaptomus oregonensis	76.0	30.0	16.3
Skistodiaptomus pallidus	2.0	22.0	0.0

Zooplankton Indices-Geographical Distribution and Ecoregion Means

Background: Minnesota Sentinel Lakes Data: Total zooplankton densities (number/liter) and biomass (µg/liter) proved to be potential indicators of lake productivity among the 24 Minnesota sentinel lakes (Hirsch 2013), where regression results showed strong relationships between both zooplankton densities and biomass with total phosphorus (Figure 2). In addition, there appeared to be a general trend among the sentinel lakes within each of the three major ecoregions, where densities and biomass were lower among lakes in the Northern Forest Ecoregion, and higher in the Great Plains Ecoregion. Species richness (number of taxa) did not show as clear of a trend between ecoregions although lakes with the highest species richness tended to be those in either the Northern Forest or Eastern Temperate Forest ecoregions (Table 2). Because of these findings, these three indices were further examined with zooplankton data collected from the 149 Minnesota NLAP lakes. Appendices C, D and E list the NLAP lakes sampled in this survey, along with zooplankton densities, biomass and species richness for each of the lakes in the three ecoregions of Minnesota.

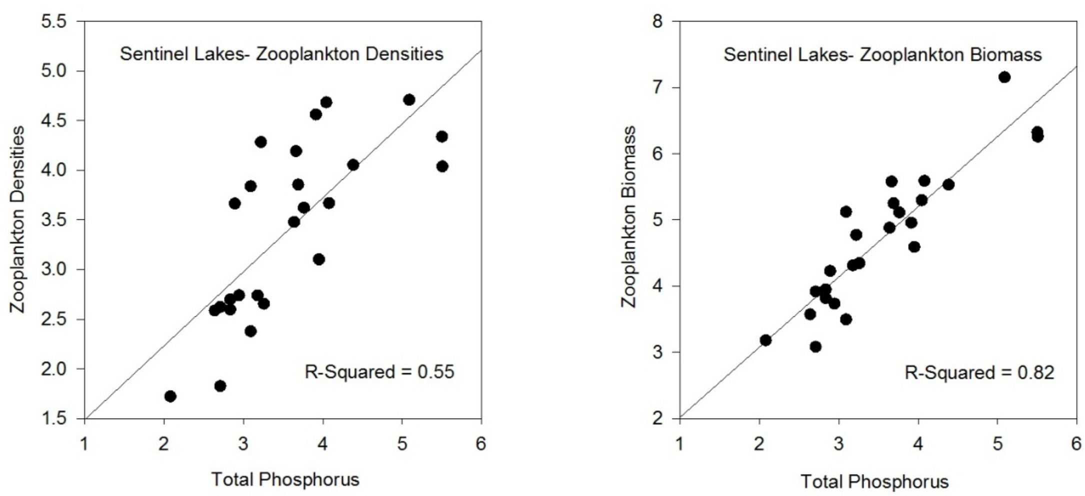


Figure 2. Simple linear regressions of total phosphorus (µg/liter) with zooplankton densities (no. /liter) and zooplankton biomass (µg/liter) from Minnesota's 24 Sentinel Lakes, 2008-2011. (Data was transformed to $\log^{e}+1$ for normality).

Table 2. Mean summer total phosphorus (μg/liter), mean annual zooplankton densities (number individuals/liter), biomass (μg/liter) and species richness for the 24 Sentinel lakes, 2008-2011. Lakes arranged by ecoregion.

Sentinel Lakes Zooplankton	**Mean Summer Total Phosphorus (2010)**	**Mean Annual Densities (2008-2011)**	**Mean Annual Biomass (2008-2011)**	**Species Richness**
Great Plains				
Artichoke	244	75.64	558.03	13
Shaokotan	161	109.97	1279.54	11
Madison	79	56.66	251.73	13
St. James	49	94.79	141.20	13
St. Olaf	38	65.23	264.00	15
Carrie	21	45.51	166.84	14
Eastern Temperate Forest				
Peltier	245	55.79	521.96	12
Belle	58	38.26	266.98	13
South Center	51	21.23	97.73	19
Pearl	39	46.28	190.12	13
Carlos	16	13.86	50.89	17
Cedar	14	12.78	49.27	11
Northern Forest				
Portage	56	107.30	199.12	13
Echo	42	36.43	165.21	14
Hill (south)	37	31.45	130.95	13
Elephant	25	13.22	76.11	14
Red Sand	24	71.58	117.36	18
Hill (north)	23	14.48	73.66	13
White Iron	21	9.79	31.95	17
Elk	18	14.50	40.83	16
South Twin	17	38.05	67.55	13
Tait	16	12.42	44.62	15
Bearhead	14	5.21	20.81	16
Ten Mile	13	12.30	34.64	16
Trout	7	4.60	23.06	15

NLAP Lakes Zooplankton Density: Mean zooplankton densities for each ecoregion are plotted in Figure 3. One-way analysis of variance (ANOVA) results showed that mean densities were significantly higher in lakes from the Great Plains Ecoregion than those from the Northern Lakes Ecoregion ($p<.05$). Although mean densities from the Eastern Temperate Forest Ecoregion were higher than those from the Northern Forest Ecoregion and lower than those from the Great Plains Ecoregion, neither of these differences was significant. These findings support results from the sentinel lakes study, where higher zooplankton densities were generally found in lakes in the southern portion of the state, and lower densities in northern Minnesota lakes.

The majority of NLAP lakes across the state had total zooplankton densities of less than 50 individuals per liter (Figure 4). Lakes that had the highest densities were generally restricted to the Great Plains and Eastern Temperate Forest ecoregions, with one exception, an Unnamed Lake in Clearwater County (DOW# 15049100), which had total densities of 418 individuals per liter. The highest densities were found in Lindgren Lake, Kandiyohi County (DOW# 34029400) with 1,748 individuals per liter.

NLAP Lakes Zooplankton Biomass: Similar to densities, mean zooplankton biomass was highest in lakes from the Great Plains Ecoregion and lowest in lakes from the Northern Forest Ecoregion (Figure 5). One-way ANOVA results showed significant differences ($p<.05$) between mean biomass in lakes from the Northern Forest and Eastern Temperate Forest Ecoregions, and the Northern Forest and Great Plains ecoregions, but no significant difference between lakes in the Eastern Temperate Forest and Great Plains Ecoregion.

Similar to densities, the majority of NLAP lakes had relatively low total zooplankton biomass, in the range of 1-102 μg per liter (Figure 6). Lakes with higher biomass were generally restricted to the Great Plains Ecoregion with a few exceptions occurring in the Eastern Temperate Forest Ecoregion. The lake with the highest zooplankton biomass was Talcot Lake, Cottonwood County (DOW# 17006000) with 2,252 μg per liter.

NLAP Lakes Zooplankton Species Richness: Mean number of taxa identified per lake (species richness) for each ecoregion was plotted in Figure 7. One-way ANOVA results showed significantly lower mean species richness ($p<.05$) in lakes in the Great Plains Ecoregion than in the other two major ecoregions of the state. There was no significant difference in mean species richness between lakes in the Northern Forest and Eastern Temperate Forest ecoregions.

General trends in species richness appear to be lower in the Great Plains Ecoregion lakes and higher in lakes in the central portion of state, with the exception of Ball Club Lake, Cook County (DOW#16018200), where 13 taxa were identified (Figure 8). Long Lake, Cass County (DOW#11048000) had the highest species richness of all the NLAP lakes, with 15 taxa identified. Findings were similar among the sentinel lakes, where lakes with the highest species richness were located in the north central portion of the state.

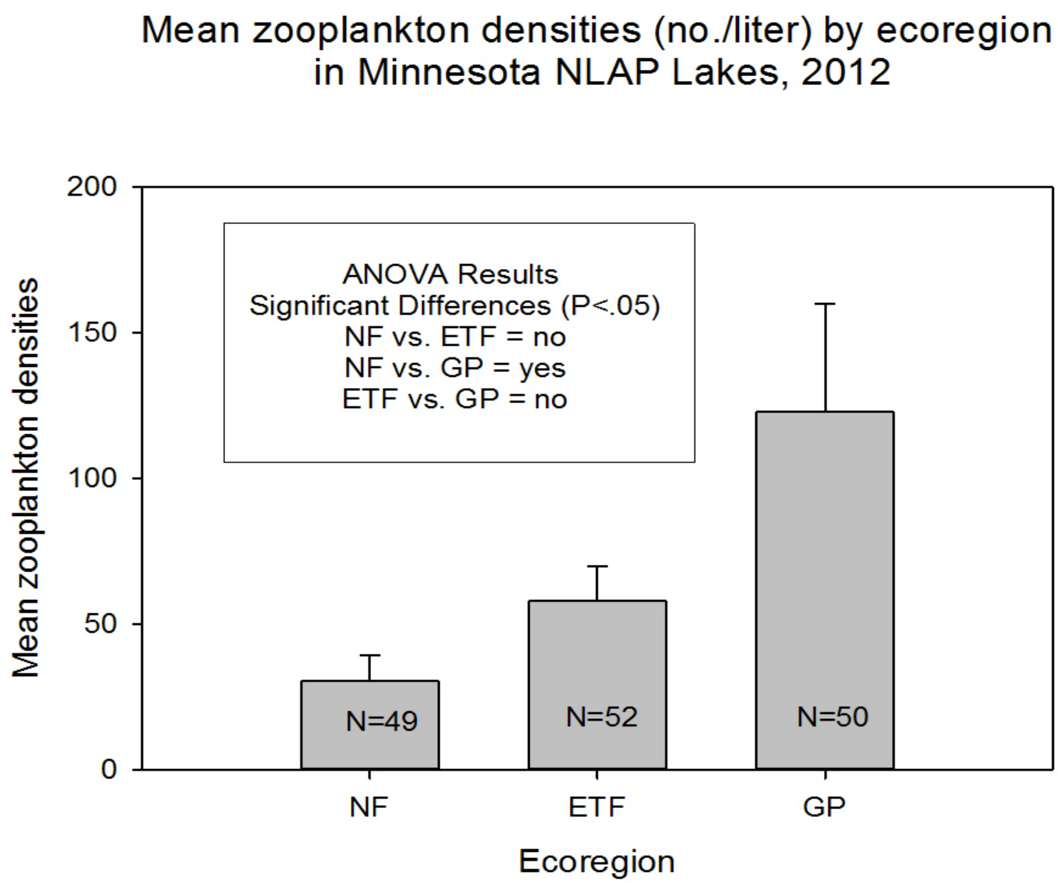


Figure 3. Mean zooplankton densities (no. /liter) by ecoregion for Minnesota NLAP lakes, 2012. Error bars represent standard error of the mean. NF= Northern Forest Ecoregion, ETF= Eastern Temperate Forest Ecoregion and GP= Great Plains Ecoregion. One way analysis of variance test (Kruskal-Wallis ANOVA and Dunn's Method) showed significant differences ($P<.05$) between NF and GP, but not between NF and ETF nor between ETF and GP. Zooplankton data was log transformed (ln+1) for statistical analysis. N= number of lakes.

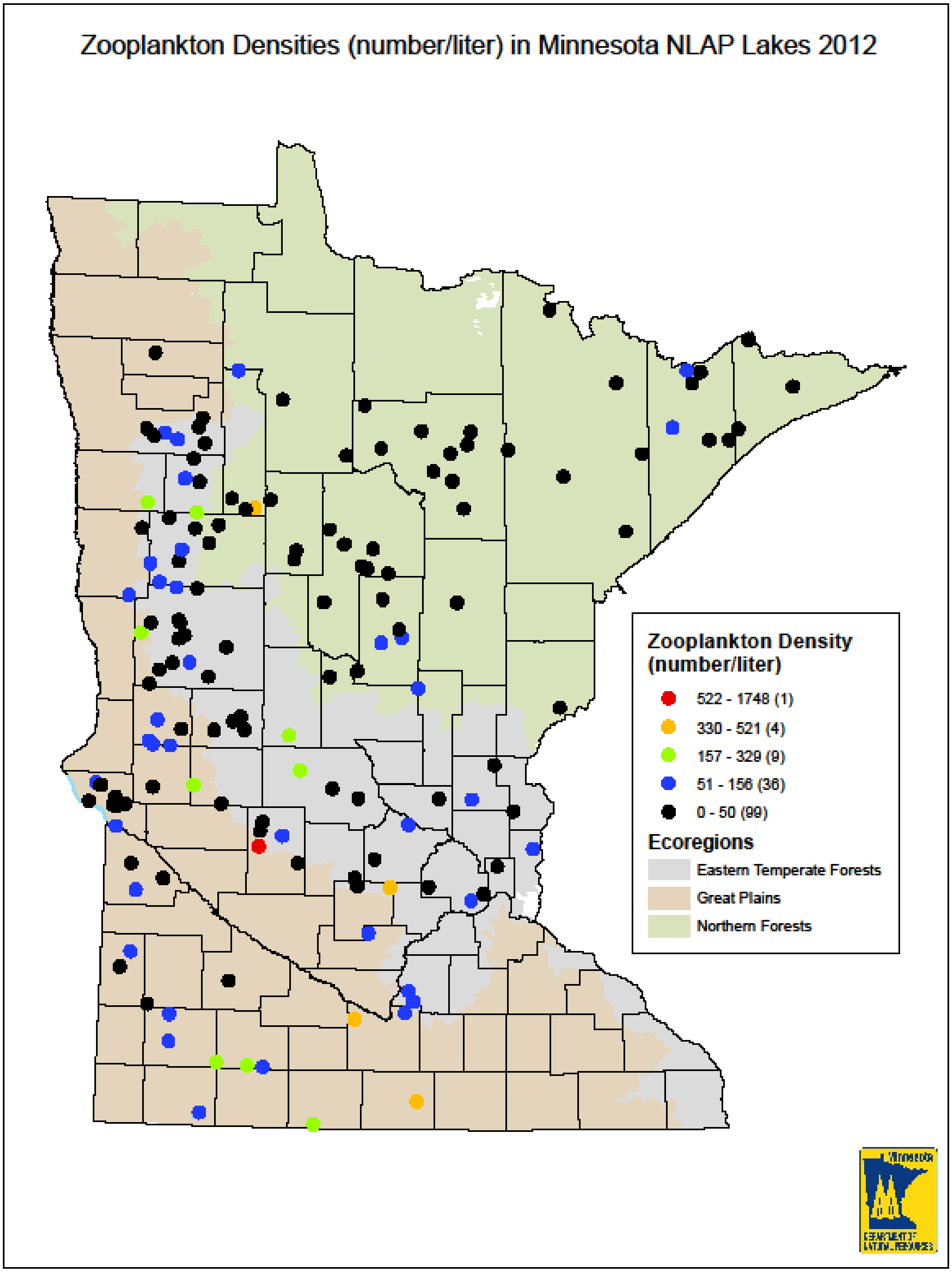


Figure 4. Zooplankton density distribution for Minnesota NLAP Lakes, 2012.

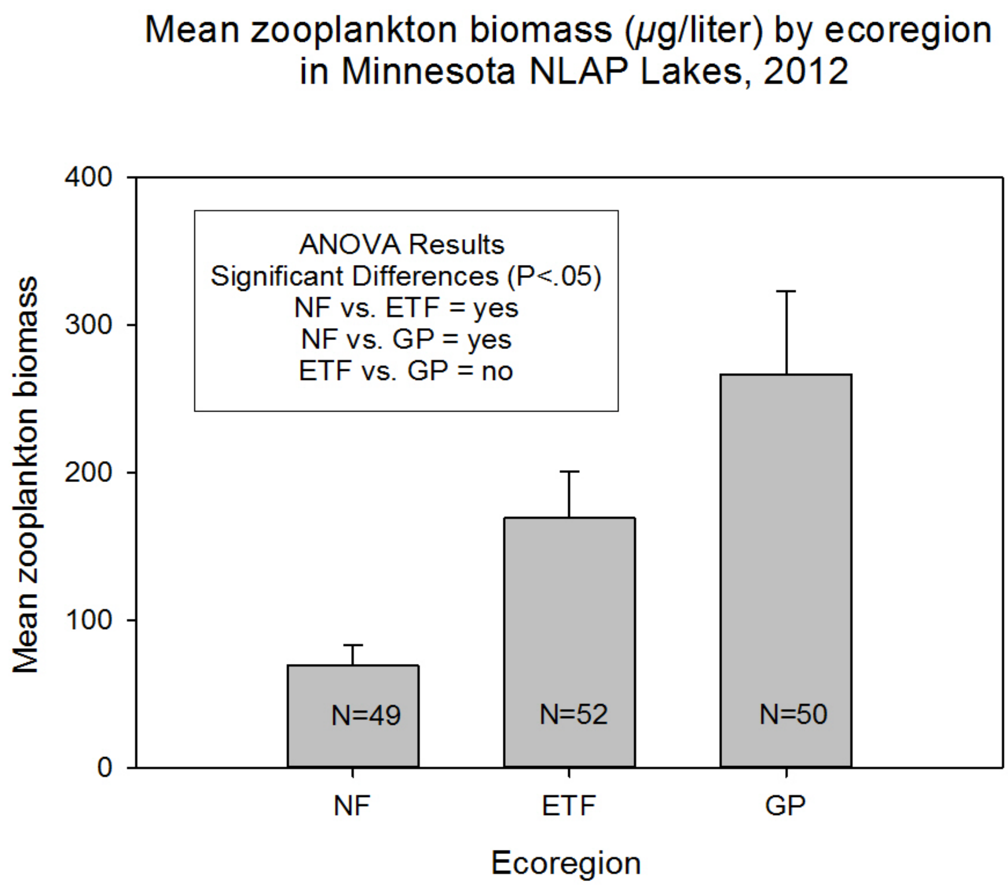


Figure 5. Mean zooplankton biomass (µg/liter) by ecoregion in Minnesota NLAP lakes, 2012. Error bars represent standard error of the mean. NF= Northern Forest Ecoregion, ETF= Eastern Temperate Forest Ecoregion and GP= Great Plains Ecoregion. One way analysis of variance tests (Kruskal-Wallis ANOVA and Dunn's Method) showed significant differences (P<.05) between NF and ETF, NF and GP, but not between ETF and GP. Zooplankton data was log transformed (ln+1) for statistical analysis. N= number of lakes.

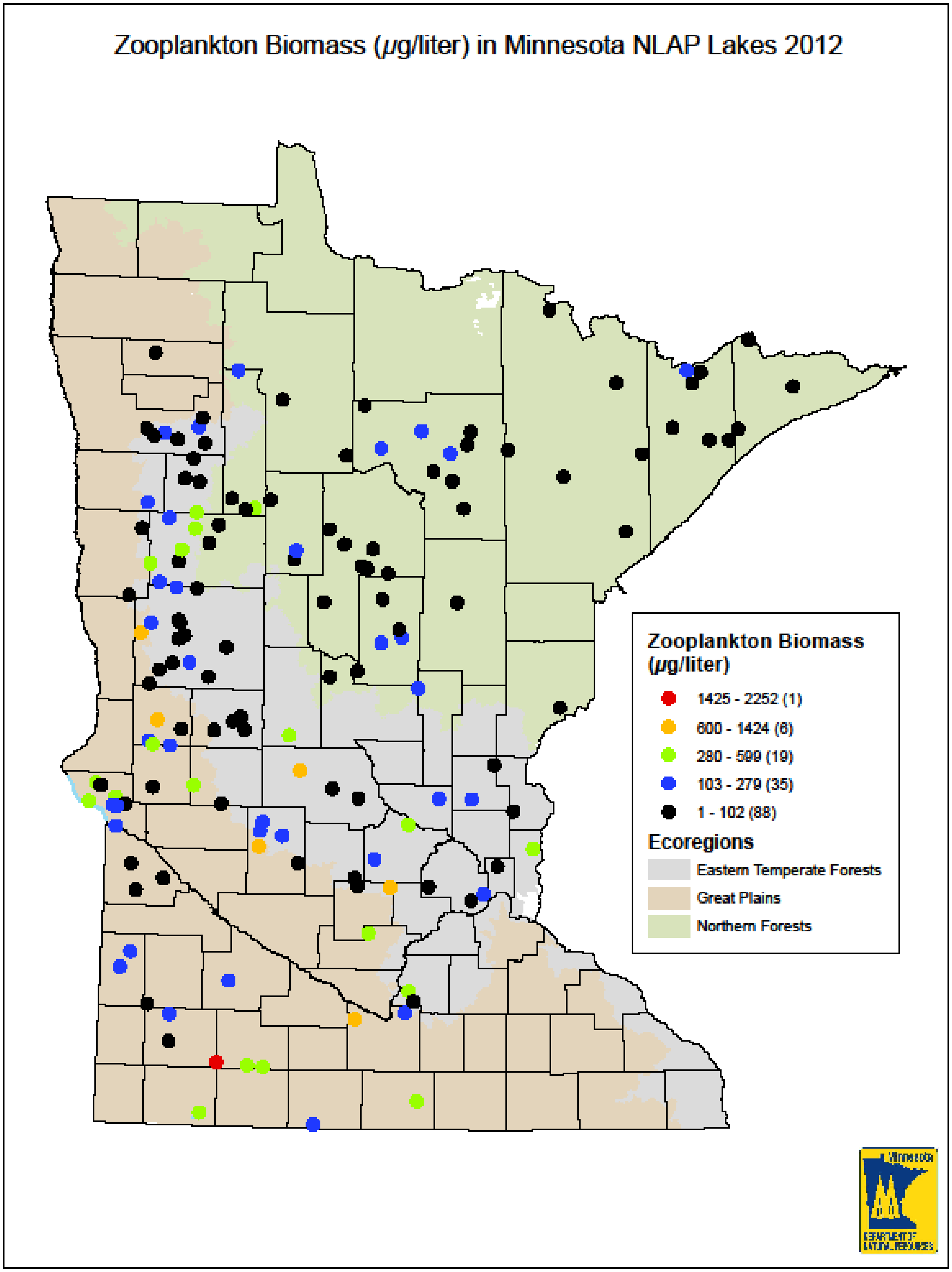


Figure 6. Zooplankton biomass distribution for Minnesota NLAP Lakes, 2012.

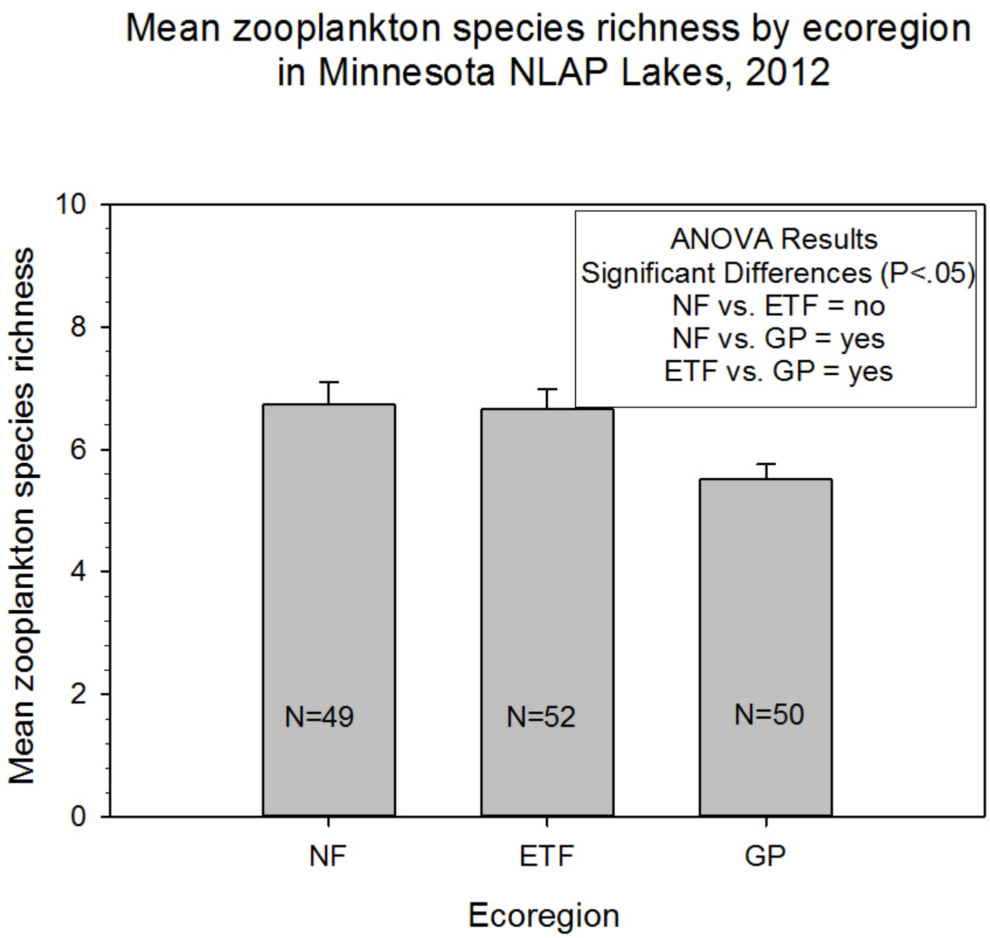


Figure 7. Mean zooplankton species richness by ecoregion in Minnesota NLAP lakes, 2012. Error bars represent standard error of the mean. NF= Northern Forest Ecoregion, ETF= Eastern Temperate Forest Ecoregion and GP= Great Plains Ecoregion. One way analysis of variance tests (Kruskal-Wallis ANOVA and Dunn's Method) showed significant differences ($P<.05$) between NF and GP, ETF and GP, but not between NF and ETF. N= number of lakes.

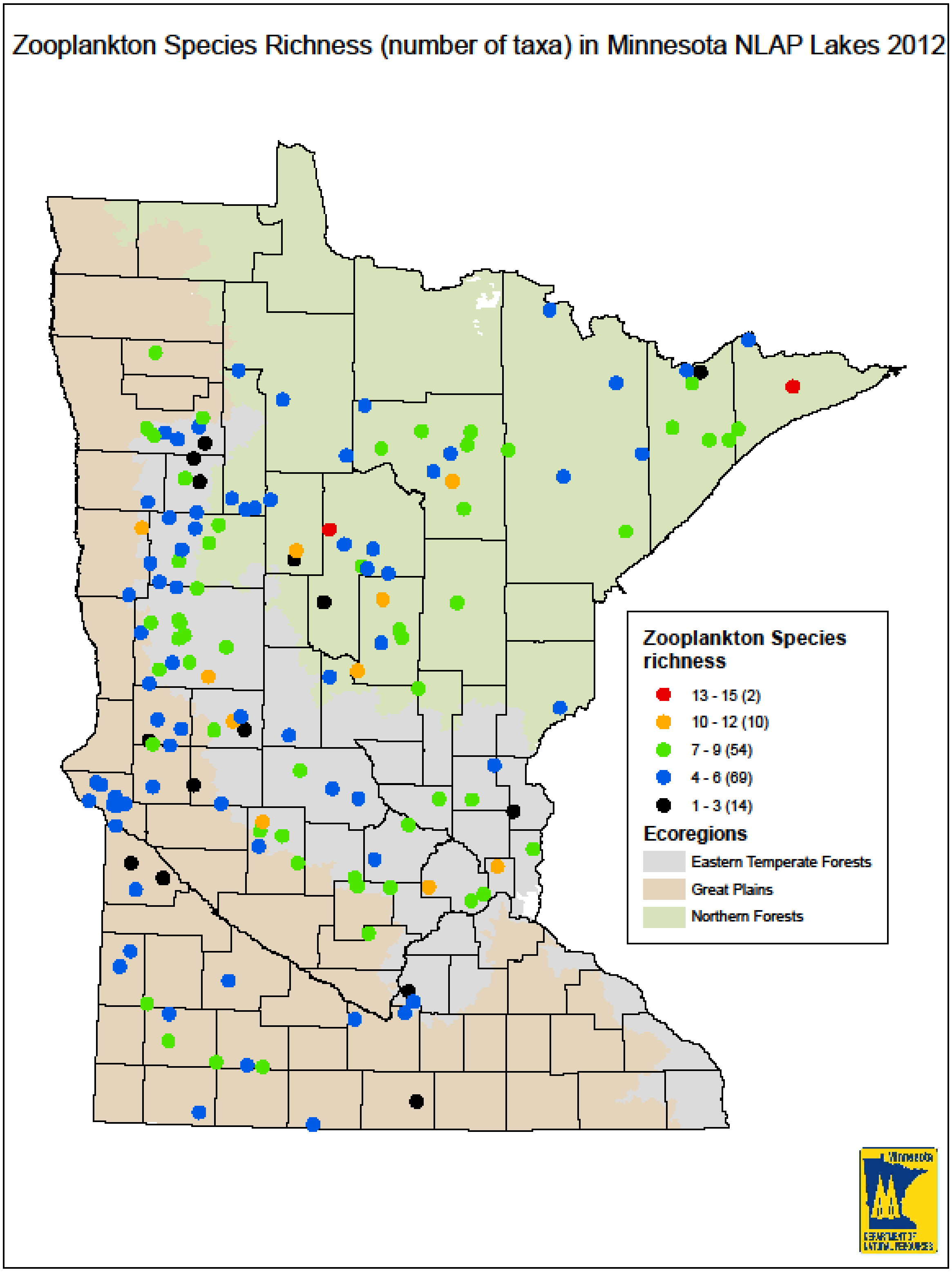


Figure 8. Zooplankton species richness distribution for Minnesota NLAP Lakes, 2012.

Zooplankton Indices-Pearson Correlation Coefficient Analysis

To further examine zooplankton indices with lake productivity, Pearson correlation coefficient analysis between lake variables and zooplankton indices was used to test hypotheses stated below (Table 3). Lake variables included total phosphorus, chlorophyll a, and Secchi depth as indices of lake productivity. Additionally, lake area and maximum depth were also included in analysis. Zooplankton indices tested included total zooplankton densities, total zooplankton biomass, species richness, percent *Daphnia* by weight, mean weight of *Daphnia* and percent calanoid copepods.

- <u>Total zooplankton densities</u>: Hypothesis: As nutrient loading increases, total zooplankton densities should increase (Pace 1986; Attayde and Bozelli 1998).

- <u>Total zooplankton biomass</u>: Hypothesis: As nutrient loading increases, total zooplankton biomass should increase (Hanson and Peters 1984; Pace 1986; Gamble et al. 2006).

- <u>Species richness</u>: Hypothesis: As nutrient loading increases, species richness should decrease (Hoffman and Dodson 2005; Barnett and Beisner 2007).

- <u>Relative abundance of large *Daphnia*</u>: Hypothesis: As nutrient loading increases, relative abundance of large *Daphnia* should decrease (Harig and Bain 1998; Carpenter et al. 2001).

- <u>Relative density of calanoid copepods</u>: Hypothesis: As nutrient loading increases, percent composition of calanoids should decrease (Gannon and Stemberger 1978).

Table 3. Pearson correlation coefficient analysis between lake variables and zooplankton indices for Minnesota NLAP lakes, 2012. Significant correlations (p<.05) are shown in red. Plus symbols (+) indicate positive correlations and minus symbols (-) indicate negative correlations. NS indicates a non-significant correlation. Listed below symbols in each cell of table is the p-value for correlation. Shaded cells represent those correlations that supported corresponding hypotheses. Zooplankton densities and biomass data were transformed to $\log^{e}$ +1 for normality.

	Total Phosphorus	**Chlorophyll a**	**Secchi Depth**	**Lake Area**	**Maximum Depth**
Zooplankton Densities	**+ 1.83E-06**	**+ 9.21E-07**	**- 7.12E-06**	**NS**	**- 1.14E-02**
Zooplankton Biomass	**+ 2.98E-07**	**+ 1.22E-06**	**- 3.93E-03**	**NS**	**NS**
Species Richness	**NS**	**- 1.43E-02**	**+ 5.99E-07**	**NS**	**+ 3.39E-06**
Percent *Daphnia* (by weight)	**NS**	**- 4.85E-02**	**+ 4.98E-07**	**+ 1.55E-02**	**+ 1.07E-07**
Mean weight *Daphnia*	**NS**	**NS**	**+ 8.23E-05**	**+ 1.42E-02**	**+ 9.28E-06**
Percent Calanoids	**NS**	**NS**	**NS**	**+ 2.731E-012**	**NS**

Total zooplankton densities and biomass were both positively correlated with total phosphorus and chlorophyll a (Table 3). As hypothesized, an increase in nutrient loading may lead to an increase in total zooplankton densities and biomass. This finding is supported by ANOVA results for both mean densities and biomass among ecoregions (Figures 3 and 5), where the more productive lakes in the southern portion of the state had significantly higher zooplankton densities and biomass than those in the north. These findings also are supported by Minnesota sentinel lakes findings, where regression results showed strong relationships with both densities and biomass and total phosphorus (Figure 2).

Species richness, in contrast, showed a less consistent response; it was negatively correlated with chlorophyll a, but not with total phosphorus. As hypothesized, an increase in nutrient loading may lead to a decrease in species richness. More productive lakes with higher nutrient loading may be dominated by non-edible blue green algae species, therefore limiting the diversity of zooplankton species in these lakes.

Both zooplankton densities and biomass were negatively correlated with Secchi depth, while species richness was positively correlated with Secchi depth. Secchi depth is a measurement of water clarity, and therefore the more productive, nutrient rich lakes which also had higher zooplankton densities and biomass, would tend to have lower water clarity. It is important to note that in many of the shallow lakes, Secchi may have been on the bottom of lake. Lakes with higher species richness tended to be those lakes with higher water clarity.

The percentage of *Daphnia* (by weight), along with mean *Daphnia* weight were used as relative indices of abundance of large *Daphnia* in the zooplankton community. Percentage of *Daphnia* were negatively correlated with chlorophyll a, and positively correlated with Secchi depth, whereas mean *Daphnia* weight had no significant correlation with chlorophyll a, but positively correlated with Secchi depth. Large *Daphnia* are efficient grazers and therefore can keep algae blooms in check, reducing chlorophyll a, and in turn help maintain higher water clarity. Percentage of *Daphnia* and mean weight of *Daphnia* were also positively correlated with lake area and maximum depth. Larger and deeper lakes often provide a refuge below the thermocline where large *Daphnia* migrate into during daylight hours to avoid fish predation.

Because there was no significant correlation between *Daphnia* indices and total phosphorus, findings from this survey may not support the hypothesis that as nutrient loading increases, the percentage of large *Daphnia* should decrease. This may be explained in part by the abundance of large *Daphnia pulicaria* in some shallow highly eutrophic lakes in southern Minnesota, where the filamentous blue green algae, *Aphanizomenon flos-aquae* is also abundant as demonstrated in Sentinel lakes (Hirsch 2013). These two species have been known to co-exist together in shallow eutrophic lakes, most likely because *D. pulicaria* do not graze on *A. flos- aquae* but rather graze on other algal species that compete with *A. flos-aquae* (Lynch 1981). Furthermore, due to the physical nature of this species of algae, producing large grass-like clippings, *A. flos-aquae* may provide a refuge for *Daphnia pulicaria* in these shallow lakes. Interestingly, lakes such as these generally have high total phosphorus, but also have higher water clarity and lower chlorophyll than expected.

The percentage of calanoid copepods in the zooplankton community was only correlated with lake area and not with any lake productivity indices. This suggests that calanoids as a whole group may not be good indicators of productivity in Minnesota lakes. Gannon and Stemberger (1978) suggested that more productive lakes should have fewer calanoid copepods. This was based on the fact that calanoids are filter feeders that tend to graze on very small particles found in less productive waters. More recent findings suggest that different calanoid species feed on specific size ranges of algal particles and species distribution is partially driven by lake productivity (Torke 2001; Van Egeren et al. 2011).

Calanoid species distribution among the NLAP lakes in Minnesota, tend to support these later findings. Principal component analysis (PCA) was used as a multivariate statistical tool (McCune and Mefford 2011) to examine the distribution of calanoid species (Diaptomidae family), with association to chlorophyll a values from NLAP lakes (Figure 9). Two species, *Leptodiaptomus siciloides* and *Aglaodiaptomus clavipes* showed a positive association with chlorophyll a values, whereas *Skistodiaptomus oregonensis* and *Leptodiaptomus minutus* showed negative associations with chlorophyll a values.

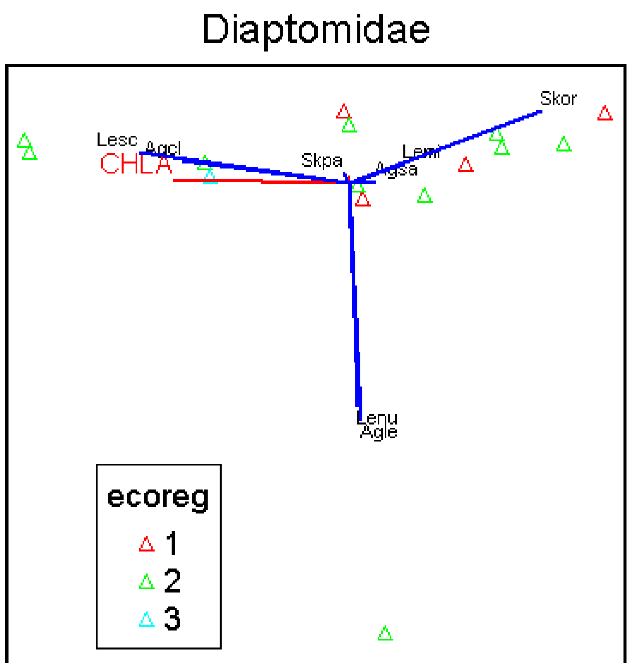


Figure 9. Principal Component Analysis (PCA) biplot of calanoid species presence among NLAP lakes with association with chlorophyll a values. Blue vector lines = species, red vector line = chlorophyll a (CHLA), and triangles = lakes. Ecoreg 1=Northern Forest, ecoreg 2= Eastern Temperate Forest and ecoreg 3=Great Plains. Species abbreviations: Lesc= *Leptodiaptomus siciloides*, Agcl= *Aglaodiaptomus clavipes*, Skpa= *Skistodiaptomus pallidus*, Agsa= *Aglaodiaptomus saskatchewanensis*, Lemi= *Leptodiaptomus minutus*, Skor= *Skistodiaptomus oregonensis*, Lenu= *Leptodiaptomus nudus* and Agle= *Aglaodiaptomus leptopus*.

Geographical Distribution of Calanoid Copepods

The geographical distribution of the species of calanoid copepods was examined in this study because they were present in over 80% of the Minnesota NLAP lakes, yet no one species appeared to be ubiquitous. Additionally, calanoid species tend to remain present in a lake throughout the season. This was particularly important in this study, where samples were collected only once between June and September and temporal data was not available.

Torke (2001) described the calanoid distribution in 499 Wisconsin lakes and concluded that lake productivity and post-glacial history are main determinants in their distribution and increasing eutrophication in lakes can change their distribution over time. Stemberger (1995) reports similar findings in northeastern U.S. lakes. Nine species of calanoid copepods were identified from the Minnesota NLAP lakes. Only one species was found in 91 lakes, two species co-existed in 31 lakes, and three species in only two lakes. Figures 10-14 illustrate the distribution of the calanoid copepod species found in the Minnesota NLAP lakes.

Skistodiaptomus oregonensis was the most common calanoid, found in 60 of the Minnesota NLAP lakes (Figure 10). This species was also the most widely distributed calanoid, occurring throughout the Eastern Temperate Forest and Northern Lakes ecoregions. Although both Torke (2001) and Van Egeren et al. (2011) described this species as being ubiquitous among Wisconsin lakes, *S. oregonensis* was not found in southern Minnesota NLAP lakes. The distribution of this species in Minnesota NLAP lakes would be consistent with the above findings in Wisconsin lakes, as Wisconsin has no Great Plains ecoregion coverage.

Leptodiaptomus minutus was one of the least common calanoids, occurring in only five of the NLAP lakes (Figure 10). Four of these lakes were located in the northeastern-arrowhead region of the state. In addition, this species was found in Long Lake (DOW# 11048000), which is a deep, clear, lake in Cass County. Balcer et al. (1984) described *Leptodiaptomus minutus* as a northern species which tends to inhabit cold, deep lakes, especially in the southern end of its range.

Skistodiaptomus pallidus occurred in 12 of the lakes, most in the central portion of the state within the Eastern Temperate Forest Ecoregion (Figure 11). This species is generally associated with more eutrophic conditions where cultural eutrophication may be expanding its range in Wisconsin (Torke, 2001).

Leptodiaptomus siciloides was the second most common calanoid species, found in 52 of the Minnesota NLAP lakes (Figure 11). The distribution of this species was restricted to the southern and western portions of the state, predominantly in the Great Plains Ecoregion. Similar to *Skistodiaptomus pallidus*, this species is also associated with eutrophic conditions. Torke (2001) suggests that because of its habitat preference, rapid growth, and its ability to utilize large blue-green algal cells (Comita 1972), this species may be a good candidate for an ecological indicator of highly productive conditions.

Aglaodiaptomus clavipes is a large calanoid that was present in 16 lakes in the south and western portion of Minnesota (Figure 12). It only occurred in lakes where *Leptodiaptomus siciloides* was present. The association of these two species is common in large impoundments in the western U.S. Torke (2001) described this species as a recent immigrant to Wisconsin lakes, where it is more commonly distributed in the western U.S.

Aglaodiaptomus leptopus is another large calanoid that was found in only five of the Minnesota NLAP lakes and with no general geographical pattern of distribution (Figure 12). This species tends to inhabit small fishless ponds or lakes that winterkill frequently, as it is very intolerant of fish predation (Torke 2001). All but one of the five NLAP lakes where it was found were very shallow, with a maximum depth of one meter.

Leptodiaptomus nudus was very uncommon, present in only one NLAP lake, Unnamed Lake (DOW#44022800) in Mahnomen County (Figure 13). This was one of the five lakes that *Aglaodiaptomus leptopus* was also found in. Smith and Fernando (1978) describe this calanoid as a rare pond species in northern Ontario, whereas Torke (2001) makes no mention of this species' presence in Wisconsin lakes.

Aglaodiaptomus saskatchewanensis was another uncommon calanoid, found in only three of the Minnesota NLAP lakes. All three of these lakes were small and shallow, and located in very close proximity to one another in Ottertail County (Figure 13). The distribution of this species is not well documented and is not included in the taxonomic keys for the Great Lakes Region (Dr. Janet Reid, PhD, personal communication). Torke (2001) described this species to be rare where it was found in only one marsh in southern Wisconsin.

Epischura lacustris was the only calanoid found in the Minnesota NLAP lakes that did not belong to the Diaptomidae family. This species occurred in five of the lakes in the northern part of the state (Figure 14). This species could be more widespread across Minnesota lakes than what we found in this survey, as Torke (2001) found this species in low densities in lakes throughout Wisconsin (Torke 2001).

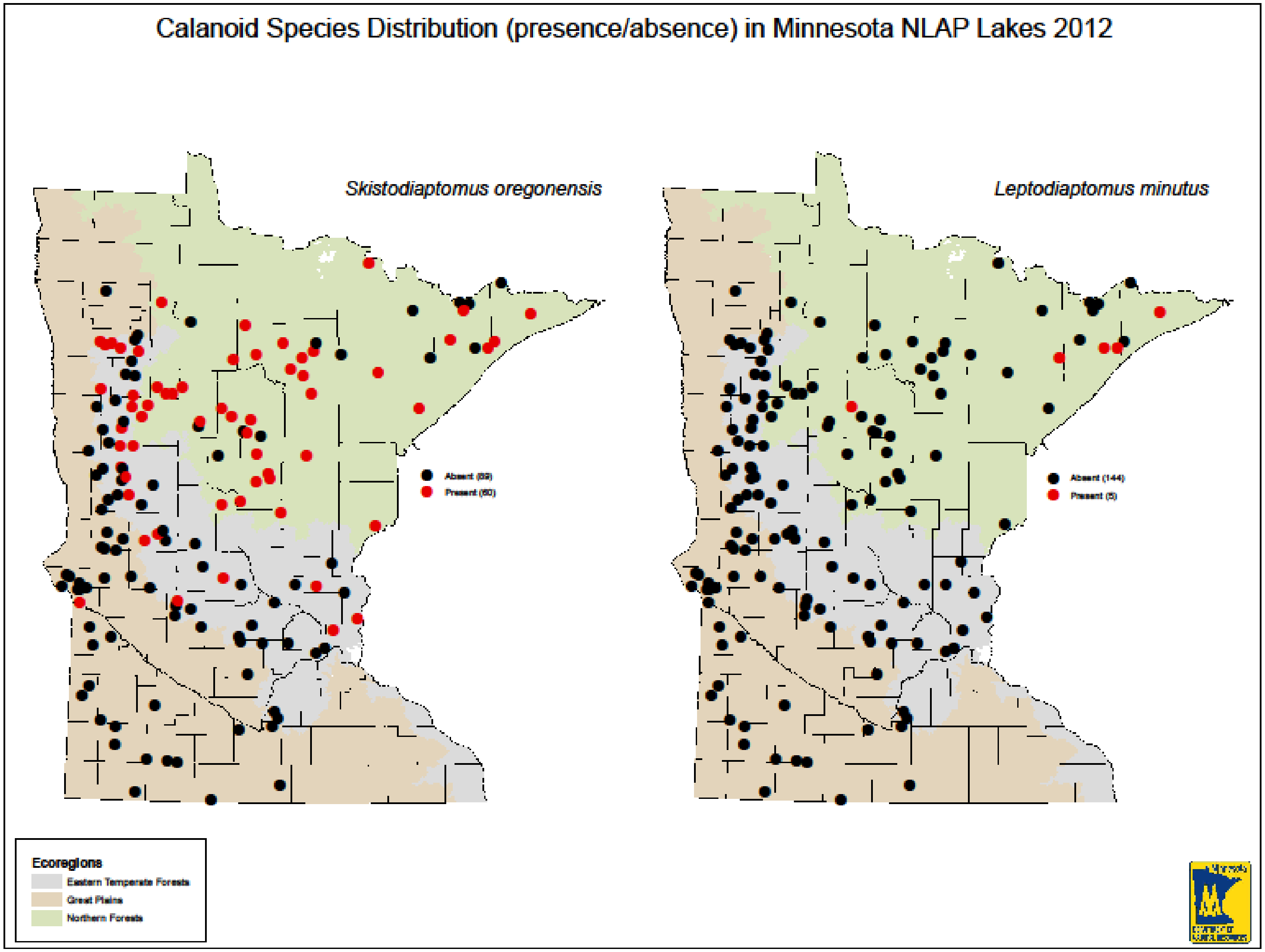


Figure 10. Distribution of *Skistodiaptomus oregonensis* and *Leptodiaptomus minutus* in MN NLAP lakes, 2012.

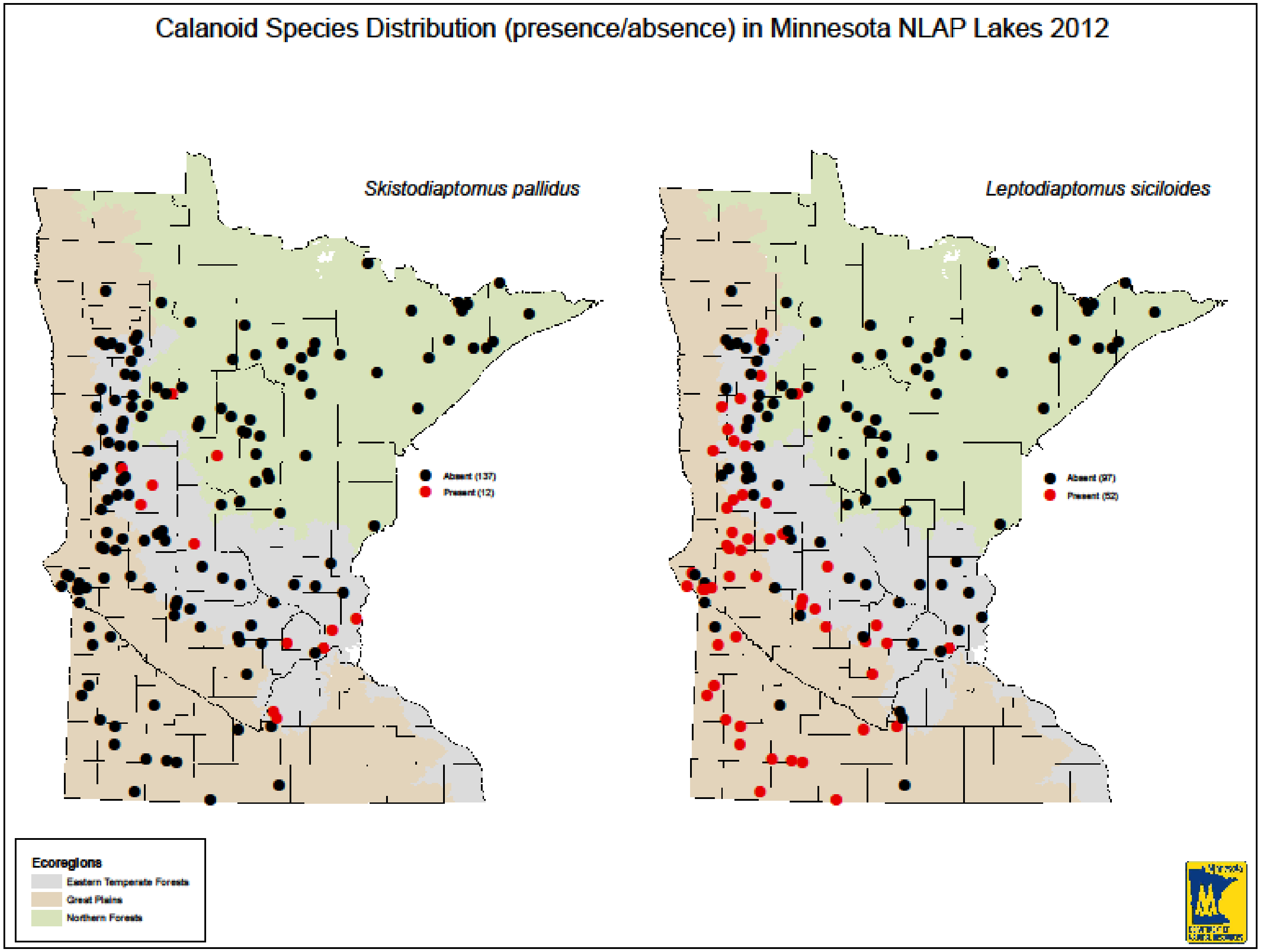


Figure 11. Distribution of *Skistodiaptomus pallidus* and *Leptodiaptomus siciloides* in MN NLAP lakes, 2012.

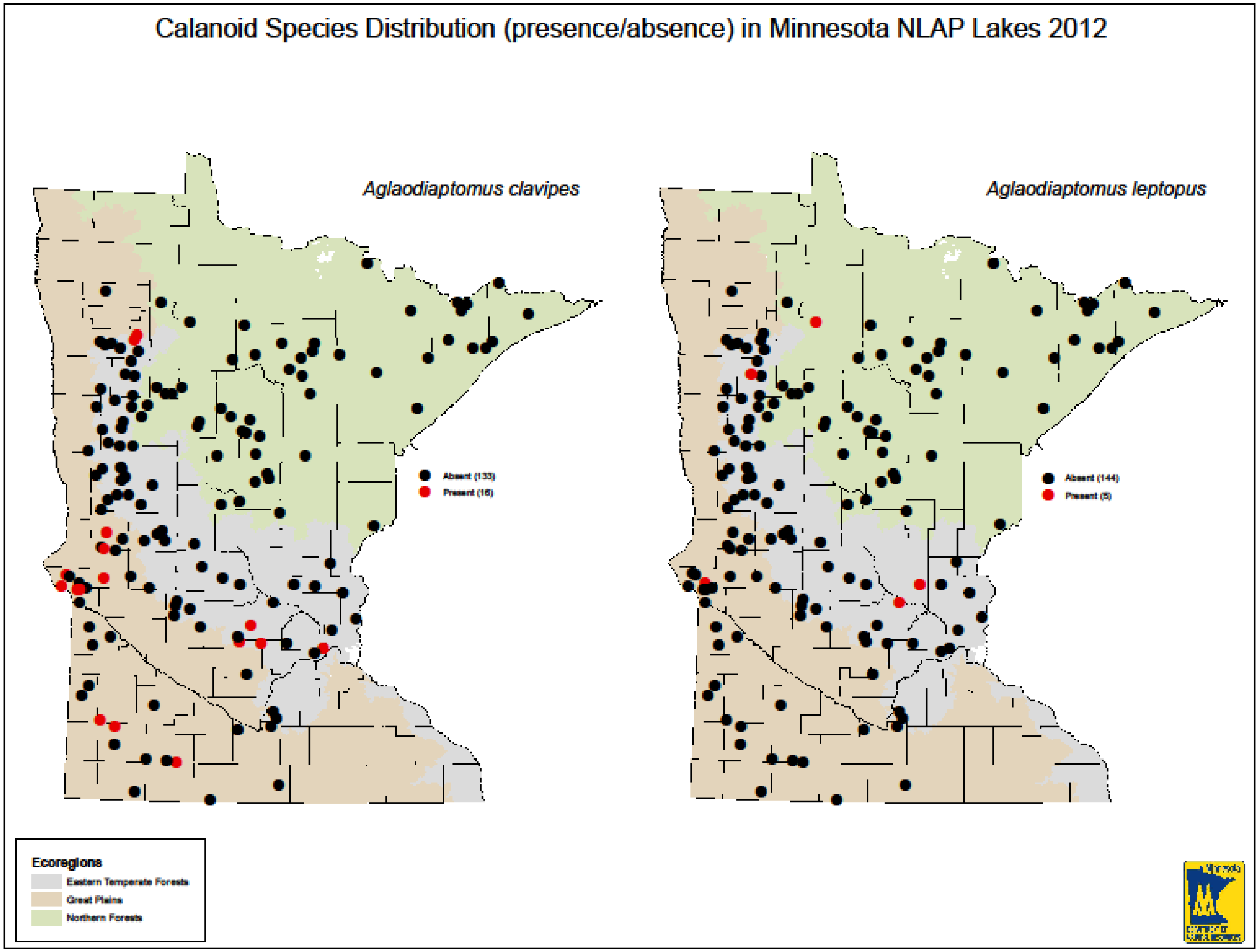


Figure 12. Distribution of *Aglaodiaptomus clavipes* and *Aglaodiaptomus leptopus* in MN NLAP lakes, 2012.

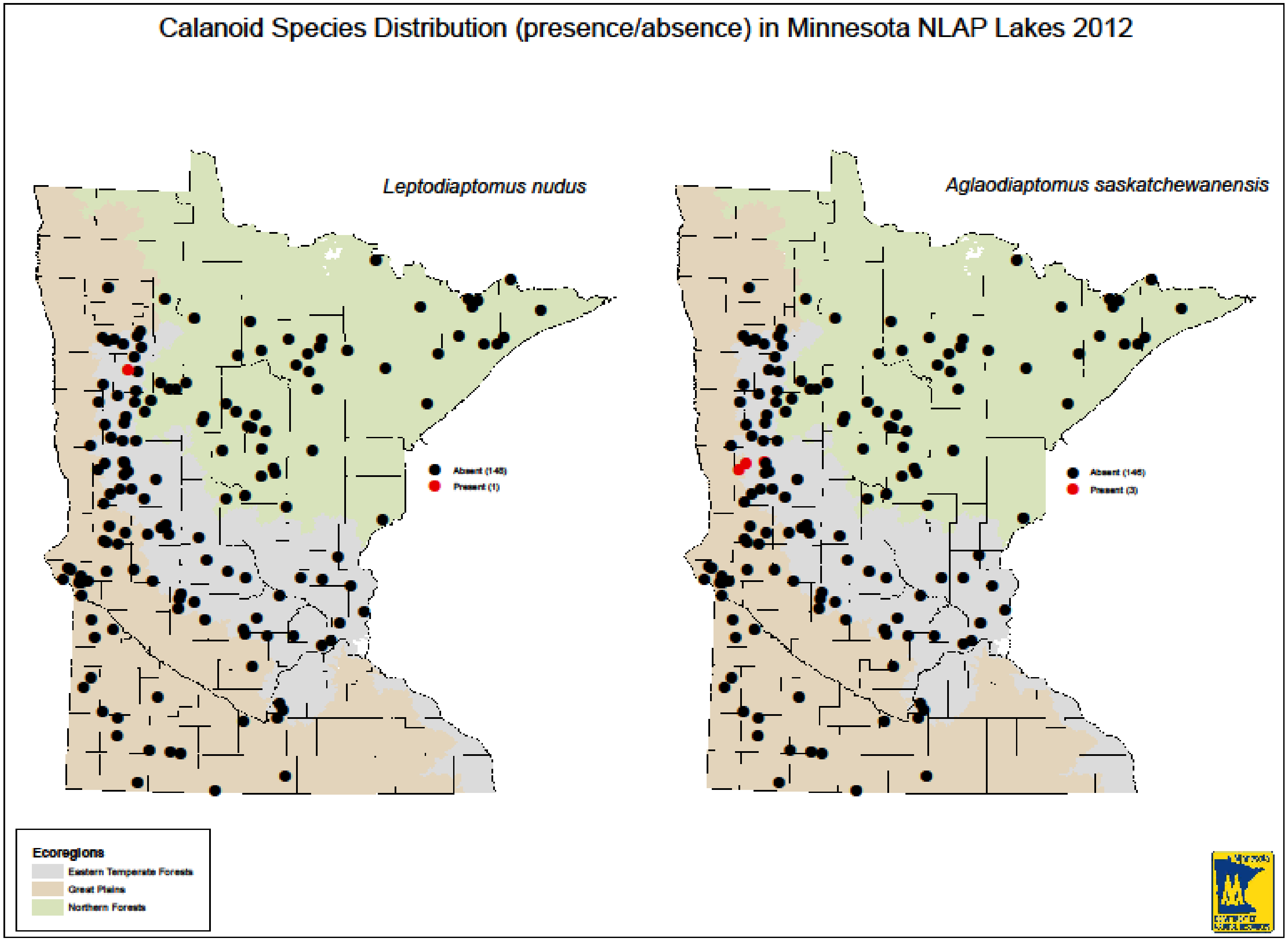


Figure 13. Distribution of *Leptodiaptomus nudus* and *Aglaodiaptomus saskatchewanensis* in MN NLAP lakes, 2012.

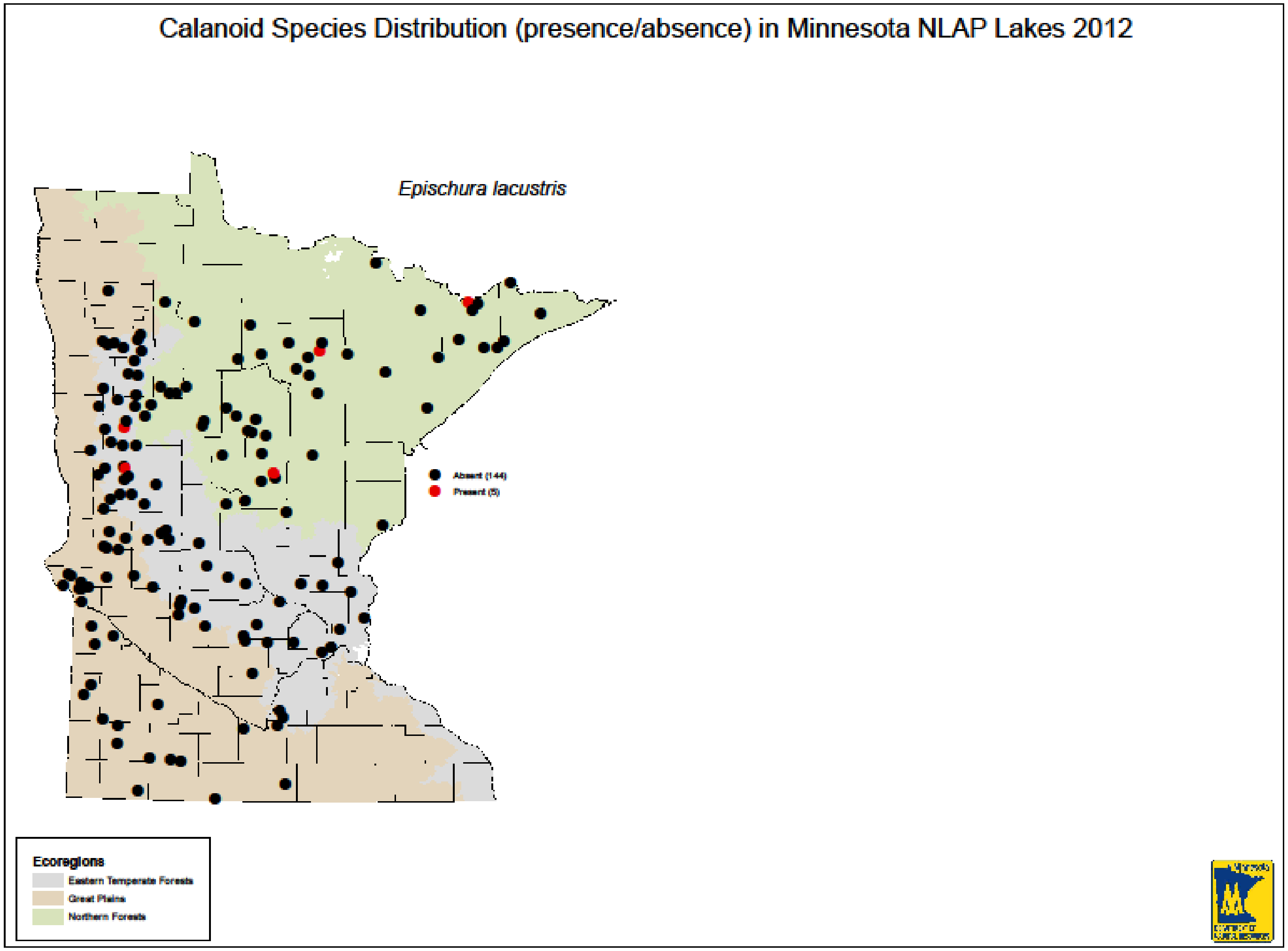
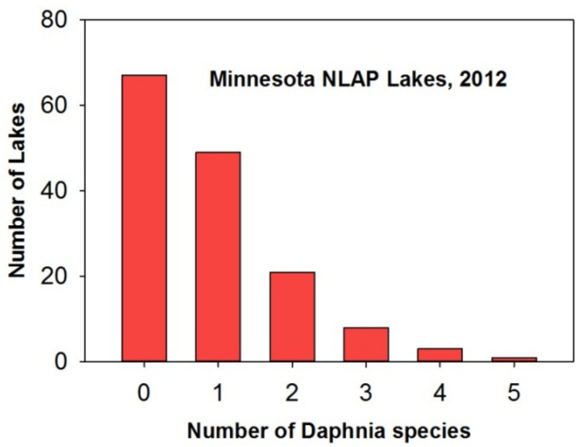


Figure 14. Distribution of *Epischura lacustris* in MN NLAP lakes, 2012.

Distribution of *Daphnia* Species

Daphnia were collected from 82 of the 149 Minnesota NLAP lakes, with eight species identified overall. The number of *Daphnia* species per lake ranged from zero in 67 of the lakes to five species in one lake (Figure 15). Since sampling was limited to one tow per lake, collected between June and September, many of the lakes sampled later in the season may have had *Daphnia* present though they were not collected in this survey. *Daphnia* tend to be more ephemeral than other zooplankton taxa, some species present earlier in the season, and then due to limited food supply and fish predation, decline in numbers by mid-summer. Others, especially smaller *Daphnia* species, tend to be more abundant later in the season. The number of *Daphnia* species per lake were similar among lakes in the Northern Forest and Eastern Temperate Forest Ecoregions, but the Great Plains Ecoregion had more lakes where there was either zero or one *Daphnia* species present and very few with two or more.



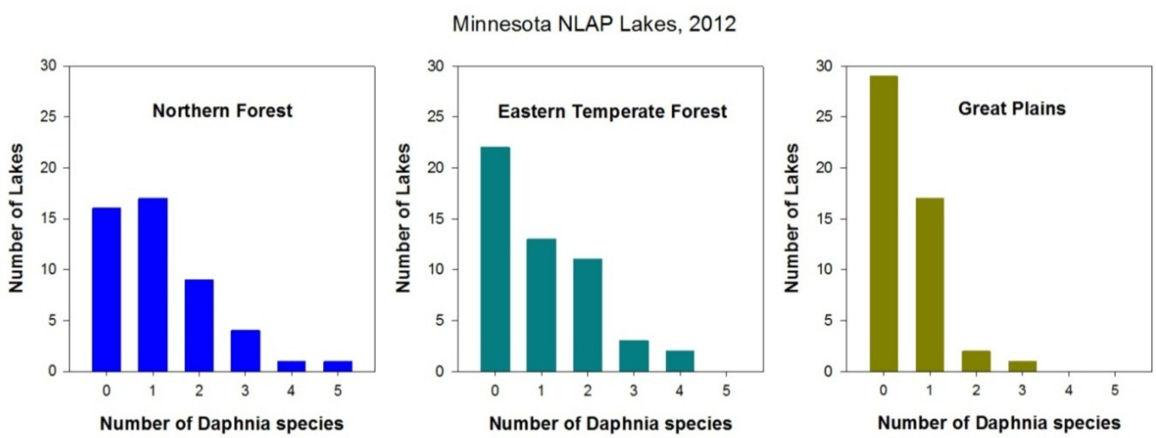


Figure 15. Distribution of the number of *Daphnia* species per lake collected from MN NLAP Lakes, 2012. (Graphs in lower half of figure show number of taxa per lake in each of the three ecoregions).

The geographical distribution of the *Daphnia* species found among the Minnesota NLAP lakes is illustrated in Figures 16-19. There appeared to be four common and ubiquitous species including *Daphnia galeata mendotae*, *Daphnia pulicaria*, *Daphnia retrocurva* and *Daphnia parvula* (Figures 16-18). *D. galeata mendotae* was the most common species, present in 43 of the lakes (Figure 16). *D. pulicaria* was also common, present in 31 lakes, although this species tends to be very temporal and may have been present in more lakes than indicated by this survey. *Daphnia ambigua* is a very small-sized *Daphnia*, sometime overlooked in studies. This species was found in 8 of the NLAP lakes located in central Minnesota (Figure 17).

Daphnia longiremis was found in only four lakes, all in fairly close proximity to one another in the north-central portion of state (Figure 18). All four of these lakes were deep (>14meters) mesotrophic or oligotrophic lakes. *D. longiremis* is described as a cold water stenotherm, usually found below the thermocline in well-oxygenated lakes (Balcer et al. 1984). *Daphnia laevis* and *Daphnia schodleri* were the least common *Daphnia* species found in the NLAP lakes. *D. laevis* was present in two shallow (<1 meter) unnamed lakes, one in Big Stone Co. and the other in Wright Co. *D. schodleri* was found only in Fox Lake, Beltrami Co., DOW # 04025100 (Figure 19).

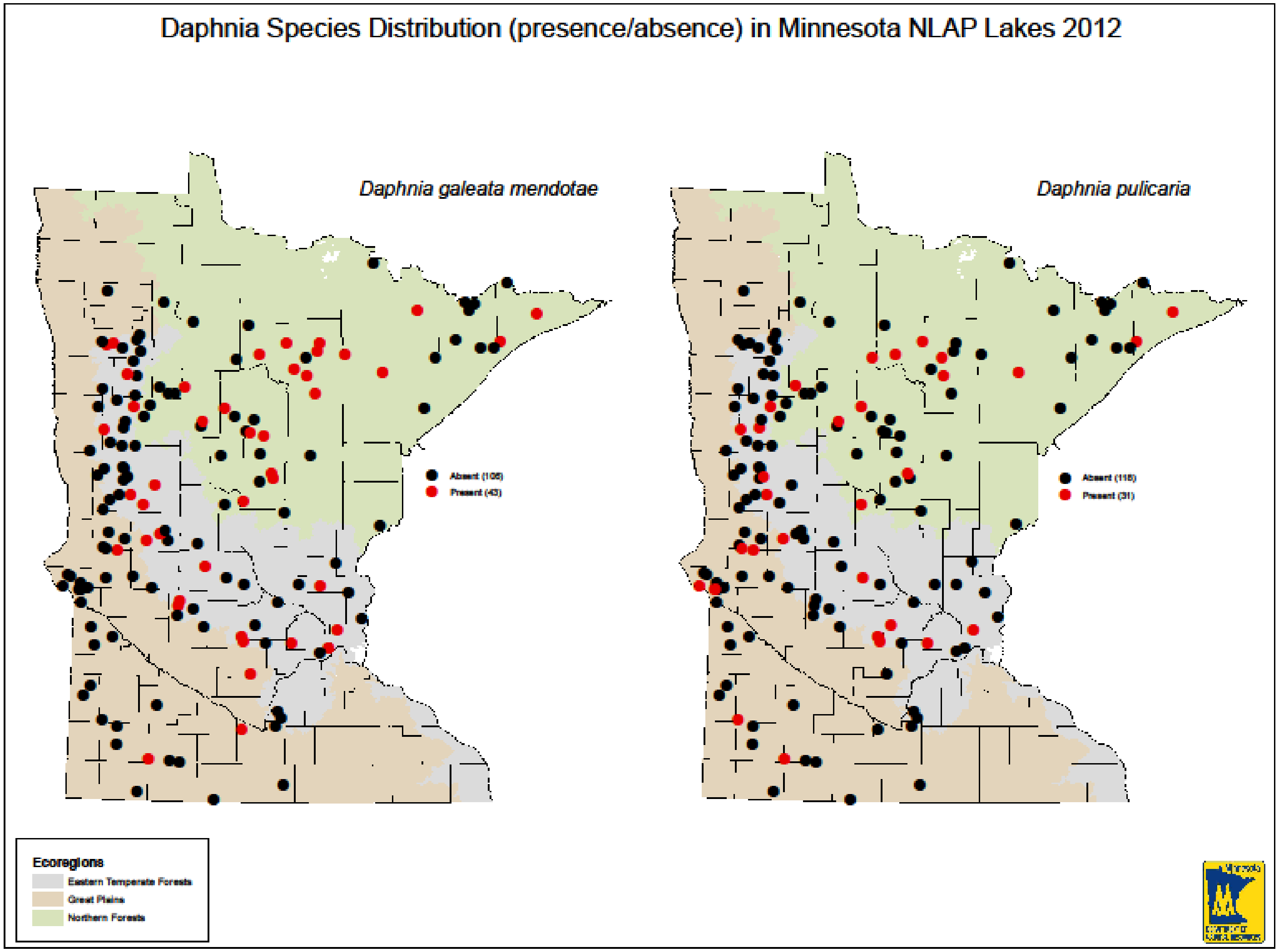


Figure 16. Distribution of *Daphnia galeata mendotae* and *Daphnia pulicaria* in MN NLAP lakes, 2012.

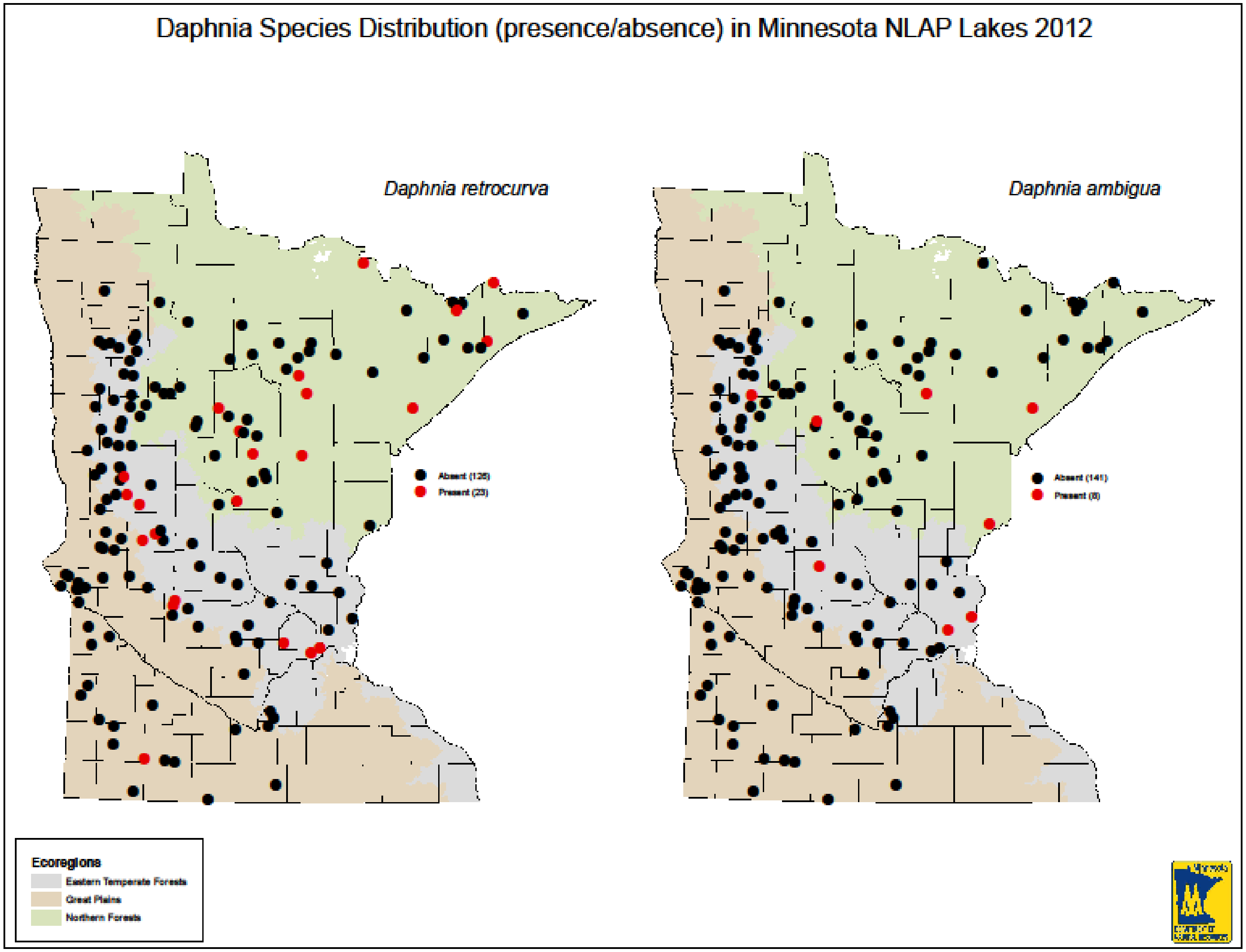


Figure 17. Distribution of *Daphnia retrocurva* and *Daphnia ambigua* in MN NLAP lakes, 2012.

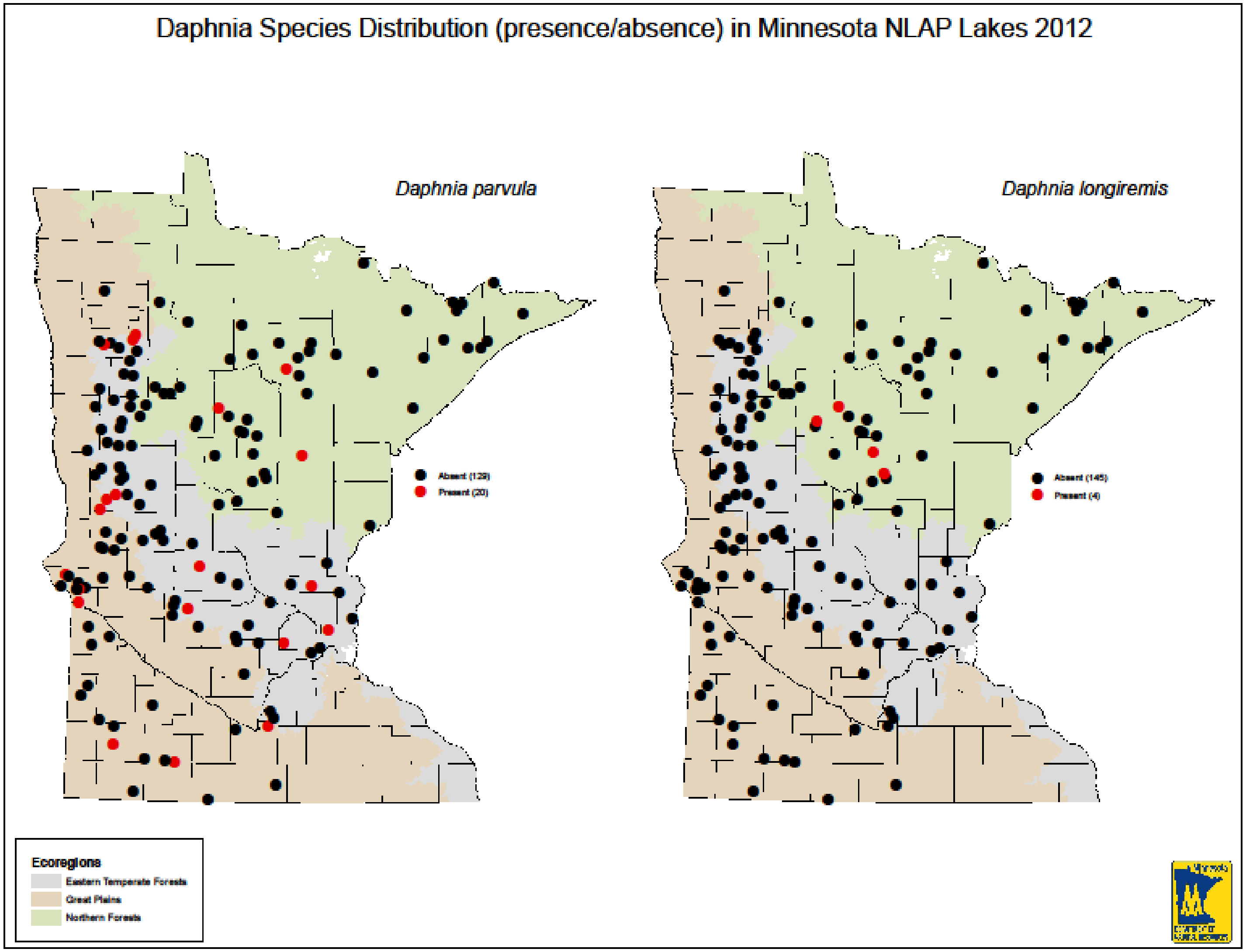


Figure 18. Distribution of *Daphnia parvula* and *Daphnia longiremis* in MN NLAP lakes, 2012.

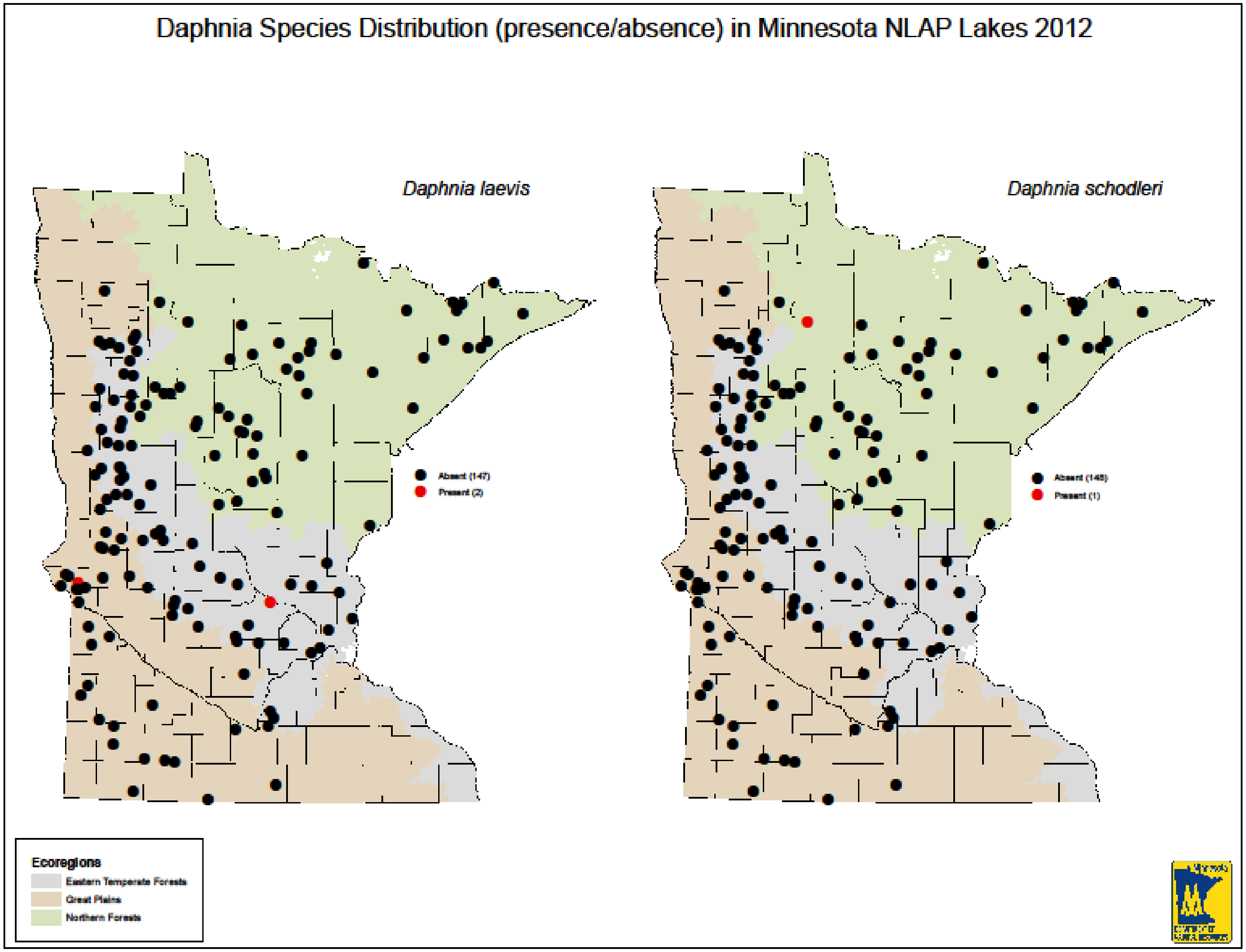


Figure 19. Distribution of *Daphnia laevis* and *Daphnia schodleri* in MN NLAP lakes, 2012.

Comparison of 2007 and 2012 EPA Zooplankton Results

Background: Minnesota participated in the National Lakes Assessment Project in 2007 where crustacean zooplankton were collected from 50 national-frame lakes using a 243 μm mesh net. In 2012, 20 of these lakes were revisited, using both 2007 methods (243μm net) and 2012 EPA methods (150μm net), as well as the method described in this report using the 80 μm net. Rotifers were also collected both years using an 80μm mesh net in 2007, and a 50μm mesh net in 2012.

Results comparing the crustacean zooplankton collected with the three mesh sizes, as well as rotifers collected with the two mesh sizes are summarized in this section of report. It should be noted that the 2012 EPA methods also differ from the other two methods where length of tow was not the full water column tow, but rather a set depth, depending upon lake depth. A complete description of these methods can be found in the 2012 EPA's NLAP Field Operations Manual:
http://water.epa.gov/type/lakes/assessmonitor/lakessurvey/upload/NLA2012_FieldOperationsManual_120517_FINAL_CombinedQRG.pdf.

Crustacean Zooplankton-Comparisons between 2007 and 2012 (243μm mesh net): Densities of crustacean zooplankton collected with the 243μm mesh were higher in 12 and lower in 8 of the 20 lakes in 2012, when compared to those collected in 2007 (Table 4). Some of these differences are quite extreme. Differences between years could be explained, in part, to temporal variability, as many of these lakes were not sampled during the same month, although the lake with the greatest difference in densities between years was North Eagle, where samples were collected during August of both years.

Species richness in most lakes was similar between years, with the following two exceptions. The number of species identified from Darling Lake increased from 8 in 2007 to 12 in 2012 while the number of species identified from Fairy Lake decreased from 7 in 2007 to 3 in 2012 (Table 5). Again, temporal variation may explain the differences in these samples, although in Fairy Lake, the samples were collected during both years only one week apart.

Table 4. Crustacean zooplankton densities (no./liter) in Minnesota NLAP revisit lakes.

Lake name	**DOW number**	**2007 sampling date**	**2012 sampling date**	**2007 densities 243µm**	**2012 densities 243µm**	**2012 densities 150µm**	**2012 densities 80µm**
Long	11048000	July 10	August 25	0.16	10.30	7.40	9.23
Flat	03024200	August 7	June 12	0.26	1.02	9.98	10.33
Spring	69012900	June 28	June 19	0.40	7.75	5.04	45.86
Long	31026600	August 15	July11	0.47	2.78	11.09	4.22
Darling	21008000	July 19	August 8	0.76	1.72	4.25	6.83
Richey	16064300	August 2	June 25	2.00	0.57	0.69	6.93
Becoosin	38047200	July 18	June 26	2.33	1.16	6.49	20.49
Round	56047600	July 25	August 8	2.41	14.14	2.45	3.72
Crow Wing	18015500	July 30	July 25	2.57	1.50	21.77	22.83
North Eagle	07006000	August 8	August 14	2.96	101.04	135.00	72.63
Lookout	18012300	August 13	August 7	3.20	13.52	29.50	60.87
Fairy	56035600	August 6	July 31	3.92	0.89	12.11	35.93
Woodcock	34014100	July 24	August 15	6.51	26.27	48.12	81.97
Nokomis	27001900	June 27	July 12	6.82	53.90	79.99	44.64
Norway	34025100	August 22	August 6	6.98	57.05	42.80	32.81
Cokato	86026300	August 20	June 13	10.81	43.46	41.78	14.90
Snail	62007300	July 18	June 7	12.88	4.86	35.07	13.88
Jennie	47001500	August 21	June 12	24.74	1.10	4.90	8.10
North Ash	41005500	July 10	July 17	75.93	31.61	27.77	49.72
South	43001400	July 23	June 7	162.54	23.63	107.39	437.57

Table 5. Crustacean zooplankton species richness in Minnesota NLAP revisit lakes.

Lake name	**DOW number**	**2007 sampling date**	**2012 sampling date**	**2007 species richness 243 μm**	**2012 species richness 243 μm**	**2012 species richness 150 μm**	**2012 species richness 80 μm**
Long	11048000	July 10	August 25	8	9	8	15
Flat	03024200	August 7	June 12	6	5	10	8
Spring	69012900	June 28	June 19	9	11	7	9
Long	31026600	August 15	July11	7	6	9	8
Darling	21008000	July 19	August 8	8	12	9	12
Richey	16064300	August 2	June 25	6	4	8	8
Becoosin	38047200	July 18	June 26	7	5	10	7
Round	56047600	July 25	August 8	4	7	5	8
Crow Wing	18015500	July 30	July 25	7	5	9	10
North Eagle	07006000	August 8	August 14	9	10	9	5
Lookout	18012300	August 13	August 7	6	7	7	7
Fairy	56035600	August 6	July 31	7	3	7	9
Woodcock	34014100	July 24	August 15	6	8	8	7
Nokomis	27001900	June 27	July 12	9	9	9	8
Norway	34025100	August 22	August 6	8	8	8	12
Cokato	86026300	August 20	June 13	5	7	7	6
Snail	62007300	July 18	June 7	7	9	10	10
Jennie	47001500	August 21	June 12	7	7	6	8
North Ash	41005500	July 10	July 17	4	4	5	6
South	43001400	July 23	June 7	6	5	4	7

Net size comparisons-crustacean zooplankton (2012): When comparing densities of crustacean zooplankton collected during 2012 with the three different mesh net sizes, the general pattern appeared to be higher densities in samples collected with the two smaller mesh nets (80μm and 150μm), although this was not the case in all lakes, and in 4 lakes (Long-Cass Co., Round, Norway and Cokato), densities were highest in the 243μm mesh net catches (Table 4). Very few lakes had similar densities collected with all three nets, but one exception was Long Lake (Cass Co.) The lake with the most extreme difference in densities between nets was South Lake, where densities were 23 individuals/liter with the 243μm mesh net, compared to 437 individuals/liter caught with the 80μm mesh net. When examining the composition of the zooplankton collected with the 80μm mesh net in South Lake, over 50% of the catch in numbers were small cyclopoids which most likely escaped capture in the larger mesh nets. South Lake is very shallow (1 m) and is the most eutrophic lake in the entire Minnesota data set.

Both densities and biomass of crustacean zooplankton collected from the 20 revisit lakes (including one additional lake sampled in 2012 with all three methods-Big Stone Lake) with the three mesh sizes are illustrated in Figure 20. Lakes are arranged on graph left to right in increasing order of total phosphorus levels. In general, some of the lakes with the higher total phosphorus levels had higher densities and biomass, regardless of net mesh size, but there were many exceptions. Nokomis Lake appeared to have higher zooplankton densities and biomass relative to its phosphorus levels, whereas North Eagle, Woodcock and North Ash appeared to have lower biomass relative to phosphorus levels.

Species richness varied between all mesh net catches with no apparent pattern (Table 5). The most extreme difference in species richness was in Long Lake (Cass Co.) where 15 taxa were identified in the 80μm mesh net samples, compared to only 8 from the 150 μm and 9 from the 243 μm mesh nets. These differences could be due again to some species being too small to be captured in the larger mesh nets. In addition, Long Lake is very deep, and therefore the 150 μm mesh net method, which did not sample the full water column, missed *Daphnia* species (including *Daphnia longiremis*) that inhabit the deeper area of lake during daylight hours.

After transforming data (Log^e +1) for normality, zooplankton densities and biomass collected with all three net meshes during 2012 were plotted with simple linear regressions to examine if extrapolations could be used to convert densities and biomass from one mesh size to another (Figure 21). Regression results showed no significant relationships between 80μm and 243μm mesh nets for both densities and biomass, with R-squared values of 0.28 and 0.45, respectively. However, regression results showed higher R-squared values with both densities and biomass from 150μm and 243 μm, and 150μm and 80μm mesh nets. The regression with the highest R-squared value was for biomass regressions with the 150μm and 80μm mesh nets, with an R-squared value of 0.65 (Figure 21).

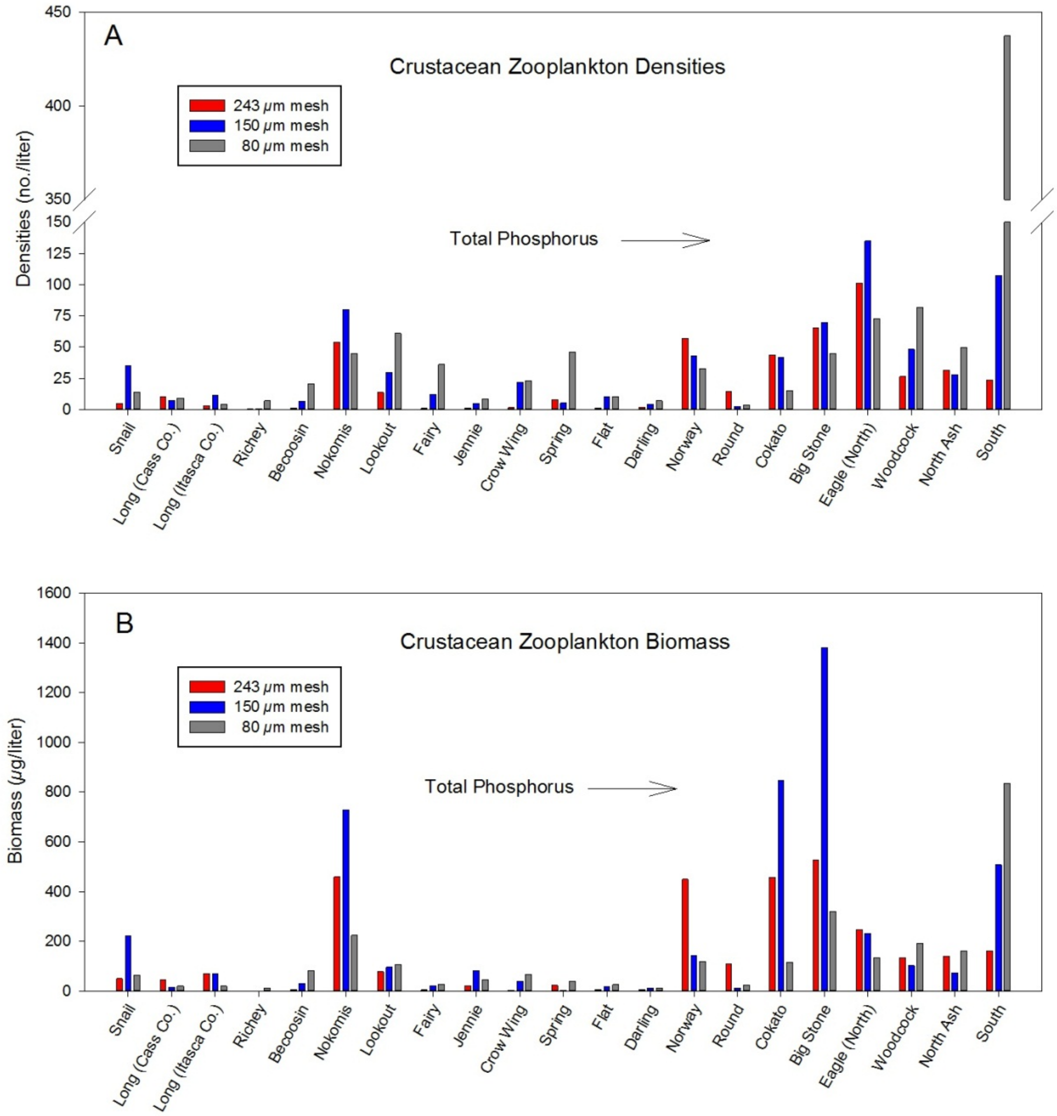


Figure 20. Crustacean zooplankton densities (A) and biomass (B) collected from 21 MN NLAP lakes with 243μm, 150μm and 80μm mesh nets, during 2012. (Lakes are arranged from left to right with increasing phosphorus levels).

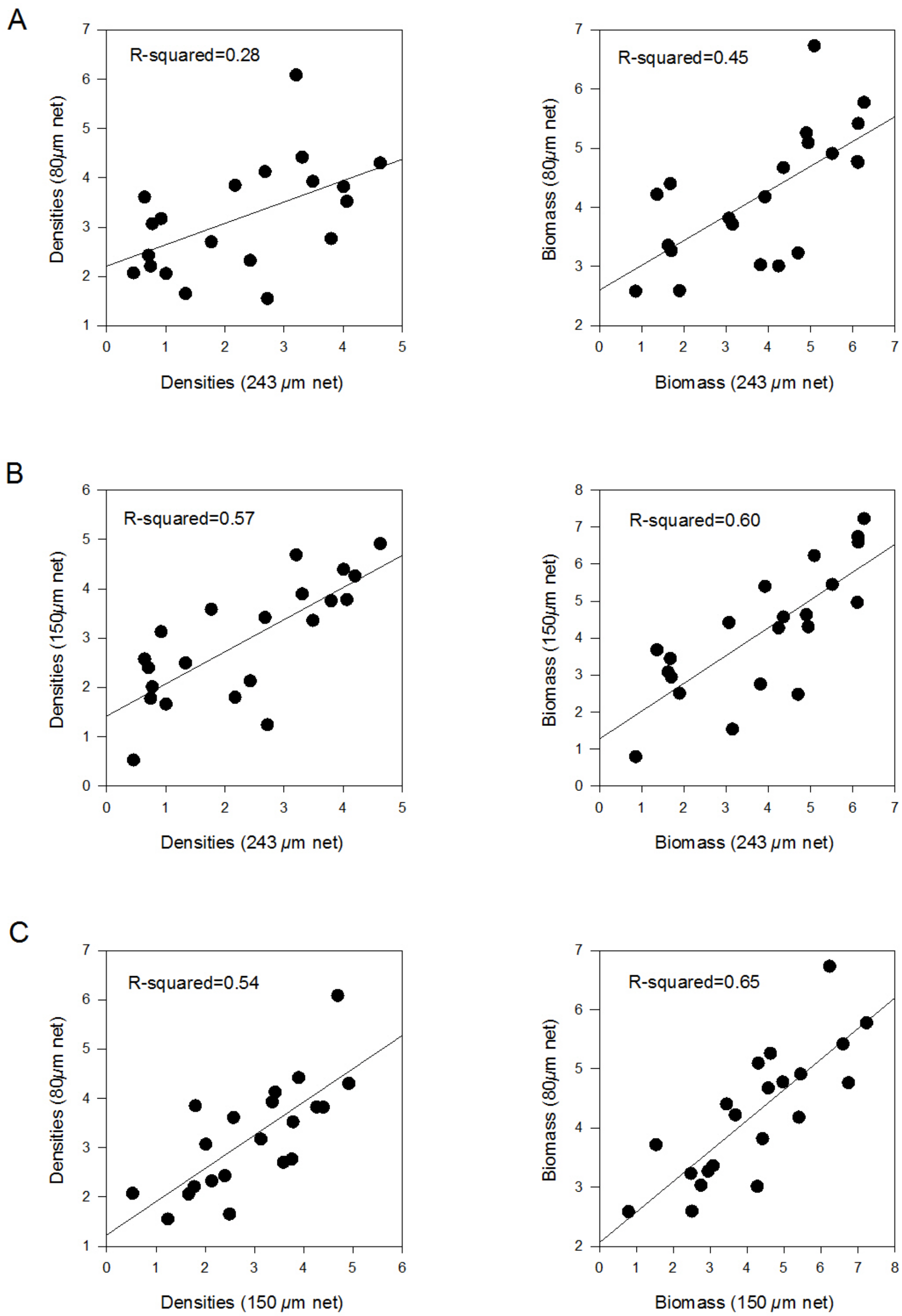


Figure 21. Simple linear regressions of zooplankton densities (no./liter) and biomass (µg/liter) between 243µm and 80µm mesh nets (A), 243µm and 150µm mesh nets (B) and 150µm and 80µm mesh nets (C), collected from the revisit MN NLAP lakes in 2012. (Data was transformed to log^e+1 for normality).

<u>Net size comparisons-rotifers (2012):</u> Rotifer densities collected with the 50μm mesh net were higher than those collected with the 80μm mesh net in all but one of the revisit lakes (Figure 22). Crow Wing Lake had very similar densities with both mesh nets. Rotifer biomass also was higher in the samples collected with the 50μm mesh net, with the exception again of Crow Wing Lake, where biomass was much higher in the 80μm mesh net samples (Figure 22). In many of the lakes, rotifer densities and biomass were considerably higher in the 50μm mesh net samples, suggesting that the 80μm mesh net was too coarse to collect rotifers accurately.

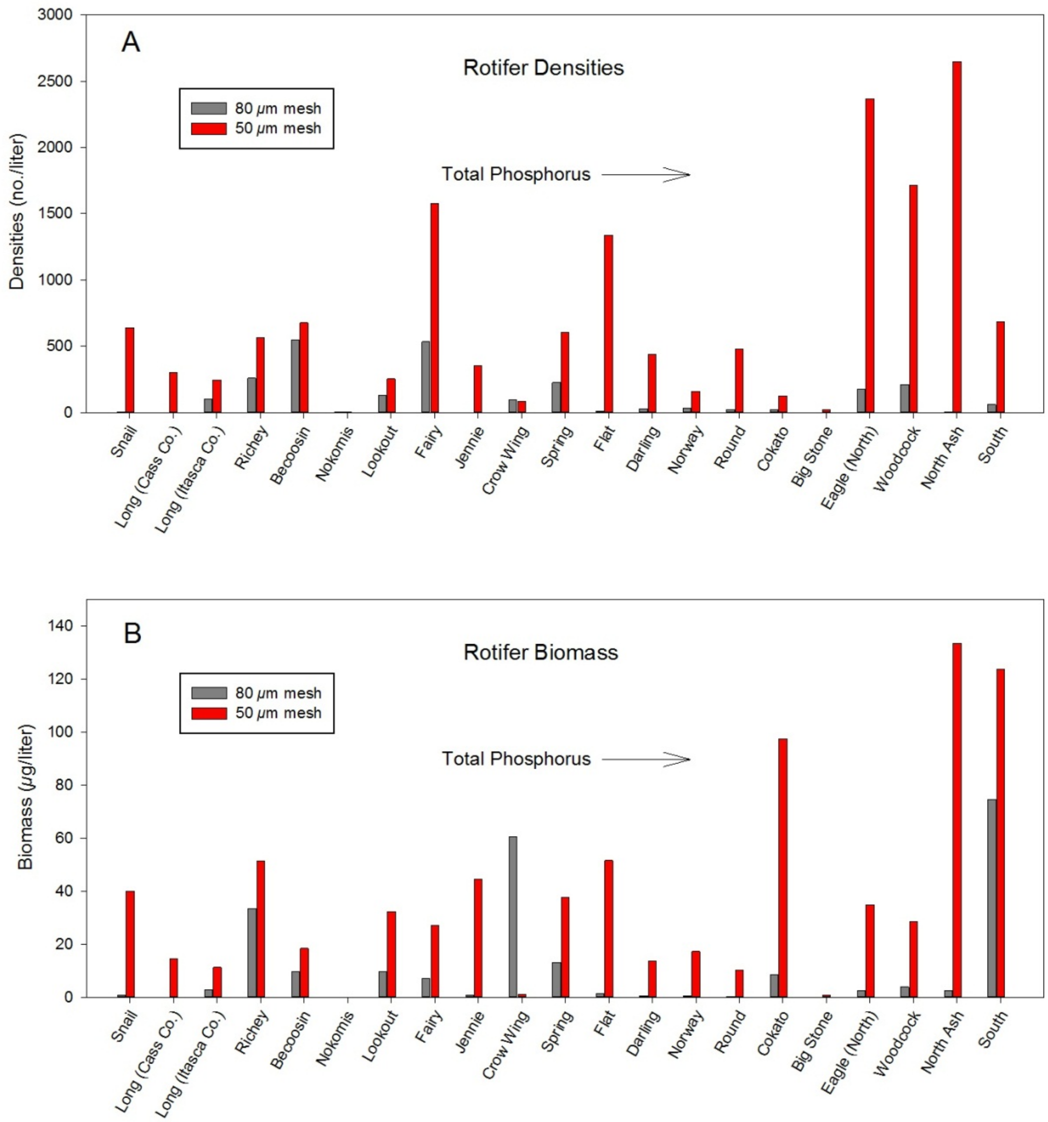


Figure 22. Rotifer densities (A) and biomass (B) collected from 21 MN NLAP lakes with 80μm and 50μm mesh nets, during 2012. (Lakes are arranged from left to right with increasing phosphorus levels).

Summary and Conclusions

The 2012 NLAP study provided an opportunity to sample crustacean zooplankton communities from 149 lakes across Minnesota. Potential zooplankton indices (densities, biomass and species richness) were examined by comparing lakes between the three major ecoregions of the state. Furthermore, correlations between zooplankton indices and lake variables were also examined for their use as indicators of lake productivity. Species geographical distribution of two major groups of zooplankton (calanoid copepods and *Daphnia*), were documented among NLAP lakes. Finally, methods used in this report were compared with both EPA'S 2007 and 2012 methods, examining differences between years and mesh sizes for a subset of revisit lakes.
From this study, the following conclusions can be made:

1. Among the 149 lakes sampled, 51 different zooplankton species were identified, where most lakes had four to eight species per lake. This statewide pattern was similar within the Northern Forest and Eastern Temperate Forest ecoregions, but in the Great Plains Ecoregion, most lakes had fewer species per lake. There appeared to be three ubiquitous cladocerans, one ubiquitous cyclopoid and no ubiquitous calanoids among the lakes.

2. Mean zooplankton densities were significantly lower in lakes from the Northern Forest Ecoregion than those from the Great Plains Ecoregion. Mean zooplankton biomass was significantly lower in the Northern Forest Ecoregion lakes when compared to both the Eastern Temperate Forest and Great Plains Ecoregion lakes. Species richness was significantly higher in the lakes of the Northern Forest and Eastern Temperate Forest Ecoregion when compared to lakes in the Great Plains Ecoregion.

3. Hypotheses were tested using correlation coefficient analysis, and based on findings from this study, these conclusions can be made:

    - <u>Total zooplankton densities</u>: Hypothesis: As nutrient loading increases, total zooplankton densities should increase. Findings from this study strongly support this hypothesis, as total phosphorus, chlorophyll a and Secchi depth were all significantly correlated to zooplankton densities.

    - <u>Total zooplankton biomass</u>: Hypothesis: As nutrient loading increases, total zooplankton biomass should increase. Findings from this study strongly support this hypothesis as well, as total phosphorus, chlorophyll a and Secchi depth were all significantly correlated to zooplankton biomass.

    - <u>Species richness</u>: Hypothesis: As nutrient loading increases, species richness should decrease. Findings from this study may support this hypothesis, but only based on chlorophyll a and Secchi depth, as total phosphorus was not significantly correlated to species richness.

- <u>Relative abundance of large *Daphnia*</u>: Hypothesis: As nutrient loading increases, relative abundance of large *Daphnia* should decrease. Findings from this study may support this hypothesis, but only using chlorophyll a and Secchi depth, as total phosphorus was not significantly correlated to percent large *Daphnia* nor mean *Daphnia* weight.

- <u>Relative density of calanoid copepods</u>: Hypothesis: As nutrient loading increases, percent composition of calanoids should decrease. Findings from this study reject this hypothesis. No lake productivity indices were significantly correlated with percent calanoids.

- In addition to the above mentioned correlations between zooplankton indices and lake productivity indices, large *Daphnia* and percent calanoid indices were both significantly correlated with lake area and large *Daphnia* indices were also correlated with maximum lake depth.

4. The documentation of the geographical distribution of calanoid copepods and *Daphnia* in the NLAP lakes in this study provided the following insights:

   - *Daphnia longiremis* and *Leptodiaptomus minutus* are species that should be monitored closely in future zooplankton surveys, as these species are restricted to cold, well oxygenated waters in oligotrophic or mesotrophic lakes in Minnesota. Their populations could decline with increasing eutrophication and/or warmer waters due to climate change.

   - Those species that seem to favor or adapt better to more eutrophic conditions include *Aglaodiaptomus clavipes*, *Leptodiaptomus siciloides, Skistodiaptomus pallidus* and possibly *Eubosmina coregoni*. These are species that also should be monitored closely in future surveys, as their populations could expand with increasing eutrophication.

   - Other species that were rare among the lakes in this survey, with distributions that may be driven by fishless conditions or specific habitats, rather than lake productivity include *Daphnia schodleri, Daphnia laevis, Aglaodiaptomus leptopus, Aglaodiaptomus saskatchewanensis* and *Leptodiaptomus nudus.* Documenting the occurrence of these species in other lakes in Minnesota should also be a priority in future zooplankton surveys.

5. Crustacean zooplankton densities and species richness from samples collected from 20 MN NLAP revisit lakes during 2012 were compared with those collected from the same lakes in 2007, using the same EPA protocol (243μm mesh net). In 12 of the lakes, densities were higher in 2012 and in 8 of the lakes higher in 2007. Differences could be due to temporal variability, as many lakes were not sampled during the same time

period each year. Species richness between years was more similar than densities in most lakes.

6. When comparing crustacean zooplankton densities and species richness from the MN NLAP revisit lakes using the three different mesh net sizes (80μm, 150μm and 243μm) during 2012, the two finer mesh nets revealed higher densities in most lakes when compared to the coarser mesh net. Species richness varied between all mesh net sizes with no apparent pattern.

7. Regressions of crustacean zooplankton densities and biomass with the three mesh sizes revealed significant relationships between the 243μm and 150μm mesh nets, and between the 150μm and 80μm mesh nets, but not between the 243μm and 80μm mesh nets. It may be possible to use regression equations to convert both densities and biomass from the 243μm to 150μm and the 150μm to 80μm mesh nets.

8. Rotifer densities and biomass were much higher in samples collected with the 50μm mesh net compared to samples collected with the 80μm mesh net in all but one of the 20 revisit lakes.

Recommendations

- Zooplankton community analysis should be included in the 2017 Minnesota National Lakes Assessment Program.

- Crustacean zooplankton should be collected with either a 150μm or 80μm mesh net. The 243μm mesh net proved to be too coarse to collect crustacean zooplankton and should not be used in future sampling. The 80μm net would be the preferred method for sampling crustacean zooplankton from lakes in Minnesota, as this is the standard size mesh net used for other studies in the state, and therefore more comparable among studies.

- Regression equations could be used to convert crustacean zooplankton densities and biomass between the 150μm and 80μm mesh nets for comparison between years.

- Rotifers should be collected with the 50μm mesh net, as the 80μm mesh net proved to be too coarse for sampling rotifers effectively.

- In future sampling, a full water column tow in the deepest location of lake is recommended over sampling a set tow depth. This method will help to insure the collection of possible indicator species that inhabit areas of lake below the thermocline. These species include but are not limited to *Daphnia longiremis* and *Leptodiaptomus minutus*, which may be susceptible to eutrophication and/or climate change.

- Because of the high temporal variability that zooplankton display, sampling should be conducted from all lakes within a tighter time frame, preferably within the same month, if at all possible.

- A subset of at least 20 revisit lakes should again be sampled for zooplankton in 2017 to compare with 2012 data.

- A set of both state-based and national-based lakes from Minnesota should again be sampled for zooplankton in 2017. Data generated from an additional 150 lakes would greatly enhance our knowledge of both the geographical distribution of zooplankton species as well as contributing more data to further test zooplankton indices (densities, biomass, species richness, large *Daphnia*, and percent calanoids) against lake productivity indices.

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Appendix A. Example of a Minnesota NLAP 2012 individual lake zooplankton report available from MDNR-Ecological and Water Resources Division.

Zooplankton Report - 38047200 Becoosin

Lake County

Sample site 1 sampled on 2012-06-26
Rep number - 1

Vertical Haul Method		Subsample		Remarks
Mouth diameter (cm)	13	Subsample volume	5	NLA12_MN-105
Mesh size (um)	80	Total volume (ml)	20	
Haul - top depth (m)	0.0	Dilution factor	0.250	
Haul - bottom depth (m)	4.0			
Volume seined (l)	53			

Species		Density # / l	Biomass ug / l	Number %	Weight %	Mean weight ug	Mean length mm	Count
Copepods								
nauplii		4.45	0.69	21.69	0.86	0.16	0.17	59
copepodites		2.18	2.29	10.66	2.84	1.05	0.41	29
Diaptomidae		8.21	64.75	40.07	80.45	7.89	1.12	109
cyclopoids		2.26	6.09	11.03	7.56	2.69	0.64	30
	Total	17.10	73.82	83.46	91.72			227
Cladocerans								
Daphnia retrocurva		2.18	5.20	10.66	6.46	2.38	0.82	29
Bosmina sp.		0.75	0.80	3.68	1.00	1.06	0.28	10
Holopedium gibberum		0.15	0.48	0.74	0.59	3.16	0.57	2
Ceriodaphnia lacustris		0.30	0.19	1.47	0.24	0.63	0.38	4
	Total	3.39	6.67	16.54	8.28			45
	Grand total	20.49	80.49	100.00	100.00			272

Ecological and Water Resources Division
Minnesota Department of Natural Resources
Fri Oct 25 08:39:11 CDT 2013

Report version 1.02 - Oct 06 2010

Appendix B. Photographs of zooplankton collected from Minnesota NLAP lakes, 2012

Examples of More Common Zooplankton Taxa in MN NLAP Lakes, 2012

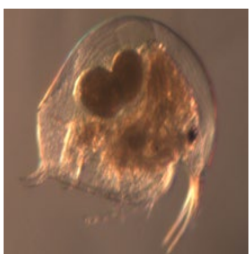
Daphnia galeata mendotae

Daphnia pulicaria

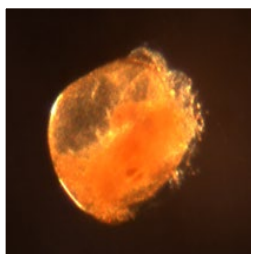
Daphnia retrocurva

Diaphanosoma birgei

Bosmina sp.



Chydorus sphaericus

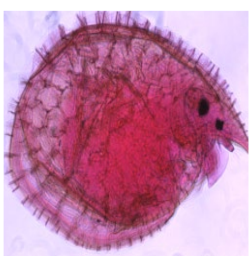


Calanoid copepod

Cyclopoid copepods

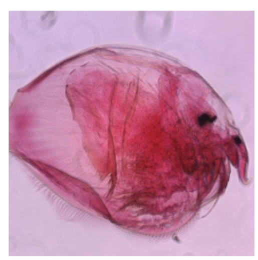
Examples of Less Common Zooplankton Taxa in MN NLAP Lakes, 2012

Chydorus faviformis



Pleuroxus denticulatus

Pleuroxus procurvatus



Appendix C. List of Minnesota 2012 NLAP lakes from the Northern Forest Ecoregion

County	DOW #	Lake Name	Date Sampled	Total Zooplankton Densities (no./liter)	Total Zooplankton Biomass (µg/liter)	Species Richness (# taxa)
Aitkin	01010000	Jenkins	6-Aug	11.75	19.82	8
Becker	03023600	Unnamed	12-Jun	34.28	99.39	7
Becker	03024200	Flat	12-Jun	10.33	25.28	8
Beltrami	04001400	Popple	8-Aug	6.48	4.58	4
Beltrami	04025100	Fox	26-Jun	4.60	15.40	6
Cass	11024100	Tamarack	6-Aug	12.21	9.96	7
Cass	11015000	Tamarack	23-Jul	14.31	32.75	5
Cass	11101300	Diamond Pond	24-Jul	19.06	59.91	6
Cass	11103300	Unnamed	6-Aug	9.64	8.58	4
Cass	11044000	Unnamed	27-Aug	19.89	12.17	3
Cass	11011000	Pistol	23-Jul	13.86	37.26	5
Cass	11048000	Long	25-Aug	9.23	19.71	15
Clearwater	15010700	Miskogineu	26-Jun	86.39	216.55	6
Clearwater	15021300	Unnamed	27-Jun	14.57	75.88	4
Clearwater	15027900	Unnamed	27-Jun	8.34	36.44	6
Clearwater	15049100	Unnamed	27-Jun	419.84	581.23	4
Cook	16064300	Richey	25-Jun	6.93	12.25	8
Cook	16061300	Tenor	28-Jun	2.44	2.05	4
Cook	16018200	Ball Club	5-Sep	3.24	14.33	13
Crow Wing	18031200	Cross Lake Reservoir	28-Aug	34.20	56.24	11
Crow Wing	18052700	Unnamed	7-Aug	110.10	109.57	4
Crow Wing	18012300	Lookout	7-Aug	60.87	105.86	7
Crow Wing	18015500	Crow Wing	25-Jul	22.83	66.89	10
Crow Wing	18043900	Pennington Pit	18-Jun	7.45	40.40	8
Hubbard	29014600	Belle Taine	18-Jun	28.33	212.91	11
Itasca	31136700	Unnamed	9-Aug	7.94	17.13	4
Itasca	31089300	Lower Pigeon	10-Jul	20.88	125.45	8
Itasca	31029800	Walters	6-Aug	11.18	37.93	8
Itasca	31041900	Charlie	2-Aug	17.08	236.85	5
Itasca	31136600	Unnamed	9-Aug	23.66	169.60	7
Itasca	31021100	Unnamed (Becker)	9-Aug	11.49	39.08	9
Itasca	31026600	Long	11-Jul	4.22	19.30	8
Itasca	31040700	Hay	10-Jul	6.31	49.85	10
Itasca	31059400	Cottonwood	10-Jul	11.82	94.28	5
Lake	38067100	Two Deer	23-Jul	56.96	60.41	7

Appendix C. (Continued)

County	DOW #	Lake Name	Date Sampled	Total Zooplankton Densities (no./liter)	Total Zooplankton Biomass (μg/liter)	Species Richness (# taxa)
Lake	38051000	Cattyman	1-Aug	6.18	3.99	3
Lake	38062300	Spree	27-Jun	84.08	167.73	4
Lake	38025600	Divide	28-Aug	5.35	12.51	7
Lake	38002400	Crooked	30-Jul	16.47	46.76	8
Lake	38047200	Becoosin	26-Jun	20.49	80.49	7
Mille Lacs	48001900	Unnamed	23-Jul	70.52	157.09	7
Morrison	49013900	Unnamed	10-Aug	14.59	70.46	5
Pine	58004500	Wilbur	10-Jul	36.77	25.80	6
St Louis	69075700	Net	21-Jun	4.04	4.01	5
St Louis	69029600	Little Crab	31-Jul	27.88	22.61	6
St Louis	69092000	Stuart	6-Sep	0.21	0.55	7
St Louis	69005000	Big	7-Aug	10.85	30.69	5
St Louis	69012900	Spring	19-Jun	45.86	40.08	9
St Louis	69065300	Long	24-Jul	7.89	29.68	6

Appendix D. List of Minnesota 2012 NLAP lakes from the Eastern Temperate Forest Ecoregion

County	DOW #	Lake Name	Date Sampled	Total Zooplankton Densities (no./liter)	Total Zooplankton Biomass (μg/liter)	Species Richness (# taxa)
Becker	03041400	Gandrud	11-Jun	71.42	306.83	6
Becker	03075100	Unnamed	1-Aug	119.94	222.13	6
Becker	03062700	Unnamed	1-Aug	54.62	294.81	6
Becker	03039300	Unnamed	11-Jun	11.15	57.59	7
Becker	03030300	Bear	28-Jun	43.40	377.83	6
Blue Earth	07006001	Eagle (North)	14-Aug	72.63	134.55	5
Chisago	13006100	Unnamed	7-Aug	0.38	2.27	2
Douglas	21006000	Kruegers Slough	20-Aug	24.49	52.85	4
Douglas	21072900	Unnamed	21-Aug	1.41	1.14	1
Douglas	21019900	Crooked	5-Sep	7.11	26.43	9
Douglas	21019900	Crooked	7-Aug	13.17	72.96	9
Douglas	21008000	Darling	8-Aug	6.83	12.35	12
Hennepin	27002900	Edina	9-Aug	55.30	97.23	8
Hennepin	27001900	Nokomis	12-Jul	44.64	224.07	8
Hennepin	27017901	North Little Long	9-Aug	4.79	42.26	10
Hubbard	29014400	Sunday	8-Aug	9.57	1.93	1
Hubbard	29030300	Lost	5-Sep	8.21	20.43	4
Isanti	30006000	Section	7-Aug	0.60	2.57	4
Isanti	30007200	Long	21-Jun	77.60	278.73	7
Kandiyohi	34014100	Woodcock	15-Aug	81.98	191.28	7
Kandiyohi	34032100	Swenson	6-Aug	38.88	112.62	7
Kandiyohi	34025100	Norway	6-Aug	32.81	117.38	12
Le Sueur	40010700	Savidge	12-Jul	113.01	327.03	3
Le Sueur	40009800	Unnamed	12-Jul	75.64	47.90	5
Mahnomen	44014000	Circle	31-Jul	12.96	44.12	3
Mahnomen	44052800	Unnamed	27-Jun	262.78	430.87	6
McLeod	43001400	South	7-Jun	437.57	835.53	7
McLeod	43007600	Bear	12-Jun	8.44	73.43	8
Meeker	47001500	Jennie	12-Jun	8.10	44.42	8
Otter Tail	56098500	Unnamed	30-Jul	284.28	1202.13	4
Otter Tail	56158200	Unnamed	20-Aug	11.45	110.34	9
Otter Tail	56011300	Unnamed	23-Jul	23.81	72.86	8
Otter Tail	56085300	Unnamed	25-Jul	19.09	73.20	7
Otter Tail	56035600	Fairy	31-Jul	35.94	27.76	9
Otter Tail	56057800	Holbrook	31-Jul	103.67	246.02	6

Appendix D. (Continued)

County	DOW #	Lake Name	Date Sampled	Total Zooplankton Densities (no./liter)	Total Zooplankton Biomass (µg/liter)	Species Richness (# taxa)
Otter Tail	56049200	Horseshoe	20-Aug	2.54	4.26	8
Otter Tail	56049000	Round	30-Jul	29.71	54.13	7
Otter Tail	56014700	Unnamed	23-Jul	34.47	61.10	10
Otter Tail	56057300	East Red River	24-Jul	0.83	1.48	7
Otter Tail	56062900	South Stang	25-Jul	2.02	7.93	6
Otter Tail	56043000	Fiske	11-Jun	55.75	234.54	8
Otter Tail	56047600	Maine (Round)	8-Aug	3.71	24.26	8
Pope	61009100	Unnamed	14-Aug	28.03	19.31	5
Ramsey	62007300	Snail	7-Jun	13.88	64.27	10
Sherburne	71004400	Little Dianne	7-Aug	27.12	117.88	9
Stearns	73031700	Unnamed	7-Aug	6.55	16.64	4
Stearns	73024100	Black Oak	11-Jun	248.12	727.29	9
Stearns	73017200	Clear	11-Jun	2.30	33.12	5
Todd	77025800	Unnamed	20-Aug	195.28	434.68	5
Washington	82003100	Terrapin	6-Jun	108.49	381.02	8
Wright	86006500	Unnamed	27-Aug	62.68	319.34	7
Wright	86026300	Cokato	13-Jun	14.90	116.00	6

Appendix E. List of Minnesota 2012 NLAP lakes from the Great Plains Ecoregion

County	DOW #	Lake Name	Date Sampled	Total Zooplankton Densities (no./liter)	Total Zooplankton Biomass (µg/liter)	Species Richness (# taxa)
Becker	03057100	Cucumber	13-Jun	38.57	158.94	5
Big Stone	06026600	Unnamed	20-Aug	5.73	5.42	4
Big Stone	06020600	Unnamed	22-Aug	23.20	300.76	6
Big Stone	06009000	Bentsen	22-Aug	44.00	173.73	5
Big Stone	06034900	Unnamed	28-Aug	6.63	9.81	4
Big Stone	06010200	Thielke	21-Aug	19.74	145.20	6
Big Stone	06025100	Taffe Pond	5-Sep	74.13	352.00	6
Big Stone	06046000	Unnamed Pool	10-Jul	147.29	227.12	5
Big Stone	06015200	Big Stone	7-Aug	44.77	320.61	6
Blue Earth	07012400	Lieberg	12-Jul	521.35	1033.06	4
Clay	14008100	Unnamed	1-Aug	17.58	44.00	12
Clay	14038900	Unnamed	30-Jul	107.89	79.13	5
Cottonwood	17002400	String	11-Jul	251.33	532.50	6
Cottonwood	17006000	Talcot	15-Aug	329.49	2251.77	7
Cottonwood	17007300	Summit	20-Aug	119.34	412.50	7
Faribault	22002200	South Walnut	15-Aug	508.09	426.14	3
Grant	26004300	Unnamed	23-Jul	8.74	24.16	5
Grant	26020500	Unnamed	23-Jul	66.30	253.68	3
Grant	26022800	Hodgson	23-Jul	150.08	718.83	5
Grant	26021700	Unnamed	21-Aug	60.57	335.95	7
Kandiyohi	34029400	Lindgren	14-Aug	1747.88	1423.61	5
Kandiyohi	34003300	Ella	9-Aug	19.65	32.09	7
Lac Qui Parle	37002600	Unnamed	9-Jul	4.52	12.19	3
Lac Qui Parle	37010000	Unnamed	9-Jul	85.89	31.55	4
Lac Qui Parle	37013400	Unnamed	9-Jul	0.60	0.61	3
Lincoln	41005500	North Ash	17-Jul	49.72	161.65	6
Lincoln	41004400	Popowski	16-Jul	67.50	215.12	5
Lyon	42007000	East Twin	20-Aug	9.45	92.76	7
Mahnomen	44024400	Unnamed	2-Aug	2.18	4.01	3
Mahnomen	44022800	Unnamed	19-Jul	88.37	101.55	7
Martin	46009800	Dutton Slough	11-Jul	212.16	158.12	5
Murray	51006800	Summit	20-Aug	102.46	97.55	7
Murray	51007900	Iron	20-Aug	53.44	280.49	6
Murray	51007900	Iron	17-Jul	55.15	252.64	6

Appendix E. (Continued)

County	DOW #	Lake Name	Date Sampled	Total Zooplankton Densities (no./liter)	Total Zooplankton Biomass (μg/liter)	Species Richness (# taxa)
Nobles	53002400	Ocheda	15-Aug	156.41	442.91	5
Norman	54001300	Home	2-Aug	168.01	253.86	5
Otter Tail	56081000	Unnamed	21-Aug	13.41	51.15	6
Pennington	57002700	Unnamed	12-Jul	5.86	16.99	8
Polk	60031900	Unnamed	31-Jul	6.25	18.46	7
Polk	60007800	Unnamed	2-Aug	5.12	30.95	3
Polk	60021100	Unnamed	30-Jul	74.21	59.11	4
Polk	60009900	Unnamed	22-Aug	21.10	126.67	6
Polk	60012900	Unnamed	21-Aug	8.30	17.27	7
Polk	60028100	Unnamed	30-Jul	6.80	21.38	7
Polk	60027500	Unnamed	2-Aug	61.78	133.36	6
Pope	61018900	Unnamed	13-Aug	277.25	559.65	3
Redwood	64009600	Unnamed	11-Jul	34.96	108.58	6
Sibley	72005000	High Island	13-Jun	141.04	598.56	7
Stevens	75016400	Silver	21-Aug	112.71	181.94	6
Stevens	75020500	Unnamed	20-Aug	11.15	48.86	5