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INTERACTIONS OF ZOOPLANKTON AND
PHYTOPLANKTON WITH CYANOBACTERIA

by

Rebecca J. Alexander

A THESIS

Presented to the Faculty of
The Graduate College at the University of Nebraska
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INTERACTIONS OF ZOOPLANKTON AND
PHYTOPLANKTON WITH CYANOBACTERIA

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University of Nebraska, 2012

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Cyanobacteria are a major concern in Nebraska reservoirs and are capable of producing toxins that can cause skin irritations and gastrointestinal problems, as well as affect the nervous system. It is important to determine the mechanisms that can cause cyanobacteria blooms due to the effect they can have on human health. The interaction of zooplankton and other phytoplankton groups with cyanobacteria is important because there is a biological component in surface waters that should be taken into consideration along with the physical and chemical parameters that have been noted to promote cyanobacteria. For example, zooplankton have the ability to alter the phytoplankton composition through their grazing and previous research has shown that cyanobacteria can have diverse effects on different zooplankton, which could promote and perpetuate cyanobacteria. Weekly samples were collected from six Nebraska reservoirs and analyzed to determine the interactions of zooplankton and phytoplankton with cyanobacteria using two generalized additive models with cyanobacteria relative percentage or cyanobacteria biovolume as explanatory variables. In most cases, cyanobacteria relative percentage and biovolume had similar effects on phytoplankton and zooplankton groups with little difference in the predicted biovolume/biomass or

density. Chemical and physical data collected from the reservoirs were analyzed with Spearman rank correlations to determine their relationships with cyanobacteria biovolume. Including biological, chemical and physical parameters to ascertain the interactions and relationships with cyanobacteria can help establish grounds for management techniques, such as biomanipulation. Biomanipulation can prove to have positive results in surface waters, but further research is needed to determine its effectiveness in Nebraska reservoirs. This study provides the first steps in helping to establish its possible effectiveness by determining the interactions of zooplankton and phytoplankton with cyanobacteria in reservoirs.

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1.0 Introduction

Cyanobacteria (blue-green algae) occur in many surface water systems and are a common component of the phytoplankton. Anthropogenic impacts on freshwater systems, such as nutrient loading, have led to cultural eutrophication of these water bodies. This eutrophication has caused cyanobacteria to form large and persistent blooms. Cyanobacteria have been known to cause minor ailments, such as skin irritation and respiratory illness (Mur et al. 1999), and findings have shown that cyanobacteria species can be harmful, and even fatal, to human health. In recent decades, it was discovered that certain species of cyanobacteria produce toxins that can affect the liver, gastrointestinal tract, and nervous system (Mur et al. 1999, Cox 2003, EPA 2010). Although many species can produce toxins, not all blooms are toxic (Carmichael 2001). In Nebraska, the concern for cyanobacteria became very apparent in 2004, when two dogs died after drinking from a small private lake near Omaha, Nebraska that had high levels of microcystin-LR (Brakhage 2009). The 2004 recreation season was not without further occurrences, which consisted of more dog and wild animal deaths, as well as human skin rashes, lesions, and gastrointestinal problems (Brakhage 2009). A monitoring program was set-up in 2004 that was designed to collect and analyze samples from lakes in Nebraska and test them for microcystin-LR.

Lentic systems are comprised of plants and animal species that may compete with cyanobacteria and may promote or suppress cyanobacteria growth. Zooplankton consume algae and have the capability to control phytoplankton abundances and affect community structure. Zooplankton are important food resources for higher trophic

levels. Haney (1987) described three possible interactions between zooplankton and cyanobacteria including direct effects (e.g., grazing and feeding interference), indirect effects (e.g., phytoplankton composition changes), and allelopathic effects (e.g., toxins produced by cyanobacteria affecting zooplankton growth and/or reproduction).

Cyanobacteria size and morphology have been related to zooplankton composition changes because of their relatively low nutritional value and mechanical interference (Ghadouani et al. 2003, Hambright et al. 2001, Haney 1987, Hansson et al. 2007, Tillmanns et al. 2008, Wilson et al. 2006), but grazing ability on cyanobacteria can be highly dependent upon the species of zooplankton (Lampert 1987). Larger cladocerans are known to eat indiscriminately, while smaller cladocerans and copepods will discriminate based on size and/or taste (DeMott 1986, DeMott and Moxter 1991). These feeding behaviors allow cyanobacteria to potentially alter zooplankton population dynamics by favoring smaller bodied cladocerans and copepods over large bodied cladocerans. Bouvy et al. (2001) observed increasing zooplankton biomass comprised mainly of copepods and rotifers leading up to and during cyanobacteria blooms in a tropical reservoir and decreasing after the blooms. Cladoceran biomass was lowest during blooms, but increased after blooms ceased while rotifers and copepod biomass decreased.

A typical zooplankton and phytoplankton cycle was described by Abrantes et al. (2006) for a shallow lake in the Mediterranean area, which described a dominance of cladocerans in the spring when Chlorophyta (green algae) was the dominant algal species. The cladocerans gave way to smaller bodied zooplankton when predation

pressure increased and edible food became harder to attain in the summer and fall. At this time, the phytoplankton biomass also shifted from a predominantly green algae composition to dominant cyanobacteria. This particular cyanobacteria assemblage was comprised of filamentous and colonial forms, which tend to be problematic for larger zooplankton to feed on. In autumn, conditions favoring cyanobacteria diminished and they were replaced by Bacillariophytes (diatoms) and larger zooplankton biomass increased again. Researchers have noted increasing amounts of zooplankton and growth even as cyanobacteria increases and replaces the dominant phytoplankton, but then a rapid decline around the time that cyanobacteria peaks (Ferra-filho et al. 2000, Ghadouani et al. 2003, Havens et al. 2009, Tillmanns et al. 2008). In many cases, the cyanobacterial dominant phases have zooplankton communities comprised mainly of small bodied cladocerans (*Bosmina*, *Ceriodaphnia*) and copepods, while *Daphnia* spp. generally decrease (Abrantes et al. 2006, Bouvy et al. 2001, Burns 1968, Ghadouani et al. 2003, Havens et al. 2009, Mayer et al. 1997). Zooplankton communities may also have the ability to “rebound” after cyanobacterial blooms subside as observed by experiments with *Daphnia* by Lampert (1982).

Phytoplankton communities almost always display strong seasonal trends throughout the year. In several studies there is generally a dominant taxon at any one point in time, seldom is abundance evenly distributed throughout the year (Abrantes et al. 2006, Murrell and Loes 2004). These natural successional cycles can be disrupted by eutrophication and subsequent long-term domination by cyanobacteria (Dokulil and Teubner 2000). Cyanobacteria generally out-compete other phytoplankters for specific

resources that promote their growth (Dokulil and Teubner 2000). For example, many species of cyanobacteria are capable of fixing nitrogen, therefore when nitrogen decreases and phosphorus increases they can attain essential nutrients for growth while other algal groups are suppressed by low nitrogen availability (Downing et al. 2001, Havens et al. 2003, Schindler 1977, Smith 1983). Cyanobacteria may also be favored under lower light conditions than other algal groups (Havens et al. 2003) and it is possible that when their abundance increases greatly that they reduce light with positive consequences for their own growth relative to other groups (Scheffer et al. 1997).

By better understanding the dynamics of zooplankton and phytoplankton with cyanobacteria, we can better understand the biological aspects of our reservoirs, so that we can better manage our aquatic resources. Most studies have focused on cyanobacteria in marine ecosystems, tropical and subtropical areas, and in natural lakes of temperate areas. There is a need to investigate the factors that affect cyanobacteria blooms in shallow, freshwater reservoirs. Most of the lentic ecosystems in eastern Nebraska are reservoirs created by the Army Corps of Engineers as water storage projects. They tend to be shallow compared to natural lakes of similar size and exhibit different fluxes and internal characteristics when compared to natural lakes. The patterns exhibited by phytoplankton and zooplankton in natural lakes may differ greatly in constructed reservoirs.

The purpose of this project was to investigate the community/population changes of zooplankton and phytoplankton in relation to cyanobacteria in Nebraska reservoirs.

What are the interactions of zooplankton and phytoplankton with cyanobacteria in Nebraska reservoirs?

- *Does using cyanobacteria relative percentage or biovolume affect the output on the response variables differently (phytoplankton biovolume or zooplankton biomass)?*
- *Which groups of phytoplankton are affected specifically by cyanobacteria and in what way?*
- *Which groups of zooplankton are affected specifically by cyanobacteria and in what way?*
- *What abiotic factors are correlated with cyanobacteria in Nebraska reservoirs?*

Chlorophytes may not be affected by cyanobacteria considering they do tend to dominate in the spring naturally decline in the seasonal succession of phytoplankton. Cyanobacteria would most likely dominate in the summer when temperatures rise and conditions become more favorable for their growth. Diatoms may be affected by cyanobacteria because they do occur at similar times (fall) when cyanobacteria may still be present. Dinoflagellates do not appear to be prominent taxa in many freshwater systems in Nebraska, which suggests that this group will most likely not be affected by cyanobacteria.

I expect different zooplankton groups to be affected by cyanobacteria, such as *Daphnia*, where previous research has shown that daphnids are one of the first groups to become hindered by high abundances of cyanobacteria. Smaller cladocerans and

copepods I expect to be affected little or possibly being positively affected (indirectly) by cyanobacteria because they tend to avoid ingesting cyanobacteria and may find alternative food sources.

2.0 Methodology

2.1 Study sites

The study sites included the following reservoirs: Bluestem, Pawnee, Olive Creek, Conestoga, Wagon Train, and Yankee Hill (Figure 1A-F). Most of the reservoirs were constructed in the 1960's under the "Flood Control Act of 1958" and the surrounding landscapes remain predominately agricultural. They are all located in the Lower Platte watershed in the Salt Creek basin. Three stations were established across each reservoir located at the dam, the inflow, and midway between the dam and inflow.

2.1.1 Bluestem reservoir

Bluestem reservoir, completed in 1963, is located in Lancaster County (latitude 40.6286136, longitude -96.7900225) and is part of the north tributary of Olive Creek Branch. It is surrounded by cropland and some small housing developments, but those are not located along its shoreline. The reservoir is 326 surface acres with six miles of shoreline and is roughly oriented in a southeast-northwest direction with the dam being located on the southeast side. This lake is often heavily used for water recreation activities (water skiing, tubing, boating, jet skiing).

2.1.2 Conestoga reservoir

Conestoga reservoir, completed in 1963, is located about eight miles south of Pawnee reservoir in Lancaster County (latitude 40.76561, longitude -96.86207). The

landscape surrounding the reservoir is predominantly agricultural. The reservoir is 230 surface acres and is oriented in an east-west direction with the dam being located on the east side. Water recreation was observed to be minimal during the field season, possibly due to its square shape. Plans to renovate Conestoga are being considered for the near future.

2.1.3 Olive Creek reservoir

Olive Creek reservoir, completed in 1964, is located in Lancaster County and was located the furthest south in relation to the other reservoirs (latitude 40.5802078, longitude -96.84494) and is part of the south tributary of Olive Creek Branch. The landscape is predominantly agricultural. It is 175 acres and arranged in a north-south orientation with the dam being on the north side. Water recreation was observed to be limited and minimal on Olive Creek during the field season due to an enforced boating speed limit of 5 mph.

2.1.4 Pawnee reservoir

Pawnee reservoir, completed in 1964, is located west outside of Lincoln, NE in Lancaster County (latitude 40.84773, longitude -96.87545). The surrounding landscape is predominantly agricultural. The reservoir is 740 surface acres and is oriented roughly north-south with the dam on the south end of the reservoir. Water recreation was observed to be moderate during the field season, primarily dominated by slow moving boats and fishing.

2.1.5 Wagon Train reservoir

Wagon Train reservoir, completed in 1963, is located east of Hickman, NE in Lancaster County (latitude 40.63298, longitude -96.58493). The surrounding landscape is predominantly agricultural, but is within 2 miles of Hickman, NE. It is 315 acres with a north-south orientation with the dam on the south end. Water recreation was observed to be moderate with slow-moving boats (fishing), due to a speed limit of 5 mph. Wagon Train was restored in 2000/2002 to stabilize shorelines, create fringe wetlands, and to help reduce sediment and nutrient loading.

2.1.6 Yankee Hill reservoir

Yankee Hill reservoir, completed in 1965, is located east of Denton, NE in Lancaster County (latitude 40.72565, longitude -96.78776). The surrounding landscape is predominantly agricultural. It is 208 surface acres with a northeast-southwest orientation, with the dam being on the northeast side. The morphometry of this reservoir is distinctly different from the other five being that it is not shaped like a rectangle or square, but in a “v” shape with two branching arms. Water recreation was observed to be minimal with slow-moving boats (fishing), due to a speed limit of 5 mph. Yankee Hill was restored in 2004/2005 to provide more open water for fish, stabilize shorelines, and to help reduce sediment and nutrient loading.

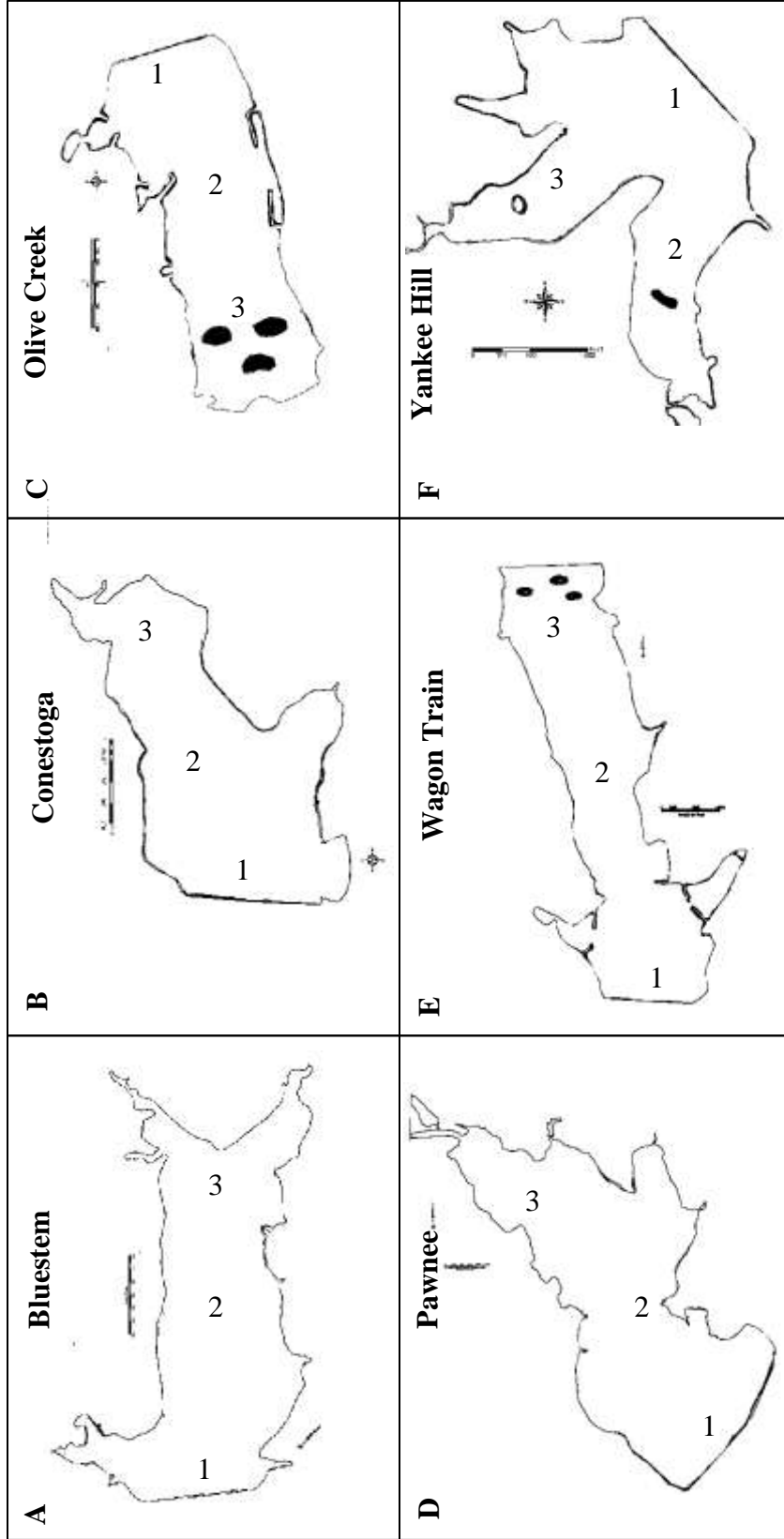


Figure 1A-F. Location of the sampling stations of the study reservoirs. Stations numbered 1 refer to dam stations, stations numbered 2 refer to mid stations, and stations numbered 3 refer to inflow stations.

2.2 Materials and methods

The six reservoirs in the Lincoln area were sampled weekly from mid-May 2011 through September 2011, and once in mid-October 2011. Physical, chemical, and biological parameters were collected at each of three stations from each reservoir. A Garmin Legend Cx Global Positioning System (Garmin, Olathe, KS) was used to mark the latitude and longitude of each station for each sampling day.

2.2.1 Field collection of physical and chemical parameters

A Van dorn bottle was lowered 0.5 meter below the water surface and 0.5 meter from the bottom to obtain epilimnion and hypolimnion samples, respectively. Total nitrogen and total phosphorus (TN and TP) samples were collected from the epilimnion and hypolimnion in 125 mL and 60 mL Nalgene bottles at each station. Chlorophyll samples were taken from the epilimnion at each station by passing a known amount of sample water through a 25 mm A/E glass fiber filter in the field. Microcystin toxin was collected from the epilimnion at each station in 60 mL amber bottles. All samples were transported on ice to the laboratory where they were refrigerated or frozen until lab analyses were completed. Secchi disk depth was taken at each station and turbidity was measured from the epilimnion and hypolimnion using a Hach 2100P Turbidometer (Hach, Loveland, CO). Dissolved oxygen and temperature were measured every 0.25 meters for the entire water profile using a YSI Pro DO probe (YSI, Yellow Springs, OH). A Li-Cor LI-250A light meter (LI-COR, Lincoln, NE) was used to measure photosynthetically active radiation (PAR), which was used to calculate a light extinction coefficient (LEC). Readings for PAR were taken every 0.1 meter up to 1 meter and then

every 0.2 meters up to 2 meters. Relative *in vivo* phycocyanin measurements were measured using a Turner Designs Aquafluor model 8000-010 fluorometer (Turner Designs, Sunnyvale, CA) with three readings taken at each station in the epilimnion and hypolimnion. Microcystin toxins were analyzed using ELISA test kits (Abraxis LLC, Warminster, PA).

2.2.2 Field collection of biological parameters

Phytoplankton samples were collected using a Van dorn bottle lowered 0.5 m below the water surface. 100 mL of unfiltered sample water was collected and preserved with 10 mL of 1% Lugol's solution in 120 mL glass jars. Zooplankton samples were collected using a 15 L Schindler-Patalas plankton trap, filtered through a 35- μ m mesh net. The samples were collected in 250-mL glass jars and preserved with a 1:1 ratio of sample water:10% neutral sugar formalin solution (Lind 1985).

2.2.3 Laboratory analysis of physical and chemical parameters

Total nitrogen and total phosphorus were analyzed using EPA standard operating procedures (EPA Method 353.2 and EPA Method 365.4) on a BIOS Lachat Quick Chem 8500 Series II (Hach, Loveland, CO). Chlorophyll *a* samples were analyzed using standard procedures (EPA Method 445, modified using ethanol instead of acetone) and fluorescence was measured using a 10-AU Fluorometer (Turner Designs, Sunnyvale, CA).

2.2.4 Laboratory analysis of biological parameters

Phytoplankton samples were inverted several times and 3 mL of subsample were allowed to settle overnight in sedimentation chambers. Ten random fields of view were

counted with a Nikon Diaphot inverted microscope (Nikon, Melville, NY) at 200x, ensuring that at least 300 organisms were counted per sample, and algae were identified to the lowest practicable taxon based on preliminary examination of live material. Samples were diluted or concentrated accordingly to attain approximately 300 organisms for every ten random fields of view. An ocular micrometer was used to measure lengths and widths of 25 cells, filaments, or colonies of each taxonomic group at 600x and 200x. Averages were calculated for each measurement and biovolumes were then calculated using formulae for simple geometric shapes according to Hillebrand et al. (1999). Phytoplankton relative percentages were calculated for each major group using the biovolume estimates.

Zooplankton samples were counted using a Sedgewick-Rafter cell with a Nikon Labophot-2 compound microscope at 100x . A maximum of 5% of the sample was counted to attain approximately 300 organisms per sample. Samples were identified to the lowest practical taxon, usually genus and/or species. Ten individuals of each taxon were measured to determine average body lengths, zooplankton dry weight was then calculated using length-weight relationships from Bottrell et al. (1976) and McCauley (1984), and relative percentages were calculated for each major group using biomass estimates.

2.2.5 Statistical analyses

Two separate generalized additive models (GAM) using the identity link-function were used to ascertain the effect of each cyanobacteria relative percent and cyanobacteria biovolume on different phytoplankton and zooplankton groups. GAM was used due to

the extreme non-linearity observed in the response variables over time and across varying levels of cyanobacteria. The GAM can compensate for this non-linearity exhibited in the data better than generalized linear models. Fixed effects of cyanobacteria relative percentage and time interaction were used in one model and cyanobacteria biovolume and time interaction in a second model, as well as random factors of time and site (reservoir). The random effects of time allow for generalizations and predictions over time and a random effect of site allows for generalizations to be made about all six reservoirs based on the GAM output. Response variables were $\ln(x+1)$ transformed to achieve a more normal distribution, which worked for all of the response variables except for dinoflagellate biovolume and rotifer biomass (probably due to a large number of zero occurrences in both cases). All t-values estimated from the coefficients of the fixed effects were compared to a critical t-value with six degrees of freedom. Six degrees of freedom were chosen because we believed it to be the most conservative value to make a comparison against, to deem significance of the fixed effects.

Spearman rank correlations were used to determine the relationships between the physical and chemical factors that may influence cyanobacteria biovolumes. Spearman rank correlations were also applied to cyanobacteria biovolume and microcystin levels and *in vivo* phycocyanin (a fast, easy, and relatively inexpensive method to measure cyanobacteria). All generalized additive modeling and spearman rank correlations were done using the statistical software program R 2.13.1 (R Development Core Team 2011).

3.0 Results

3.1 Phytoplankton

Figures 2A–2F depict the weekly relative percentage based on the biovolume means of five groups of phytoplankton over the course of the 2011 growing season. There appears to be a general pattern exhibited by the six reservoirs primarily between bacillariophytes (diatoms) and cyanobacteria. In several cases, spikes of cyanobacteria occurred after diatom crashes (Figures 2A, 2B, 2E, 2F) throughout the growing season. This pattern was not as distinct in Olive Creek and Pawnee reservoirs (Figures 2C and 2D). Diatoms and cyanobacteria generally comprised the majority of the biovolume in all of the reservoirs. In general, chlorophytes, euglenophytes, and dinoflagellates remained relatively low throughout the season with small spikes (except in Pawnee and Wagon Train). Euglenoid and dinoflagellate maxima tended to occur later in the growing season (Figures 2C, 2D, 2E), while chlorophytes tended to have higher percentages in the early summer and late spring, then declined quickly (Figures 2A, 2B, 2C, 2D, 2F). Bacillariophytes was comprised mainly of *Aulacoseira*, *Cyclotella*, *Stephanodiscus*, and *Nitzschia*. Cyanobacteria was comprised mainly of *Anabaena* sp. and *Anabaena spiroides* early in the season at all of the sites with additions of *Oscillatoria* and *Cylindrospermopsis* during mid-summer. Several sites had other cyanobacteria compositions later in the summer, such as *Planktothrix* and *Spirulina* (Pawnee and Wagon Train) or *Microcystis* (Yankee Hill). Euglenophytes were made up mainly of *Euglena*, but sometimes with considerable contributions from *Phacus* and *Trachelomonas*. Dinoflagellates were comprised solely of *Ceratium*. Chlorophyte

composition was variable and highly dependent on the reservoir; with most biovolume from *Pediastrum*, *Oocystis*, *Eudorina*, *Cosmarium*, *Closterium*, and *Staurastrum* and with contributions from other genera.

Table 1. Statistical results of the GAM for the major algal taxa for the three fixed effects: Time, Cyanobacteria percentage, the interaction of time and cyanobacteria percentage ($\alpha=0.05$).

Taxa	Coefficient	SE	df	t-value	p-value
Diatoms					
<i>Time</i>	0.251	0.079	6	3.175	0.0192
<i>Cyano. %</i>	-0.44	0.11	6	-4.104	0.0063
<i>Interaction</i>	0.0006	0.0009	6	0.697	0.5119
Chlorophytes					
<i>Time</i>	0.212	0.091	6	2.335	0.0582
<i>Cyano. %</i>	-0.017	0.008	6	-2.169	0.0732
<i>Interaction</i>	0.001	0.001	6	0.932	0.387
Dinoflagellate					
<i>Time</i>	0.015	0.108	6	0.135	0.897
<i>Cyano. %</i>	0.003	0.008	6	0.046	0.9468
<i>Interaction</i>	-0.001	0.001	6	-1.568	0.168
Euglenoid					
<i>Time</i>	0.242	0.070	6	3.445	0.0137
<i>Cyano. %</i>	0.014	0.007	6	2.104	0.08
<i>Interaction</i>	-0.003	0.001	6	-5.75	0.0012

Table 2. Statistical results of the GAM for the major algal taxa for the three fixed effects: Time, Cyanobacteria biovolume (BV), the interaction of time and cyanobacteria biovolume ($\alpha=0.05$).

Taxa	Coefficient	SE	df	t-value	p-value
Diatoms					
<i>Time</i>	0.2425	0.1606	6	1.51	0.1818
<i>Cyano. BV</i>	3.39×10^{-4}	1.15×10^{-4}	6	2.947	0.0257
<i>Interaction</i>	-2.64×10^{-5}	9.26×10^{-6}	6	-2.855	0.029
Chlorophytes					
<i>Time</i>	0.2499	0.09671	6	2.584	0.0415
<i>Cyano. BV</i>	5.43×10^{-5}	6.28×10^{-5}	6	0.866	0.4198
<i>Interaction</i>	-2.76×10^{-6}	4.99×10^{-6}	6	-0.553	0.6002
Dinoflagellate					
<i>Time</i>	-0.03327	0.1045	6	-0.318	0.7613
<i>Cyano. BV</i>	1.04×10^{-4}	6.50×10^{-5}	6	1.602	0.1603
<i>Interaction</i>	-9.97×10^{-6}	5.02×10^{-6}	6	-1.985	0.0944
Euglenoid					
<i>Time</i>	0.1775	0.0854	6	2.078	0.083
<i>Cyano. BV</i>	2.87×10^{-5}	6.09×10^{-5}	6	0.471	0.6543
<i>Interaction</i>	-1.27×10^{-5}	4.93×10^{-6}	6	-2.579	0.0418

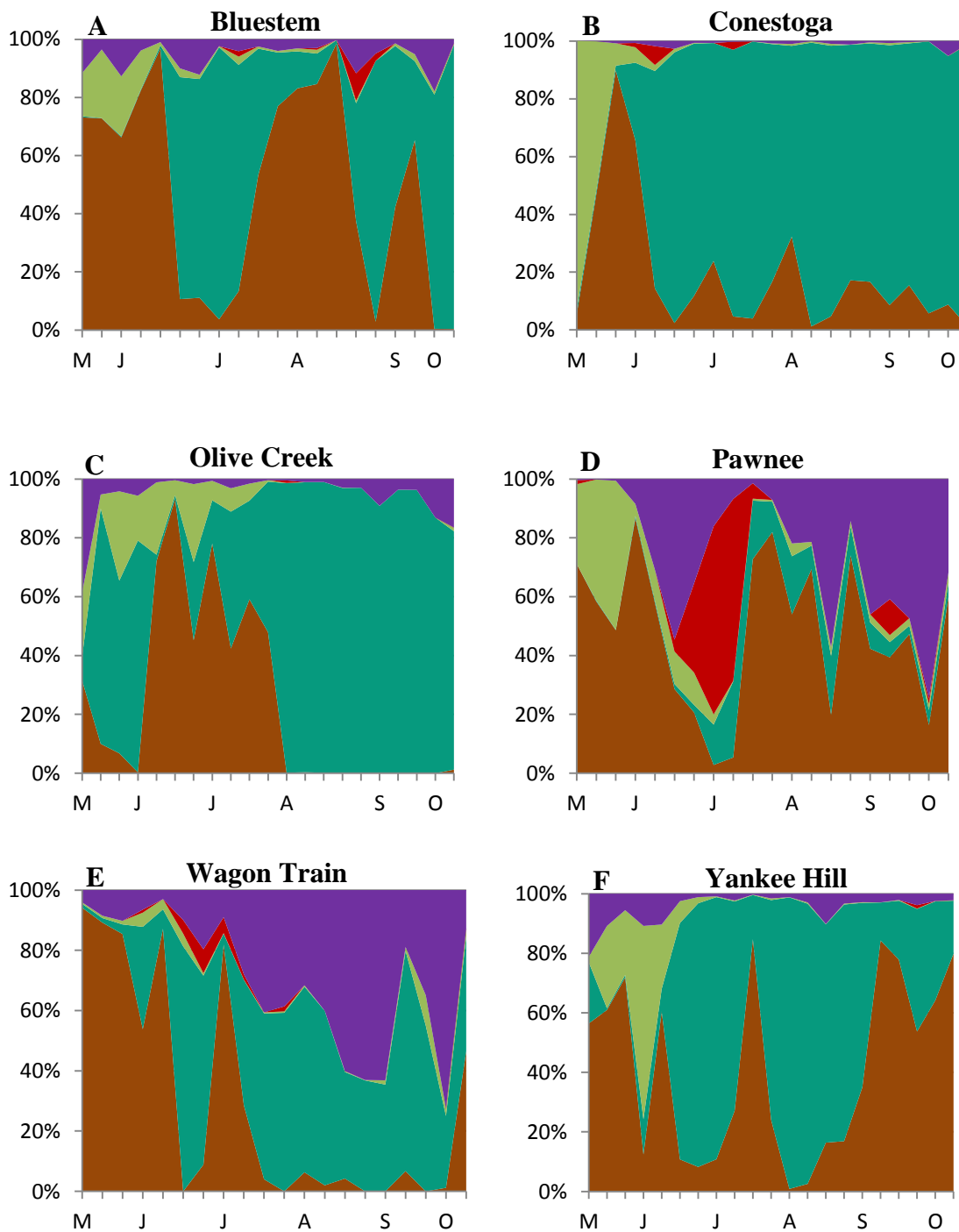


Figure 2A-F. Weekly relative percentage based on biovolume means of the five phytoplankton groups across the 2011 growing season for the six study sites

■ Euglenophyte
 ■ Dinoflagellate
 ■ Chlorophyte
 ■ Cyanobacteria
 ■ Diatoms

Diatom results showed that cyanobacteria percentage had a significant negative effect on biovolume (Table 1). Time also had a significant positive effect on diatom biovolume (Table 1). The interaction of time and cyanobacteria relative percentage was not significant (Table 1). Figure 3A presents observed values with predicted lines of diatom biovolumes at varying levels of cyanobacteria relative percentages based on the model. The model showed that when cyanobacteria makes up 5% of the total phytoplankton biovolume, diatoms were relatively high, there were some effects of time as the biovolume fluctuated across the season. At 50% and 95% of the total phytoplankton biovolume, there was a significant drop in the overall amount of diatoms. Cyanobacteria biovolume had a significant positive effect on predicted diatom biovolume estimates (Table 2). Time did not have a significant effect on diatom biovolume when cyanobacteria biovolume was used as a fixed effect (Table 2). The interaction of time and cyanobacteria biovolume had a significant negative effect on diatom biovolumes (Table 2). Figure 3B presents the observed values with predicted lines of diatom biovolumes at varying levels of cyanobacteria biovolumes based on the model. The significant positive effect of cyanobacteria biovolume on diatoms can be seen early in the season in Figure 3B. The model shows when cyanobacteria biovolume was high ($25,000 \text{ } 10^6 \mu\text{m}^3 \text{ L}^{-1}$), diatoms were relatively high. The interaction effect became apparent later in the season when overall diatom biovolume decreased over time and at higher cyanobacteria biovolumes. In general, higher cyanobacteria biovolumes predicted greater diatom biovolume, which was the opposite of high cyanobacteria percentages that predicted lower diatom biovolume. Figure 3B did begin to portray a similar pattern to

cyanobacteria percent near the end of the growing season when the lower cyanobacteria biovolumes predicted higher diatom biovolume.

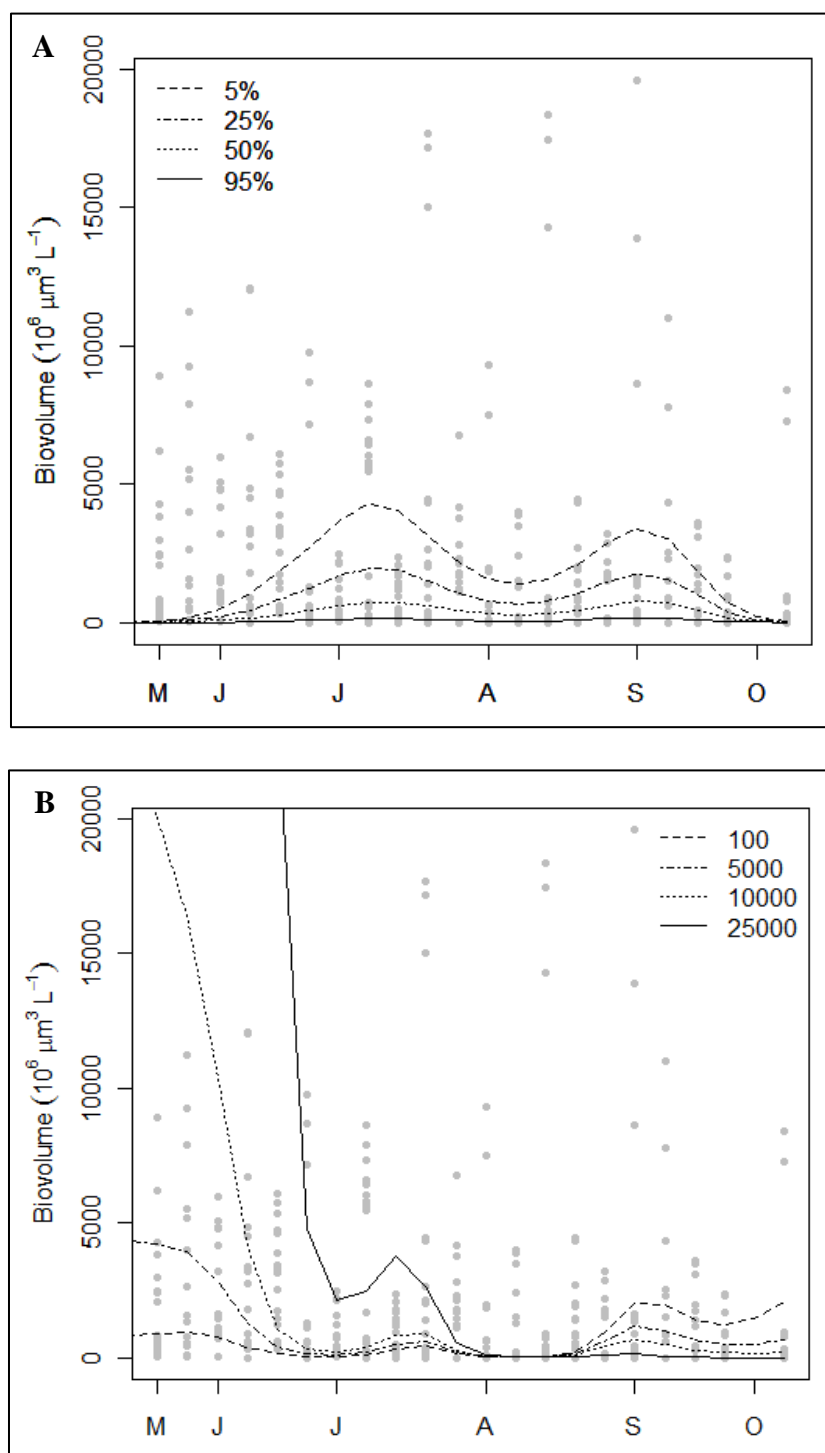


Figure 3. **A)** Bacillariophyte biovolume over the 2011 growing season. Lines indicate predicted biovolumes at different cyanobacteria percentages. Designated site for graph Conestoga. **B)** Bacillariophyte biovolume over the 2011 growing season. Designated site for graph Yankee hill. Lines indicate predicted biovolumes at different cyanobacteria biovolumes. Observed values are demarked in gray dots.

Cyanobacteria percentage did not have a significant effect on chlorophyte biovolume (Table 1). Time did not have a significant effect on chlorophyte biovolume (Table 1). The interaction of time and cyanobacteria relative percentage did not have a significant effect on chlorophyte biovolume (Table 1). Figure 4A depicts the results of the model with predicted lines of chlorophyte biovolume at different levels of cyanobacteria relative percentage. There was some divergence among the predicted lines of chlorophyte biovolume early in the season, but converged later in the season to have minimal difference among the predicted lines. Cyanobacteria biovolume did not have a significant effect on chlorophyte biovolume (Table 2). Time had a significant positive effect on chlorophyte biovolume when cyanobacteria biovolume was used as a fixed effect (Table 2). The interaction of time and cyanobacteria biovolume did not have a significant effect on chlorophyte biovolume (Table 2). A similar pattern was exhibited as an exponential decline in chlorophyte biovolume, which was evident when either cyanobacteria relative percent or biovolume was used as an explanatory variable. One difference being that at higher cyanobacteria biovolumes, a greater chlorophyte biovolume was predicted (Figure 4B). This was opposite of high cyanobacteria relative percentages, which predicted lower chlorophyte biovolumes.

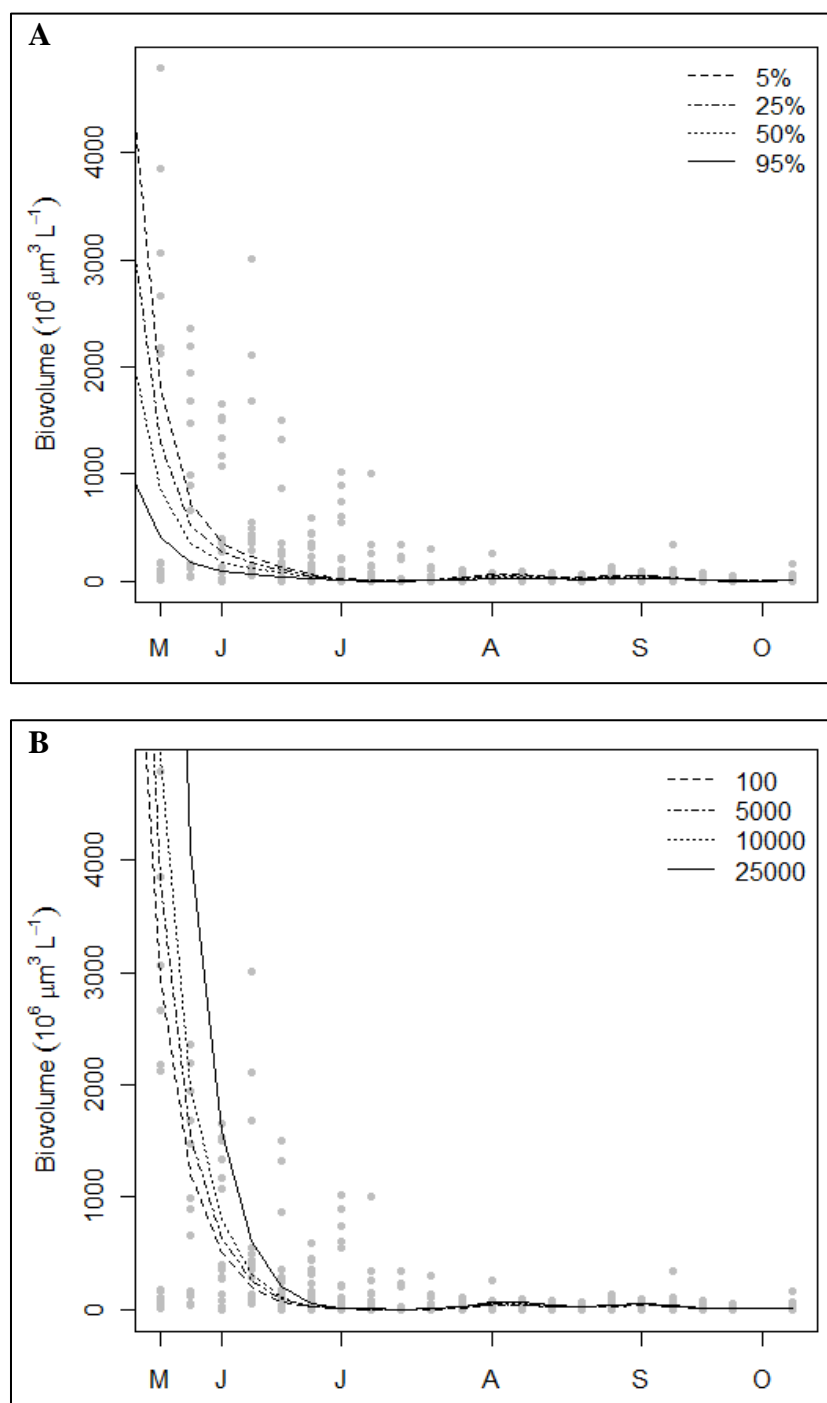


Figure 4. **A)** Chlorophyte biovolume over the 2011 growing season. Lines indicate predicted biovolumes at different cyanobacteria percentages. Designated site for graph Conestoga. **B)** Chlorophyte biovolume over the 2011 growing season. Designated site for graph Conestoga. Lines indicate predicted biovolumes at different cyanobacteria biovolumes. Observed values are demarked in gray dots.

Cyanobacteria relative percentage did not have a significant effect on dinoflagellate biovolume (Table 1). Time also did not have a significant effect on the dinoflagellate biovolume (Table 1). The interaction of time and cyanobacteria relative percentage also did not have a significant effect on dinoflagellate biovolume (Table 1). Figure 5A shows the predicted lines of dinoflagellates at varying levels of cyanobacteria relative percentage based on the model. In general, dinoflagellate biovolume is non-existent most of the season with a small spike in July mainly due to high biovolume estimates observed in Pawnee. Cyanobacteria biovolume did not have a significant effect on dinoflagellate biovolume (Table 2). Time did not have a significant effect on dinoflagellate biovolume when cyanobacteria biovolume was used as a fixed effect (Table 2). The interaction of time and cyanobacteria biovolume did not have a significant effect on dinoflagellate biovolume (Table 2). Figure 5B shows the predicted biovolumes of dinoflagellates at different levels of cyanobacteria biovolume. The pattern seen in figure 5B was very similar to the one observed in figure 5A. One difference between the two GAMs, was that at higher cyanobacteria biovolumes predicted greater dinoflagellate biovolume; this was opposite high cyanobacteria percentages, which depict lower dinoflagellate biovolumes.

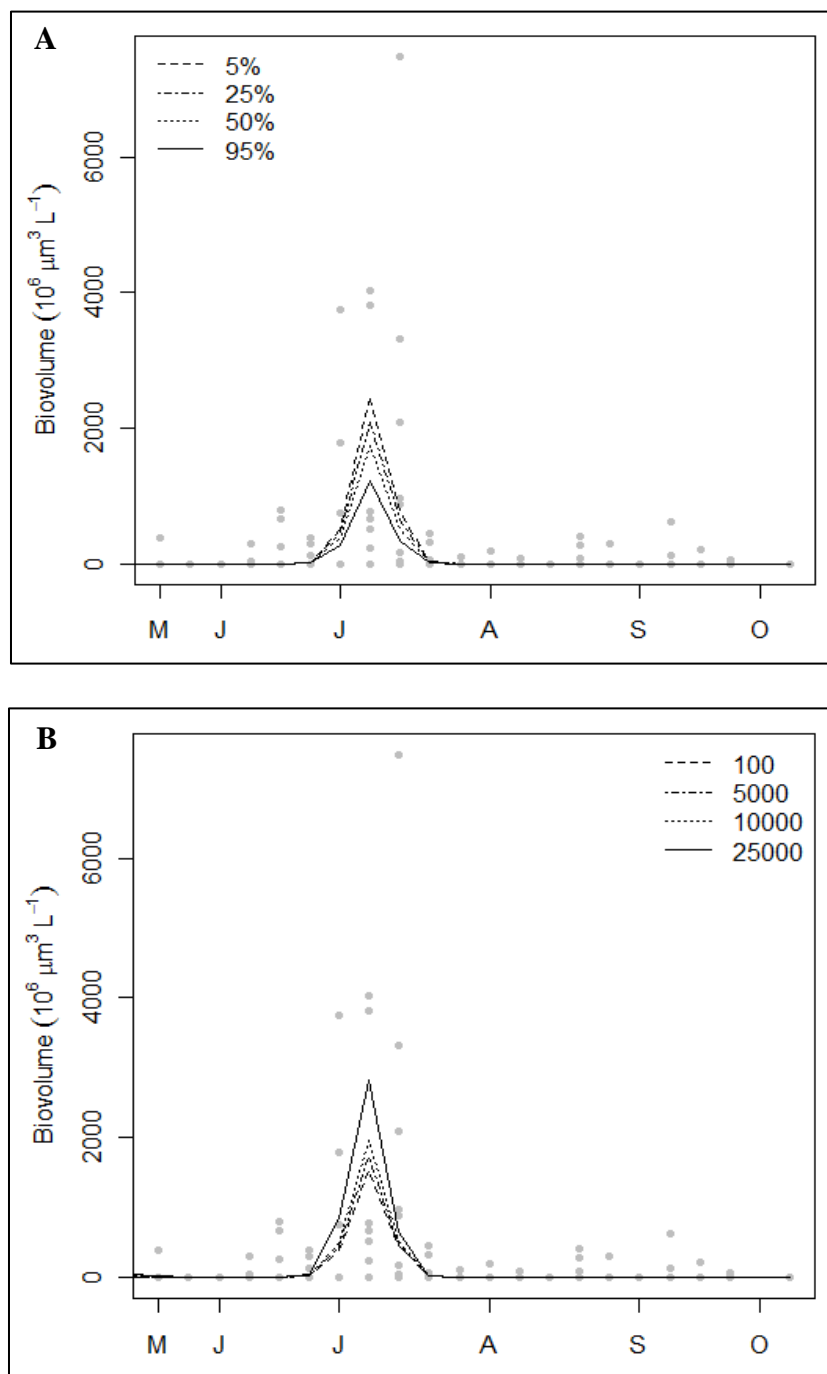


Figure 5. **A)** Dinoflagellate biovolume over the 2011 growing season. Lines indicate predicted biovolumes at different cyanobacteria percentages. Designated site for graph Pawnee. **B)** Dinoflagellate biovolume over the 2011 growing season. Designated site for graph Pawnee. Lines indicate predicted biovolumes at different cyanobacteria biovolumes. Observed values are demarked in gray dots.

Euglenoid results show that cyanobacteria relative percentage was not a significant effect (Table 1). Time had a significant positive effect on euglenoid biovolume (Table 1). The interaction of time and cyanobacteria relative percentage had a significant negative effect on the euglenoid biovolume (Table 1). Figure 6A shows the predicted biovolumes of euglenophytes at varying levels of cyanobacteria relative percentages. Overall there was a positive trend in the euglenoid biovolume over time, but the interaction of cyanobacteria relative percentage caused the euglenoid biovolume to diminish as cyanobacteria made up a greater portion of the total phytoplankton. Cyanobacteria biovolume did not have a significant effect on euglenoid biovolume (Table 2). Time did not have a significant effect on euglenoid biovolume when cyanobacteria biovolume was used as a fixed effect (Table 2). The interaction of time and cyanobacteria biovolume had a significant negative effect on euglenoid biovolume (Table 2). The negative interaction effect was shown in figure 6B in which higher cyanobacteria biovolumes predict lower euglenoid biovolumes compared with lower cyanobacteria biovolumes. The pattern seen in cyanobacteria biovolume (Figure 6B) and cyanobacteria percent (Figure 6A) are fairly similar to each other. At high cyanobacteria percentages, lower euglenoid biovolume was predicted, which is similar when high cyanobacteria biovolumes predict lower euglenoid biovolume. There were also similar increases and decrease between both figures at similar times of the season, and a general increase in euglenoid biovolume at the end of the season.

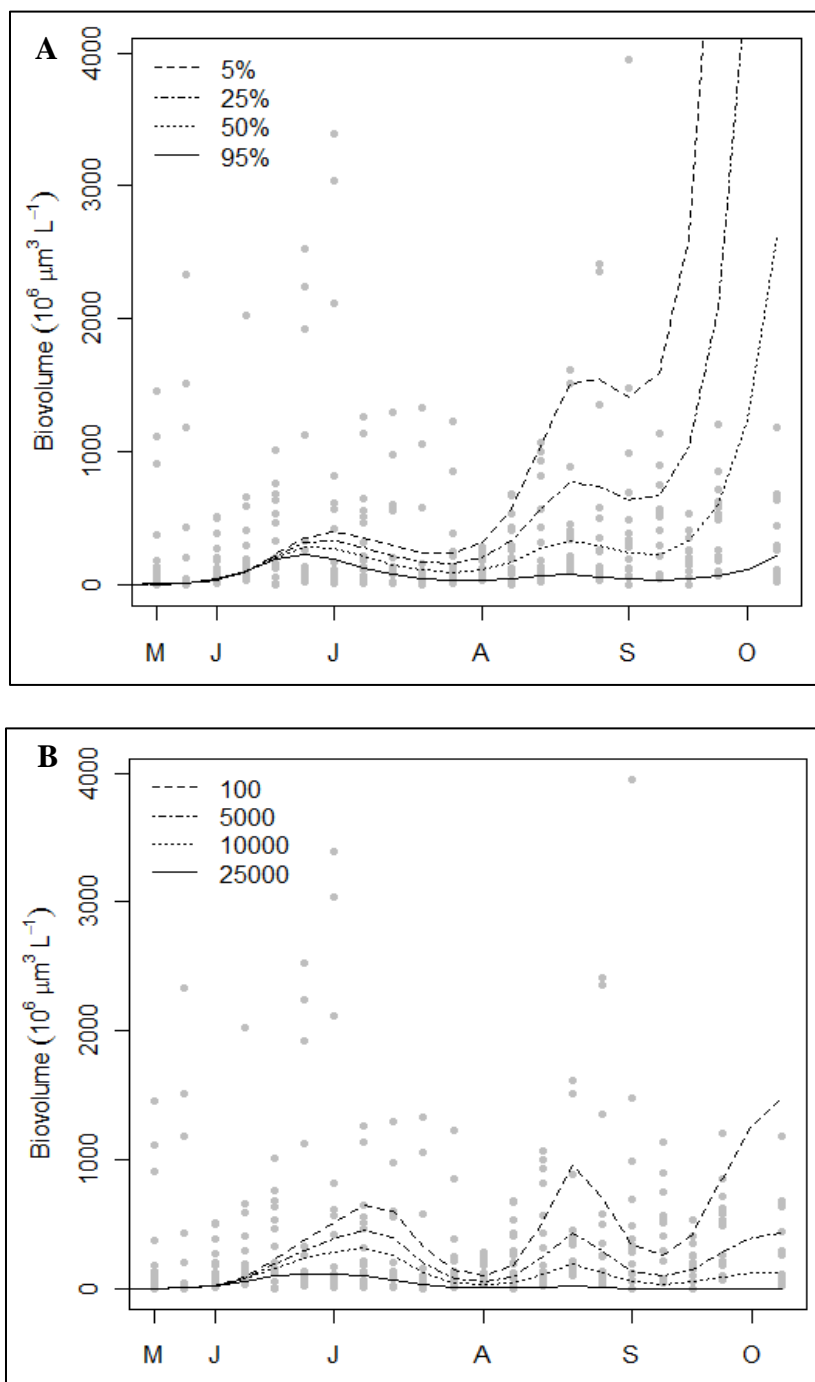


Figure 6. **A)** Euglenoid biovolume over the 2011 growing season. Lines indicate predicted biovolumes at different cyanobacteria percentages. Designated site for graph Conestoga. **B)** Euglenoid biovolume over the 2011 growing season. Designated site for graph Conestoga. Lines indicate predicted biovolumes at different cyanobacteria biovolumes. Observed values are demarked in gray dots.

3.2 Zooplankton

Figures 7A-7F depict the weekly biomass means for two general groups of zooplankton, cladocera and copepods, over time with cyanobacteria relative percentage. Rotifer biomass is not shown on these graphs due to the extremely low biomass estimates compared to cladocerans and copepods (rotifers were between 0.00- 0.09 $\mu\text{g}\cdot\text{L}^{-1}$). Although rotifer biomass may be small compared to cladocerans and copepods, rotifer density was usually greater than copepods and cladocerans during the season. Patterns exhibited by cladocerans were seldom ubiquitous among the reservoirs. In some cases, there were relatively medium biomasses of cladocerans early in the season and then a decline in that biomass in June prior to cyanobacteria relative percentage increases (Figures 7B, 7C, 7E). This pattern was not seen in the other study sites (Figures 7A, 7D, 7F). Towards late summer and fall, cladoceran biomass seemed to track with the increases and decreases in the cyanobacteria relative percentage (Figures 7A, 7B, 7D, 7F). This pattern seemed peculiar until closer examination of the generic composition; in general, *Daphnia* spp. made up most of the biomass early on in the season and then declined in June (Figures 8A, 8B, 8D, 8E, 8F). *Ceriodaphnia*, *Alonella*, *Diaphanosoma*, and *Bosmina* generally made up the cladoceran composition later in the season when cladocerans biomass tracked cyanobacteria (Figures 8A, 8B, 8C, 8D, 8F). Copepod biomass showed variable increases when the relative percentage of cyanobacteria increased (Figures 7A-7F). There are also increases in copepods when the relative percentage of cyanobacteria decreased. Copepod biomass composition mainly consisted of nauplii and calanoids, with a small contribution from cyclopoids (Figures 8A-8F).

Table 3. Statistical results of the major cladoceran taxa for the three fixed effects: Time, Cyanobacteria percentage, the interaction of time and cyanobacteria percentage ($\alpha=0.05$).

Taxa	Coefficient	SE	df	t-value	p-value
Cladoceran					
<i>Time</i>	0.092	0.061	6	1.48	0.1894
<i>Cyano. %</i>	0.011	0.01	6	2.080	0.0827
<i>Interaction</i>	0.001	0.0004	6	2.267	0.0639
<i>Daphnia</i>					
<i>Time</i>	0.239	0.094	6	2.531	0.0446
<i>Cyano. %</i>	-0.004	0.008	6	-0.542	0.6073
<i>Interaction</i>	0.002	0.001	6	3.125	0.0205
<i>Bosmina</i>					
<i>Time</i>	-0.017	0.086	6	-0.193	0.8533
<i>Cyano.%</i>	0.039	0.008	6	4.963	0.0025
<i>Interaction</i>	-0.002	0.001	6	-2.571	0.0423
Other cladoceran					
<i>Time</i>	-0.103	0.073	6	-1.407	0.2091
<i>Cyano. %</i>	0.005	0.007	6	0.761	0.4755
<i>Interaction</i>	0.001	0.0006	6	2.577	0.0458

Table 4. Statistical results of the major cladoceran taxa for the three fixed effects: Time, cyanobacteria biovolume (BV), the interaction of time and cyanobacteria biovolume ($\alpha=0.05$).

Taxa	Coefficient	SE	df	t-value	p-value
Cladoceran					
<i>Time</i>	0.1697	0.08324	6	2.039	0.0876
<i>Cyano. BV</i>	3.00×10^{-6}	6.09×10^{-5}	6	0.049	0.9625
<i>Interaction</i>	9.84×10^{-6}	4.95×10^{-6}	6	1.987	0.0941
<i>Daphnia</i>					
<i>Time</i>	0.2532	0.09429	6	2.685	0.0363
<i>Cyano. BV</i>	2.23×10^{-5}	6.21×10^{-5}	6	0.358	0.7326
<i>Interaction</i>	9.77×10^{-6}	5.02×10^{-6}	6	1.947	0.0995
<i>Bosmina</i>					
<i>Time</i>	-0.0621	0.08686	6	-0.715	0.5015
<i>Cyano. BV</i>	1.46×10^{-4}	6.71×10^{-5}	6	2.176	0.0725
<i>Interaction</i>	-8.36×10^{-6}	5.29×10^{-6}	6	-1.579	0.1654
Other cladoceran					
<i>Time</i>	0.02413	0.06822	6	0.354	0.7354
<i>Cyano. BV</i>	-8.40×10^{-5}	5.43×10^{-5}	6	-1.547	0.1728
<i>Interaction</i>	1.38×10^{-5}	4.54×10^{-6}	6	3.038	0.0229

Table 5. Statistical results of the major copepod taxa for the three fixed effects: Time, Cyanobacteria percentage, the interaction of time and cyanobacteria percentage ($\alpha=0.05$).

Taxa	Coefficient	SE	df	t-value	p-value
Copepod					
<i>Time</i>	0.180	0.033	6	5.483	0.0015
<i>Cyano. %</i>	0.004	0.003	6	1.264	0.2531
<i>Interaction</i>	-0.0002	0.0003	6	-0.646	0.5422
Cyclopoid					
<i>Time</i>	1.445×10^{-2}	6.542×10^{-1}	6	0.238	0.8198
<i>Cyano. %</i>	5.792×10^{-3}	5.315×10^{-3}	6	1.090	0.3175
<i>Interaction</i>	-3.229×10^{-5}	4.615×10^{-4}	6	-0.070	0.8198
Calanoid					
<i>Time</i>	0.108	0.044	6	2.460	0.0491
<i>Cyano. %</i>	-0.004	0.006	6	-0.725	0.4957
<i>Interaction</i>	0.001	0.001	6	1.423	0.2046
Nauplii					
<i>Time</i>	0.123	0.040	6	3.111	0.0208
<i>Cyano. %</i>	0.011	0.003	6	3.376	0.0149
<i>Interaction</i>	-0.001	0.0003	6	-3.176	0.0192

Table 6. Statistical results of the major copepod taxa for the three fixed effects: Time, cyanobacteria biovolume (BV), the interaction of time and cyanobacteria biovolume ($\alpha=0.05$).

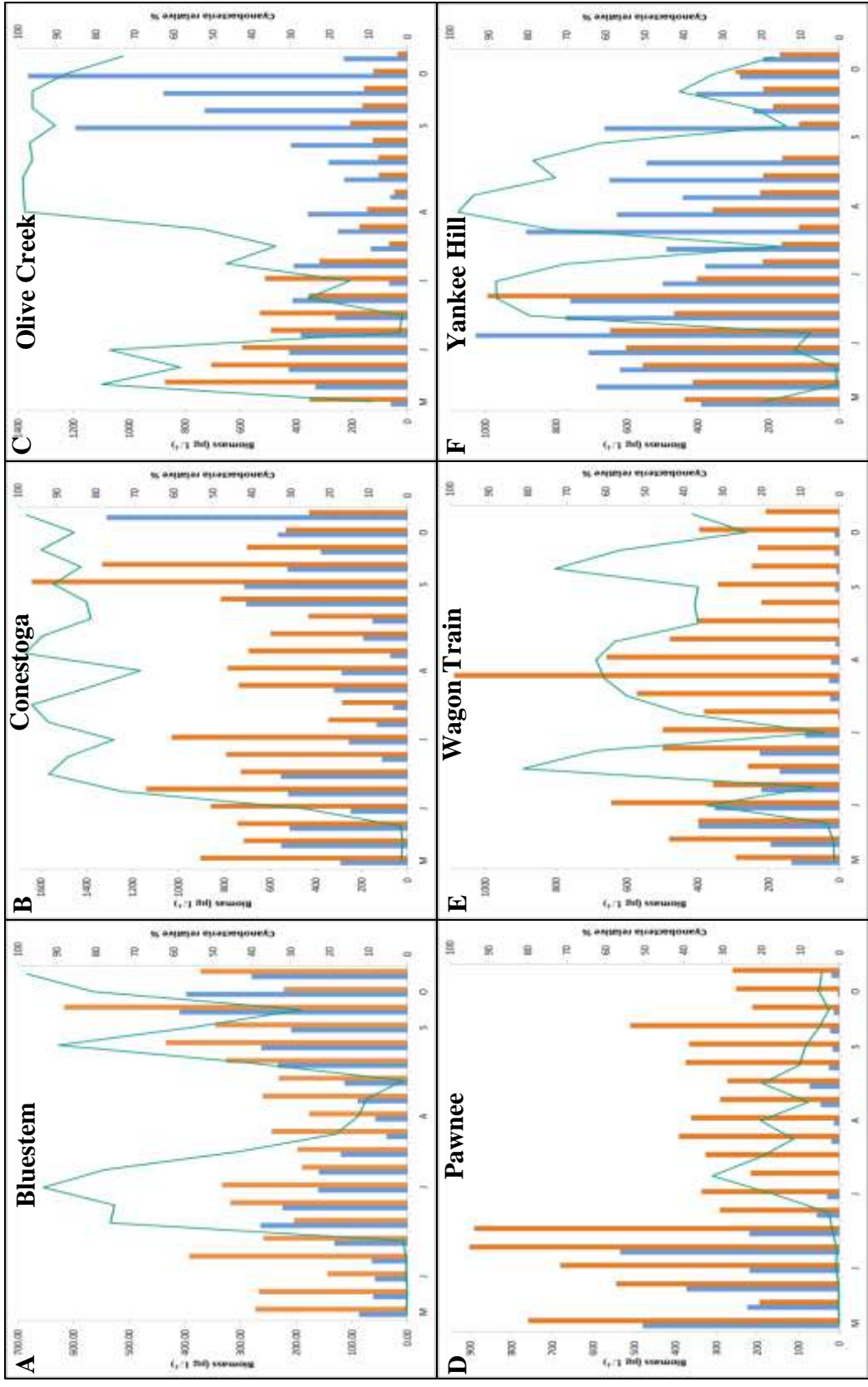
Taxa	Coefficient	SE	df	t-value	p-value
Copepod					
<i>Time</i>	0.193	0.04214	6	4.579	0.0038
<i>Cyano. BV</i>	1.97×10^{-4}	3.06×10^{-5}	6	6.454	0.0007
<i>Interaction</i>	-1.28×10^{-5}	2.43×10^{-6}	6	-5.25	0.0019
Cyclopoid					
<i>Time</i>	0.051	0.07349	6	0.694	0.5136
<i>Cyano. BV</i>	1.70×10^{-4}	5.39×10^{-5}	6	3.154	0.0197
<i>Interaction</i>	-7.34×10^{-6}	4.34×10^{-6}	6	-1.691	0.1418
Calanoid					
<i>Time</i>	0.1269	0.05678	6	2.236	0.0667
<i>Cyano. BV</i>	8.24×10^{-5}	5.96×10^{-5}	6	1.382	0.2162
<i>Interaction</i>	-3.96×10^{-6}	4.70×10^{-6}	6	-0.843	0.4315
Nauplii					
<i>Time</i>	0.1569	0.04288	6	3.659	0.0106
<i>Cyano. BV</i>	3.08×10^{-4}	3.37×10^{-5}	6	9.144	0.0001
<i>Interaction</i>	-2.21×10^{-5}	2.71×10^{-6}	6	-8.172	0.0002

Table 7. Statistical results of the rotifers for the three fixed effects: Time, Cyanobacteria percentage, the interaction of time and cyanobacteria percentage ($\alpha=0.05$).

Taxa	Coefficient	SE	df	t-value	p-value
Rotifer					
<i>Time</i>	-2.138×10^{-4}	7.896×10^{-4}	6	-0.271	0.7955
<i>Cyano. %</i>	-5.87×10^{-5}	5.83×10^{-5}	6	-1.006	0.3532
<i>Interaction</i>	7.947×10^{-6}	4.994×10^{-6}	6	1.591	0.1627

Table 8. Statistical results of the rotifers for the three fixed effects: Time, cyanobacteria biovolume (BV), interaction of time and cyanobacteria biovolume ($\alpha=0.05$).

Taxa	Coefficient	SE	df	t-value	p-value
Rotifer					
<i>Time</i>	-3.13×10^{-4}	7.80×10^{-4}	6	-0.402	0.7016
<i>Cyano. BV</i>	-2.35×10^{-7}	3.87×10^{-7}	6	-0.606	0.5667
<i>Interaction</i>	3.54×10^{-8}	3.45×10^{-8}	6	1.028	0.3436



Figures 7A-7F. Weekly biomass means for two major zooplankton groups and relative percentage of cyanobacteria over the 2011 field season for the six study sites. ■ Cladoceran ■ Copepod — Cyanobacteria %

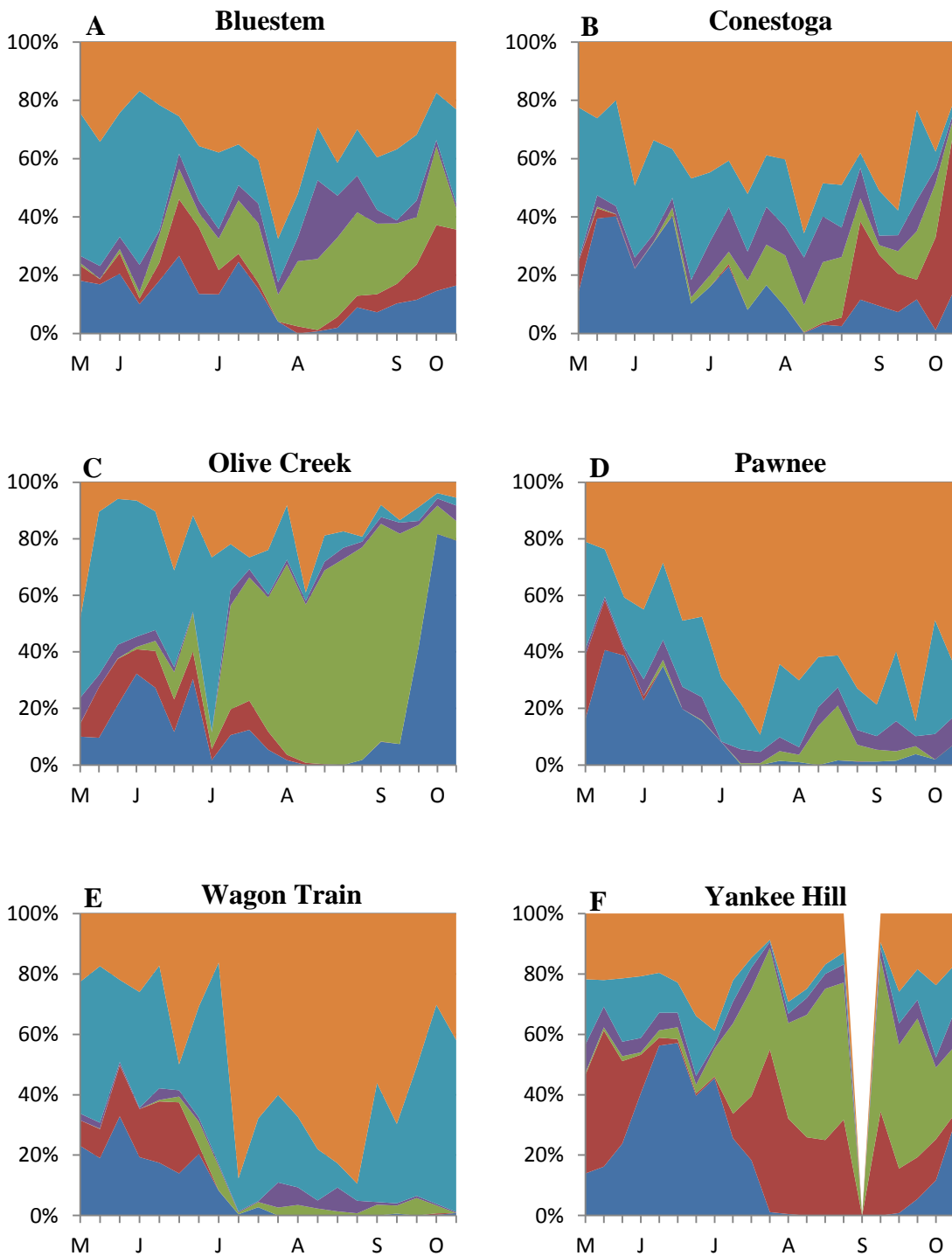


Figure 8A – 8F. Weekly relative percentages based on biomass estimates for specific groups of zooplankton over the 2011 field season for the six study sites.

■ Daphnia ■ Bosmina ■ Other clad ■ Cyclopoid ■ Calanoid ■ Nauplii

The relative percentage of cyanobacteria did not have a significant effect on overall cladoceran biomass (Table 3). Time did not have a significant effect on cladoceran biomass (Table 3). The interaction of time and cyanobacteria relative percentage also did not have a significant effect on cladoceran biomass (Table 3). Figure 9A shows predicted cladoceran biomass estimates for varying levels of cyanobacteria relative percentages. The graph shows that low percentages of cyanobacteria had low cladoceran biomass, while high percentages of cyanobacteria had higher cladoceran biomass. Although the p-value was not significant at the $\alpha = 0.05$ level, the p-value of 0.08 may reflect the pattern exhibited in figure 9A. Cladoceran biomass was not significantly affected by cyanobacteria biovolume (Table 4). Time did not have a significant effect on cladoceran biomass (Table 4). The interaction of time and cyanobacteria biovolume did not have a significant effect on cladoceran biomass (Table 4). Figure 9B (cyanobacteria biovolume) shows a similar pattern to figure 9A (cyanobacteria relative percentage) that used cyanobacteria relative percentage. Higher amounts of cyanobacteria, whether higher percentages or biovolumes, predicted a greater biomass of cladocerans in either models. The overall pattern was also similar between the two models, with increasing cladoceran biomass into June and July, followed by a decline, and then increasing again in September and October.

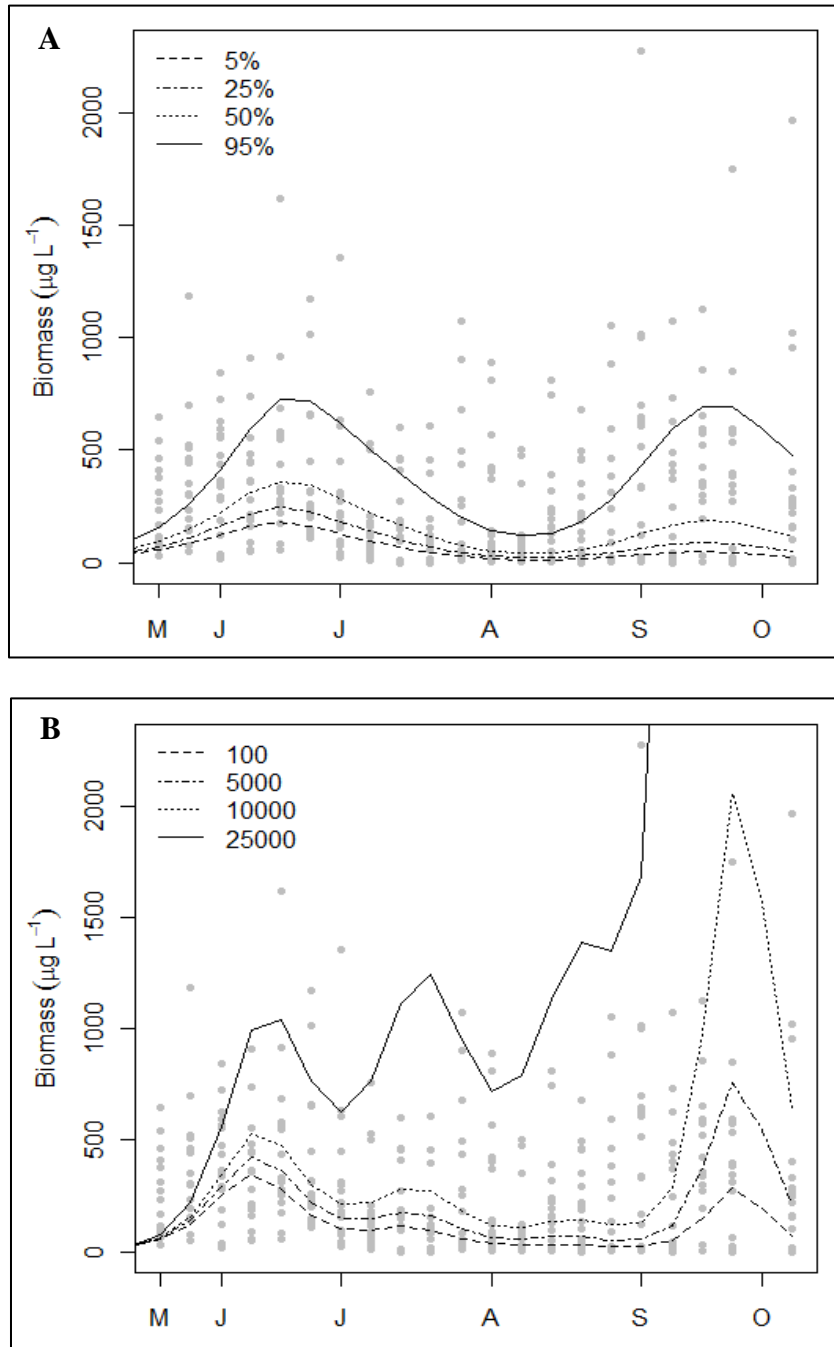


Figure 9. **A)** Cladoceran biomass over the 2011 growing season. Lines indicate predicted biomasses at different cyanobacteria percentages. Designated site for graph Olive Creek. **B)** Cladoceran biomass over the 2011 growing season. Designated site for graph Olive Creek. Lines indicate predicted biomasses at different cyanobacteria biovolumes. Observed values are demarked in gray dots.

Cyanobacteria relative percentages did not have a significant effect on *Daphnia* biomass (Table 3). Time had a significant positive effect on *Daphnia* biomass (Table 3). The interaction of time and cyanobacteria relative percentage had a significant positive effect on *Daphnia* biomass (Table 3). The differences among the predicted lines of *Daphnia* biomass are relatively small early in the summer, but in later summer the higher cyanobacteria percentages (95%) had higher biomass estimates than lower cyanobacteria percentages (Figure 10A). *Daphnia* biomass was not significantly affected by cyanobacteria biovolume (Table 4). A significant positive effect of time was seen in *Daphnia* biomass (Table 4). The interaction of time and cyanobacteria biovolume did not have a significant effect on *Daphnia* biomass (Table 4). Figure 10B depicts the GAM when using cyanobacteria biovolume and it had a strikingly similar pattern to that of figure 10A. At high cyanobacteria biovolumes there was greater *Daphnia* biomass predicted than at lower cyanobacteria biovolumes (Figure 10B), which was similar to higher cyanobacteria percentages that predicted greater *Daphnia* biomass (Figure 10A). Also the overall pattern in both GAMs shows increasing biomass in June, followed by a decline in biomass, and then an increase in biomass at relatively high cyanobacteria biovolumes and relative percentages near the end of the season.

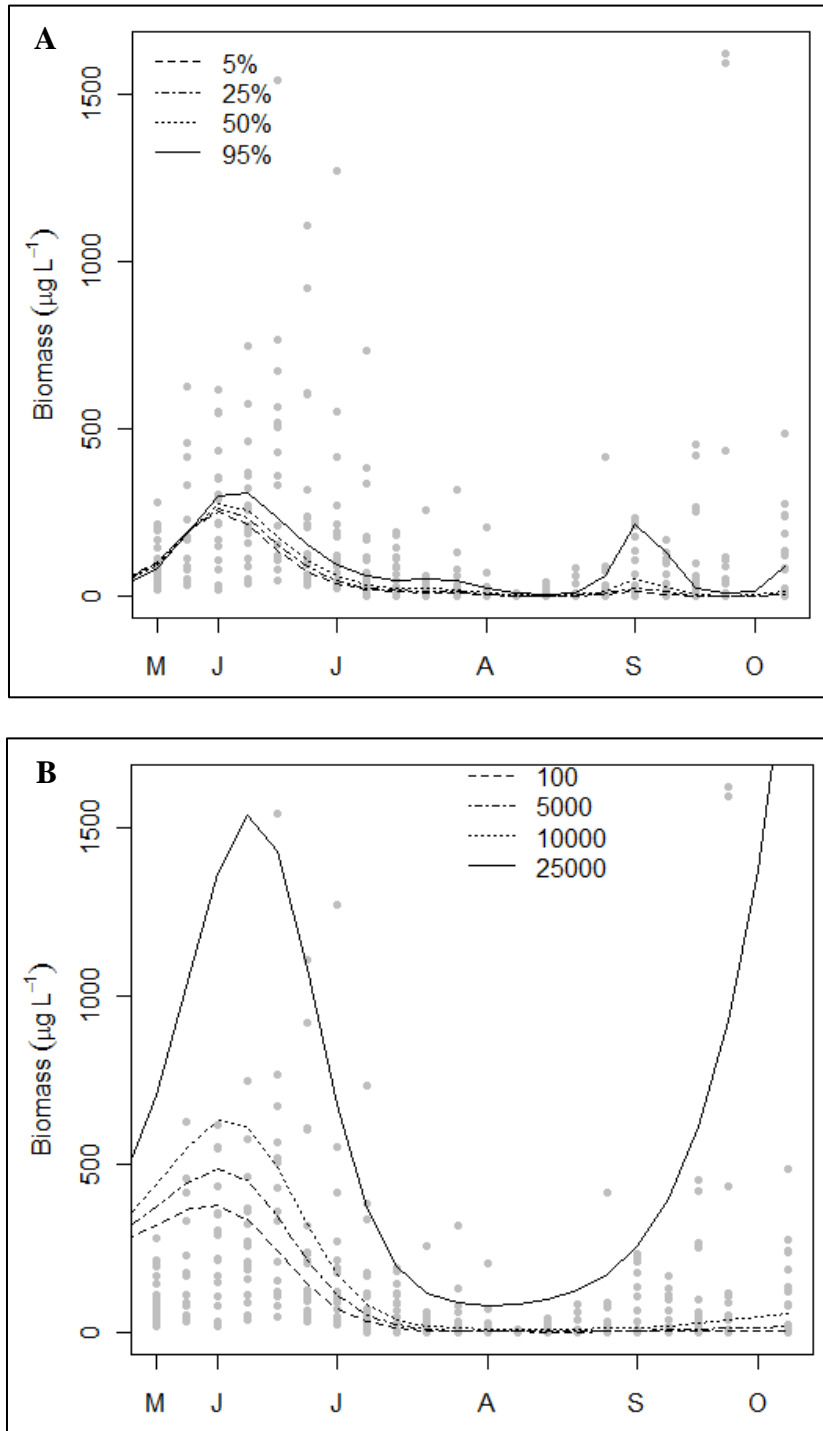


Figure 10. **A)** *Daphnia* biomass over the 2011 growing season. Lines indicate predicted biomasses at different cyanobacteria percentages. Designated site for graph Conestoga. **B)** *Daphnia* biomass over the 2011 growing season. Designated site for graph Wagon Train. Lines indicate predicted biomasses at different cyanobacteria biovolumes. Observed values are demarked in gray dots.

Cyanobacteria relative percent had a significant positive effect on *Bosmina* biomass (Table 3). Time did not have a significant effect on *Bosmina* biomass (Table 3). The interaction of time and cyanobacteria relative percentage had a significant negative effect on *Bosmina* biomass (Table 3). The greater *Bosmina* biomass observed at higher cyanobacteria percentages was much greater than at lower cyanobacteria percentages (Figure 11A). The negative interaction of time and cyanobacteria percentage can also be observed in figure 11A as the biomass declined later in the growing season. *Bosmina* biomass was not significantly affected by cyanobacteria biovolume (Table 4). Time did not have a significant effect on *Bosmina* biomass (Table 4). The interaction of time and cyanobacteria biovolume did not have a significant effect on *Bosmina* biomass (Table 4). Figure 11B shows that higher cyanobacteria biovolumes predicted higher *Bosmina* biomass early in the season and then biomass overall declining throughout the season. This pattern was similar to figure 11A, which looks at the effects of cyanobacteria relative percentage.

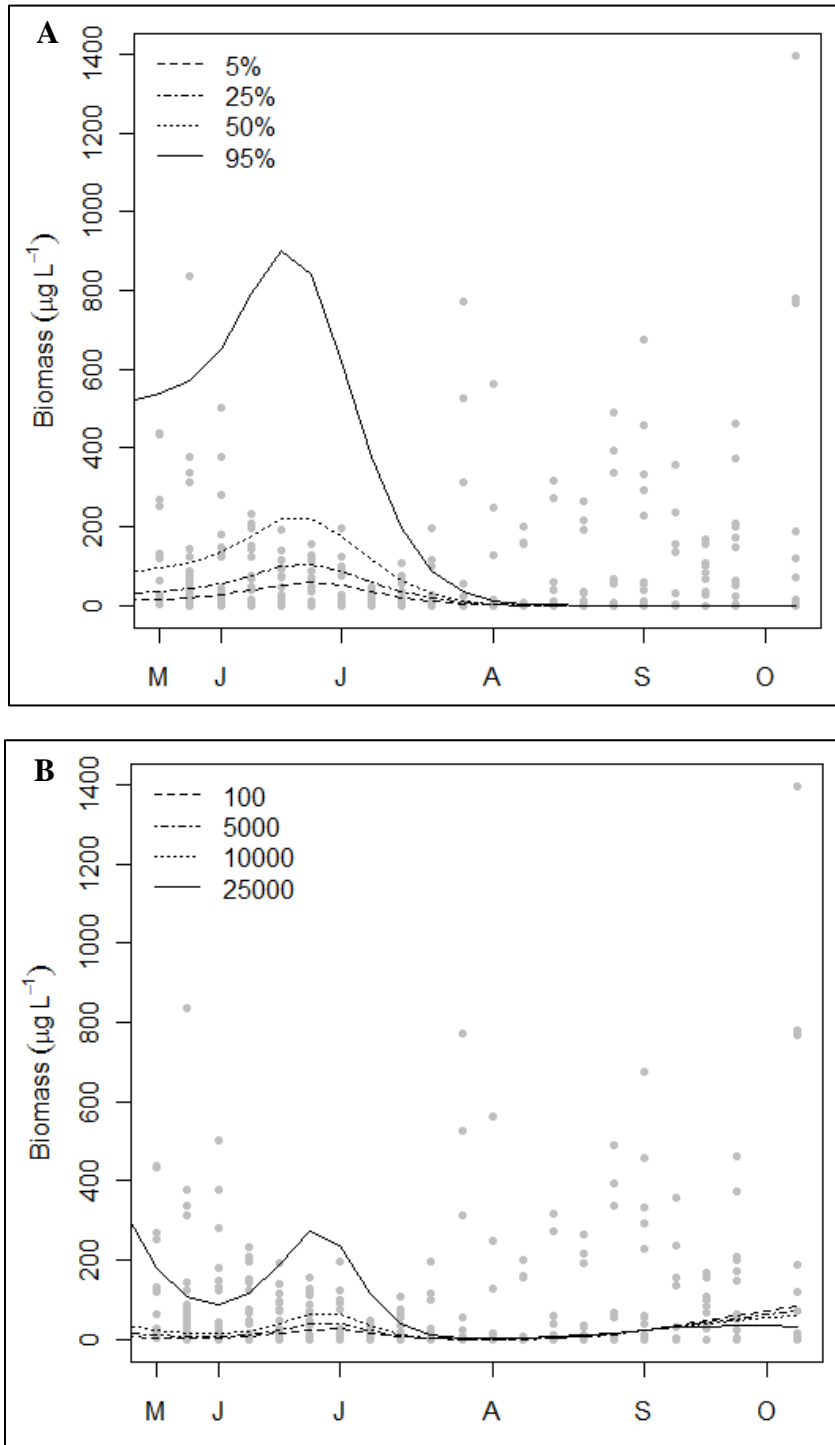


Figure 11. A) *Bosmina* biomass over the 2011 growing season. Lines indicate predicted biomasses at different cyanobacteria percentages. Designated site for graph Olive Creek. **B)** *Bosmina* biomass over the 2011 growing season. Designated site for graph Bluestem. Lines indicate predicted biomasses at different cyanobacteria biovolumes. Observed values are demarked in gray dots.

Cyanobacteria relative percentage did not have a significant effect on biomass of other cladocerans (*Ceriodaphnia*, *Diaphanosoma*, *Alonella*, *Leptodora kindtii*) (Table 3). Time also did not have a significant effect on other cladoceran biomass (Table 3). The interaction of time and cyanobacteria percentage did have a significant positive effect on other cladoceran biomass (Table 3). In figure 12A, there was no difference among the predicted biomass lines until later in the season where there was a positive increase in most levels. Higher levels of cyanobacteria percentage showed slightly greater biomass than lower cyanobacteria percentages. Other cladoceran biomass was not significantly affected by cyanobacteria biovolume (Table 4). Time did not have a significant effect on other cladoceran biomass (Table 4). The interaction of time and cyanobacteria biovolume had a significant positive effect on other cladoceran biomass (Table 4). In figure 12B, the positive interaction effect became clear when the other cladoceran biomass increased throughout the season at all of the different cyanobacteria biovolumes and also when the higher cyanobacteria biovolumes predicted higher biomass estimates of other cladocerans. The pattern exhibited over time (increasing biomass) and at the different degrees of cyanobacteria percentages and biovolumes (greater biomass at higher cyanobacteria percentages and biovolumes) were seen in both graphs (Figures 12A and 12B).

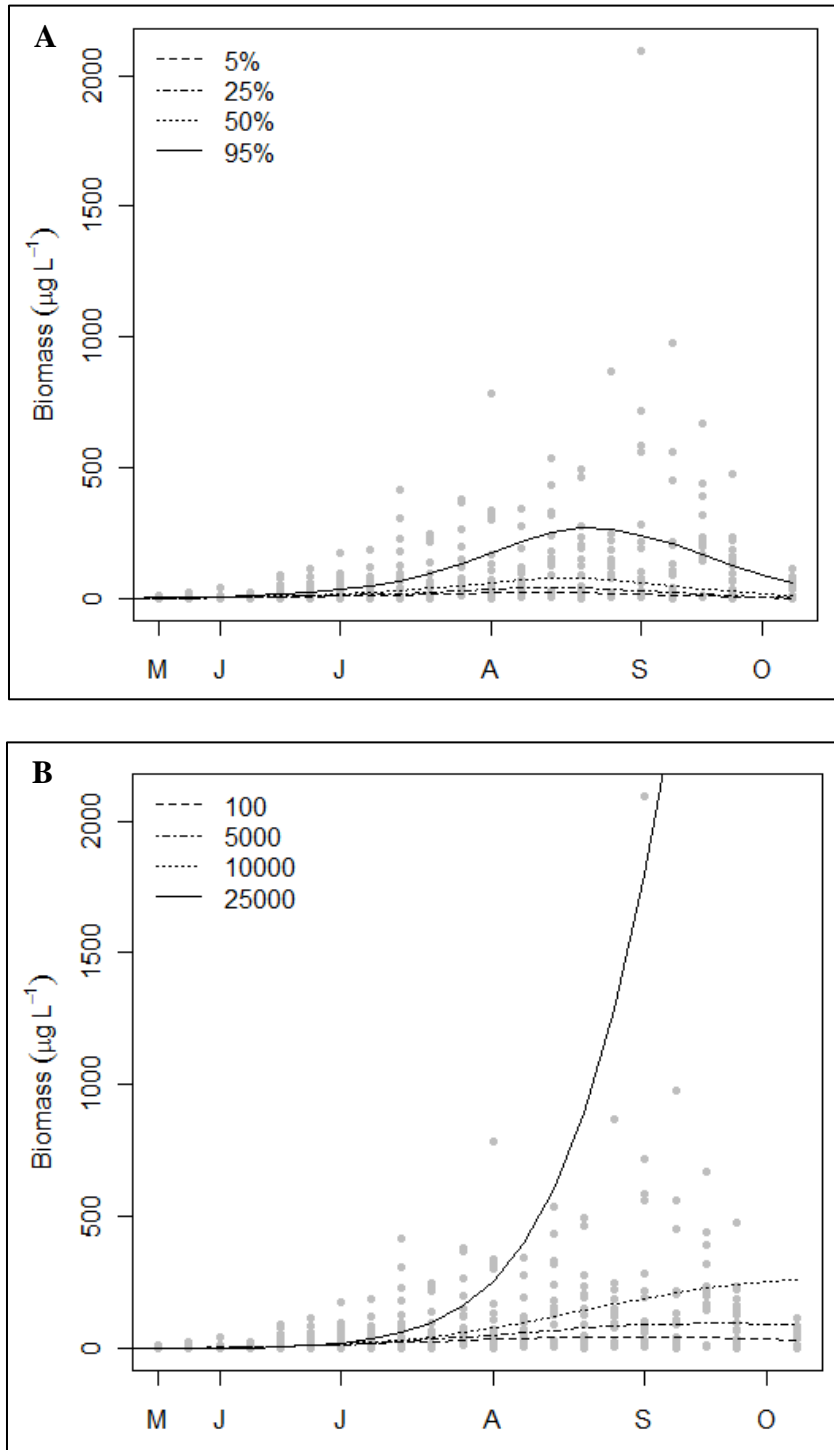


Figure 12. **A)** Other cladoceran biomass over the 2011 growing season. Lines indicate predicted biomasses at different cyanobacteria percentages. Designated site for graph Bluestem. **B)** Other cladoceran biomass over the 2011 growing season. Designated site for graph Bluestem. Observed values are demarked in gray dots.

Copepod results show that cyanobacteria relative percentage did not have a significant effect on overall copepod biomass (Table 5). Time did have a significant effect on copepod biomass (Table 5), whereas, the interaction of time and cyanobacteria relative percentage did not have a significant effect on copepod biomass (Table 5).

Figure 13A shows the predicted lines of copepod biomass for different levels of cyanobacteria relative percentages based on the model. There was minimal difference among the lines, indicating that the percentage of cyanobacteria is not significant.

Cyanobacteria biovolume had a significant positive effect on copepod biomass (Table 6). Time had a significant positive effect on copepod biomass (Table 6). The interaction of time and cyanobacteria biovolume had a significant negative effect on copepod biomass (Table 6). Early in the season the copepod biomass was predicted to be greater at higher cyanobacteria biovolumes than at lower cyanobacteria biovolumes (Figure 13B). The negative interaction effect of cyanobacteria and time became more apparent later in the season when biomass began to decrease and higher cyanobacteria biovolumes predicted lower copepods biomass (Figure 13B). The strong divergence among the cyanobacteria biovolume lines (Figure 13B) suggests that it has a strong effect on copepod biomass, more so than the relative percentage of cyanobacteria (Figure 13A).

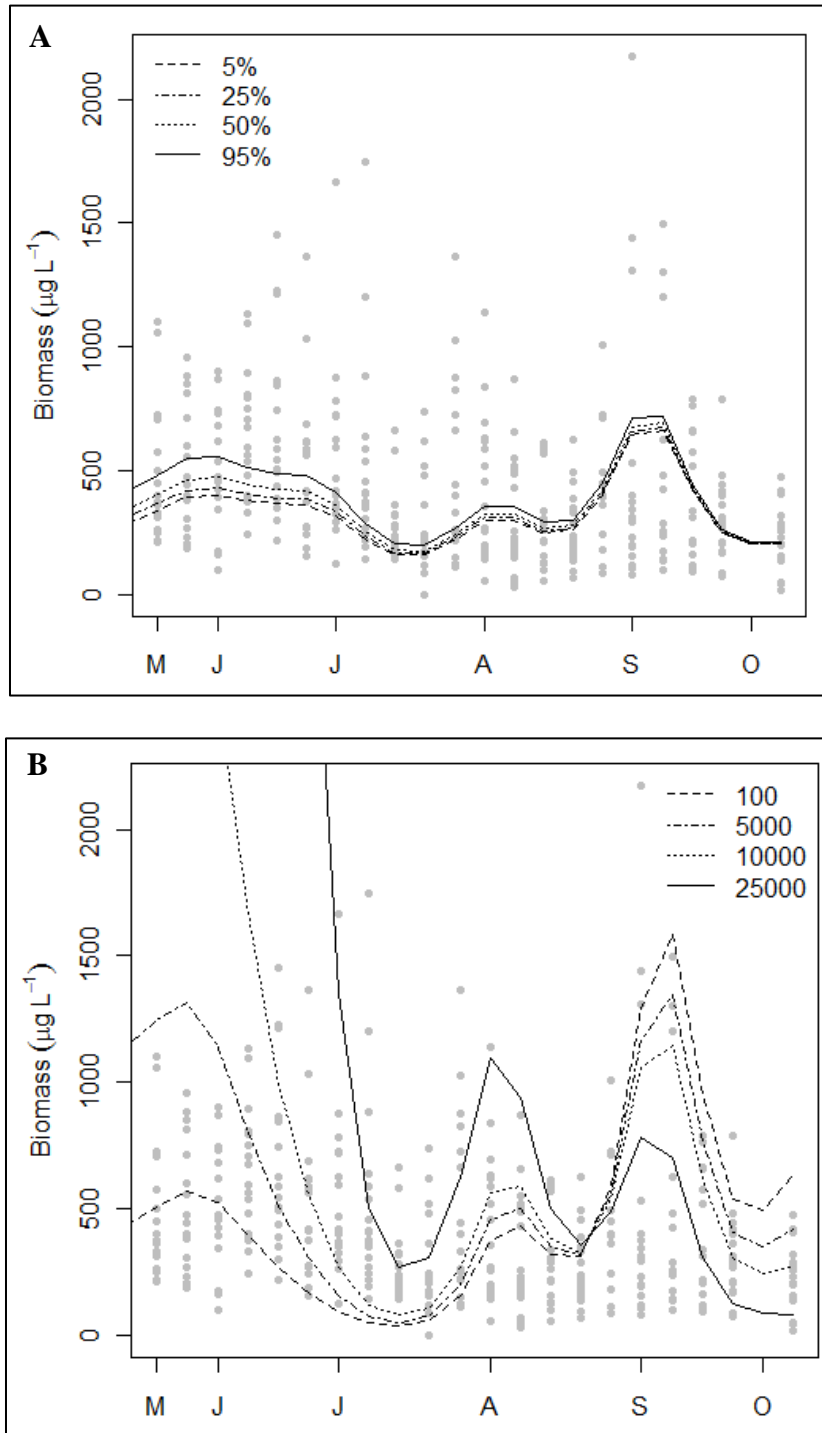


Figure 13. **A)** Copepod biomass over the 2011 growing season. Lines indicate predicted biomasses at different cyanobacteria percentages. Designated site for graph Conestoga. **B)** Copepod biomass over the 2011 growing season. Designated site for graph Conestoga. Lines indicate predicted biomasses at different cyanobacteria biovolumes. Observed values are demarked in gray dots.

Cyanobacteria relative percentage did not have a significant effect on cyclopoid copepod biomass (Table 5). Time did not have a significant effect on cyclopoid copepod biomass (Table 5). The interaction of time and cyanobacteria percentage did not have a significant effect on cyclopoid copepod biomass (Table 5). The non-significance of time and cyanobacteria relative percentage can be seen in Figure 14A. There was little difference among the predicted biomass estimates at different levels of cyanobacteria and little difference over time. Cyanobacteria biovolume had a significant positive effect on cyclopoid copepod biomass (Table 6). Time did not have a significant effect on cyclopoid copepod biomass (Table 6). The interaction of time and cyanobacteria biovolume did not have a significant effect on cyclopoid copepod biomass (Table 6). The significant effect of cyanobacteria biovolume can be seen in Figure 14B. The higher cyanobacteria biovolumes predicted greater cyclopoid copepod biomass than lower cyanobacteria biovolumes. Although cyanobacteria percent was not a significant effect, the pattern was similar to that of cyanobacteria biovolume (higher cyanobacteria percentages predicted greater cyclopoid copepod biomass).

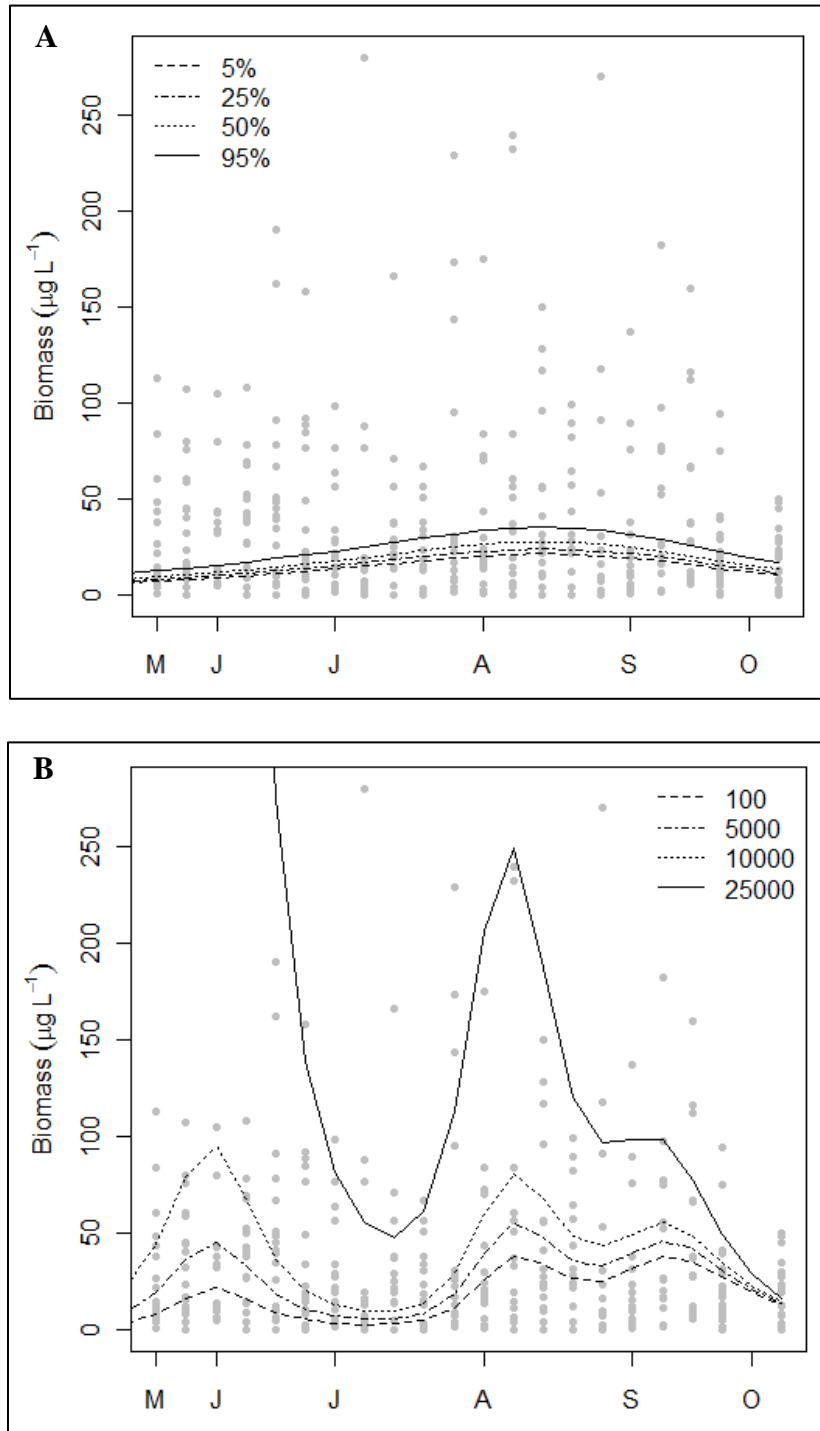


Figure 14. **A)** Cyclopid biomass over the 2011 growing season. Lines indicate predicted biomasses at different cyanobacteria percentages. Designated site for graph Conestoga. **B)** Cyclopid biomass over the 2011 growing season. Designated site for graph Conestoga. Lines indicate predicted biomasses at different cyanobacteria biovolumes. Observed values are demarked in gray dots.

Cyanobacteria relative percentage was not a significant effect on calanoid copepod biomass (Table 5). Time did have a significant positive effect on calanoid copepod biomass (Table 5). The interaction of time and cyanobacteria relative percentage was not significant (Table 5). In Figure 15A, there is minimal difference among the predicted lines of calanoid copepod biomass for different percentages of cyanobacteria confirming that it is not a significant effect. There was a distinct difference in predicted biomass over time. Cyanobacteria biovolume did not have a significant effect on calanoid copepod biomass (Table 6). Time did not have a significant effect on calanoid copepod biomass (Table 6). The interaction of time and cyanobacteria biovolume did not have a significant effect on calanoid biomass (Table 6). Cyanobacteria biovolume had a strong effect on calanoid copepod biomass due to the larger divergence among the predicted lines (Figure 15B). The pattern between the two GAMs was similar, higher cyanobacteria percentages/biovolumes predicted higher calanoid biomass early in the season, followed by an overall decline in biomass, and then an increase in calanoid biomass later in the season with lower cyanobacteria percentages/biovolumes that predicted greater biomass of calanoid copepods (Figures 15A and 15B).

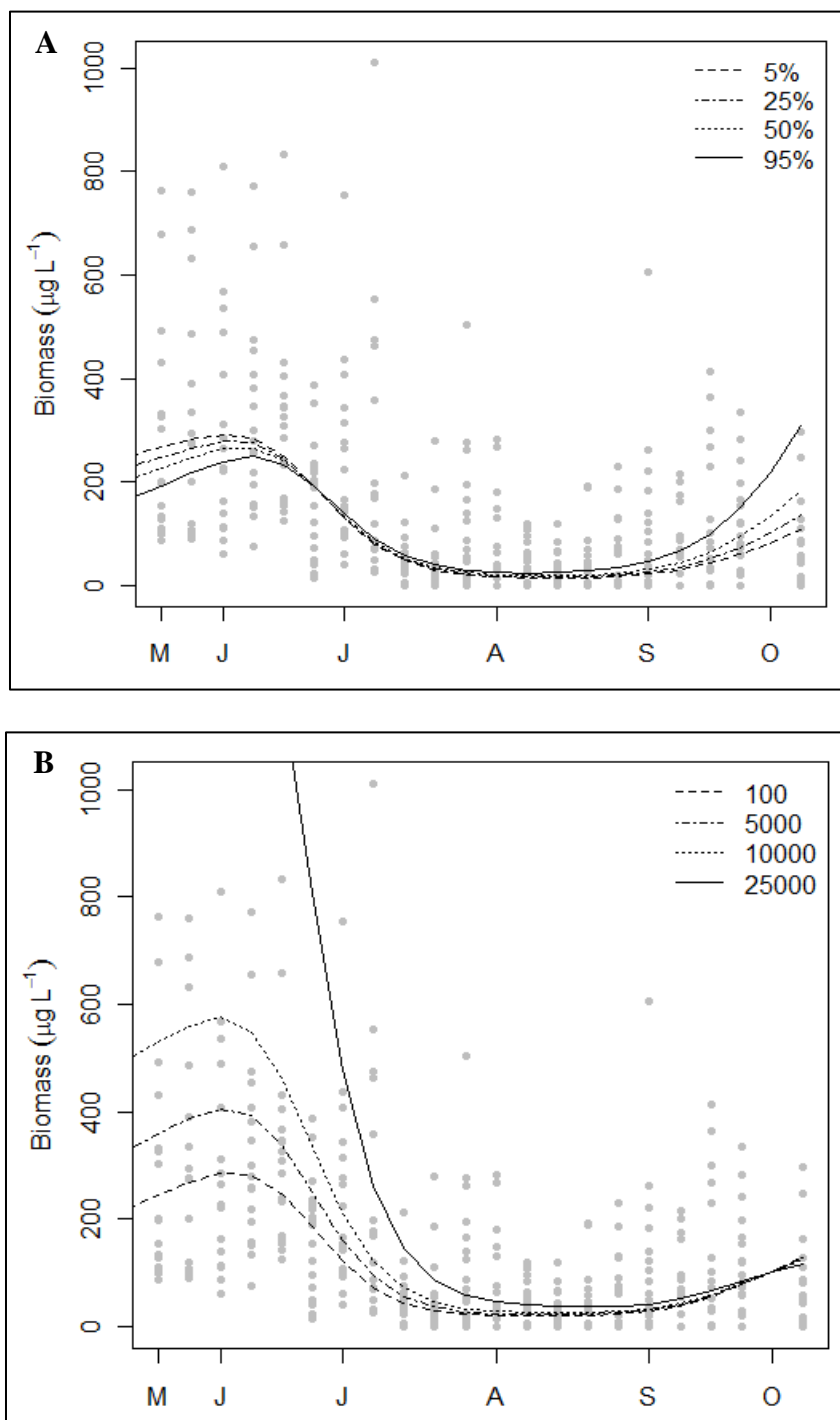


Figure 15. **A)** Calanoid biomass over the 2011 growing season. Lines indicate predicted biomasses at different cyanobacteria percentages. Designated site for graph Yankee Hill. **B)** Calanoid biomass over the 2011 growing season. Designated site for graph Yankee Hill. Lines indicate predicted biomasses at different cyanobacteria biovolumes. Observed values are demarked in gray dots.

Cyanobacteria relative percentage had a significant positive effect on nauplii biomass (Table 5). Time had a significant positive effect on nauplii biomass (Table 5). The interaction of time and cyanobacteria relative percentage had a significant negative effect on nauplii biomass (Table 5). In Figure 16A, the effect of the time and cyanobacteria percentage interaction is evident. Increasing overall biomass was seen and early in the season the higher cyanobacteria percentages had greater predicted nauplii biomass and low cyanobacteria percentages had low predicted biomass, but later in the season the situation was reversed and there was a decreasing trend in biomass overall. Cyanobacteria biovolume had a significant positive effect on nauplii biomass (Table 6). Time had a significant positive effect on nauplii biomass (Table 6). The interaction of time and cyanobacteria biovolume had a significant negative effect on nauplii biomass (Table 6). The significant positive effect of cyanobacteria biovolume was evident early in the season when higher cyanobacteria biovolumes predicted greater nauplii biomass (Figure 16B). The interaction of time and cyanobacteria biovolume also became apparent later in the season when overall nauplii biomass decreased over time and the lower cyanobacteria biovolumes predicted greater nauplii biomass (Figure 16B). The pattern seen in both figures (16A and 16B) was similar to each other over the season, but cyanobacteria biovolume had a stronger effect on nauplii biomass due to the larger divergence among the predicted lines than cyanobacteria relative percentage.

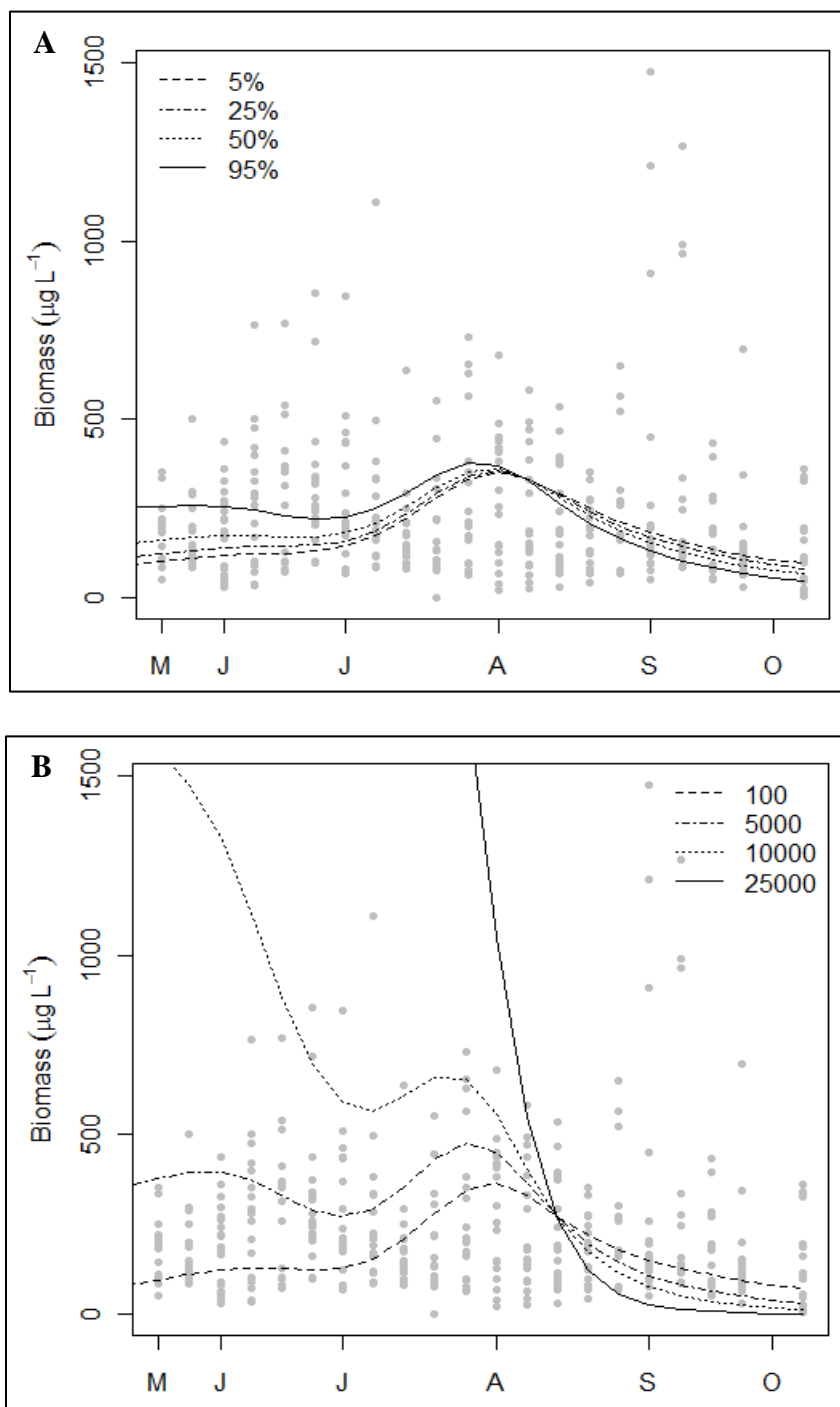


Figure 16. A) Copepod nauplii biomass over the 2011 growing season. Lines indicate predicted biomasses at different cyanobacteria percentages. Designated site for graph Wagon Train. B) Copepod nauplii biomass over the 2011 growing season. Designated site for graph Wagon Train. Lines indicate predicted biomasses at different cyanobacteria biovolumes. Observed values are demarked in gray dots.

Cyanobacteria relative percentage did not have a significant effect on rotifer biomass (Table 7). Time did not have a significant effect on rotifer biomass (Table 7). The interaction of time and cyanobacteria relative percentage also did not have significant effects on rotifer biomass (Table 7). Figure 17A shows the predicted lines of rotifer biomass at different levels of cyanobacteria relative percentages. There was very little difference among the lines indicating that cyanobacteria did not have a significant effect on rotifer biomass. Cyanobacteria biovolume did not have a significant effect on rotifer biomass (Table 8). Time did not have a significant effect on rotifer biomass (Table 8). The interaction of time and cyanobacteria biovolume did not have a significant effect on rotifer biomass (Table 8). The non-significant effect of cyanobacteria biovolume was evident in figure 17B as there was little difference among the predicted lines. The pattern seen in figures 17A and 17B are nearly identical to each other, suggesting that regardless of the factor being cyanobacteria relative percentage or biovolume, cyanobacteria did not affect rotifer biomass.

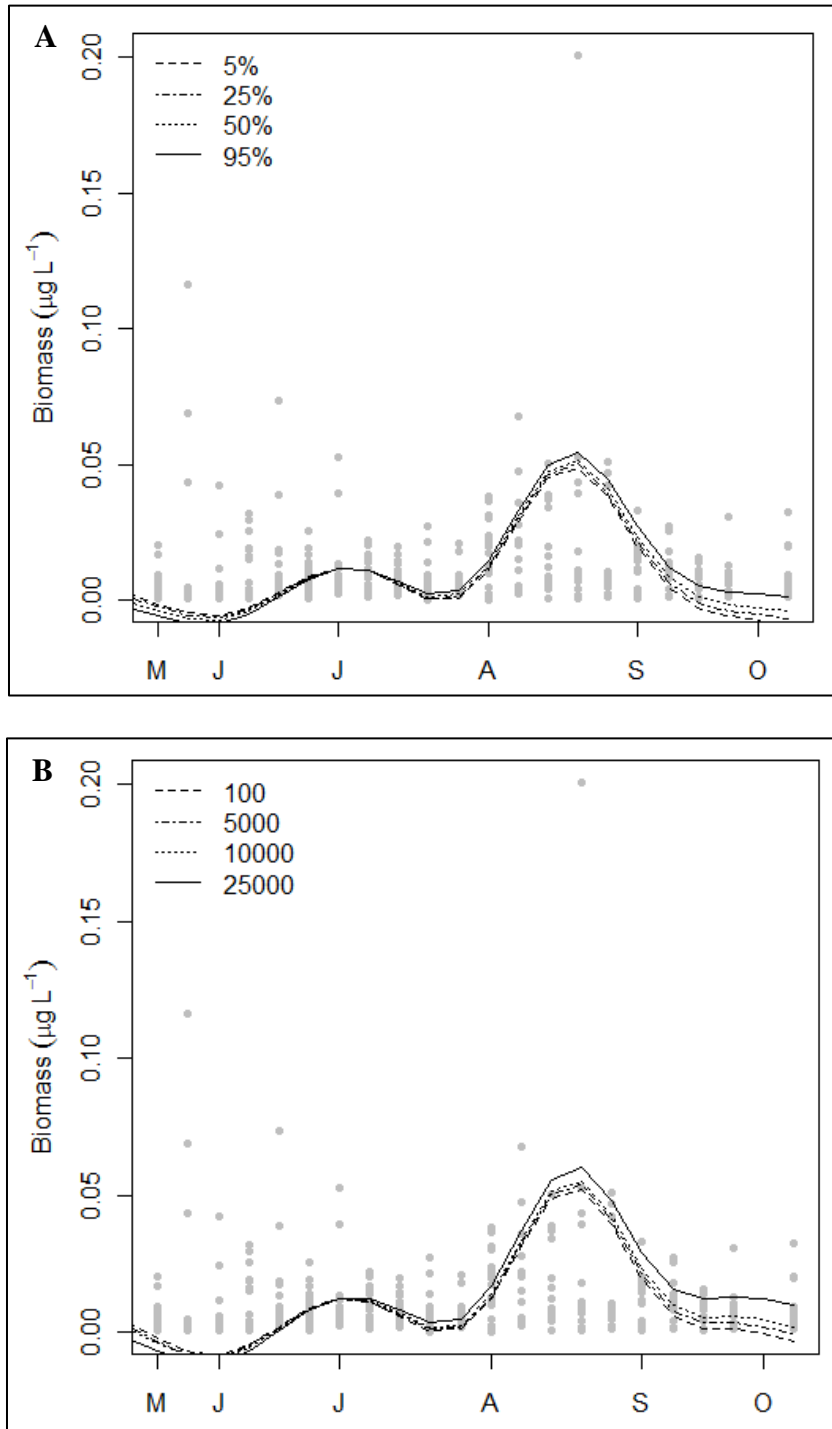


Figure 17. **A)** Rotifer biomass over the 2011 growing season. Lines indicate predicted biomasses at different cyanobacteria percentages. Designated site for graph Olive Creek. **B)** Rotifer biomass over the 2011 growing season. Designated site for graph Olive Creek. Lines indicate predicted biomasses at different cyanobacteria biovolumes. Observed values are demarked in gray dots.

3.3 Physical and chemical parameter correlations

Cyanobacteria biovolume was significantly correlated with total nitrogen (TN), total phosphorus (TP), TN:TP ratio, epilimnion temperature, turbidity, LEC, extracted chlorophyll α , nitrate, and SRP (Table 9). Total nitrogen ($r = 0.45$), total phosphorus ($r = 0.38$), extracted chlorophyll α ($r = 0.38$), and epilimnion temperature ($r = 0.37$) were most highly correlated with cyanobacteria biovolume.

Table 9. Spearman rank correlations (r_s) between cyanobacteria biovolume and physical and chemical parameters.

Variable	Cyanobacteria biovolume		
	<i>n</i>	<i>r_s</i>	<i>p</i>
TN	321	0.45	<0.001
TP	335	0.38	<0.001
TN:TP	310	-0.17	0.0027
Secchi Depth	365	-0.067	0.2
Epilim. Temp	364	0.37	<0.001
Turbidity	365	0.2	<0.001
LEC	261	0.11	0.07
Extracted Chl α	361	0.38	<0.001
Nitrate	125	-0.05	0.569
SRP	195	-0.18	0.0103

TN:TP ratio was negatively correlated with cyanobacteria biovolume and a majority of the cyanobacteria biovolume did occur at TN:TP ratios under 29:1 (Figure 18). The ratio of TN:TP has been established in the literature since Smith (1983), stating that cyanobacteria are more prevalent at TN:TP ratios <29:1 (Figure 19).

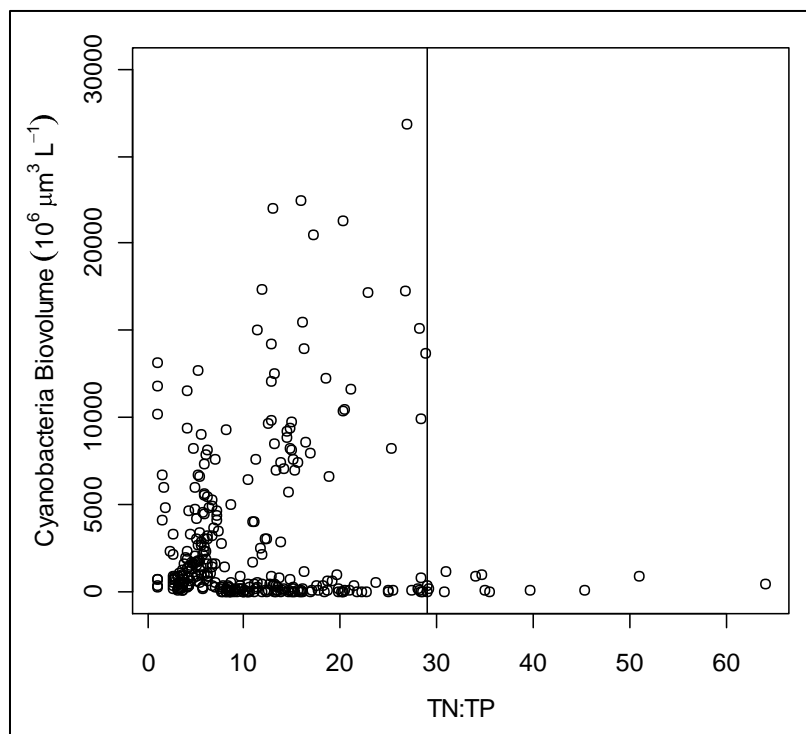


Figure 18. Cyanobacteria biovolume as a function of the ratio of total nitrogen to total phosphorus (TN:TP), vertical line represents a ratio of 29:1 for the six Nebraska study sites.

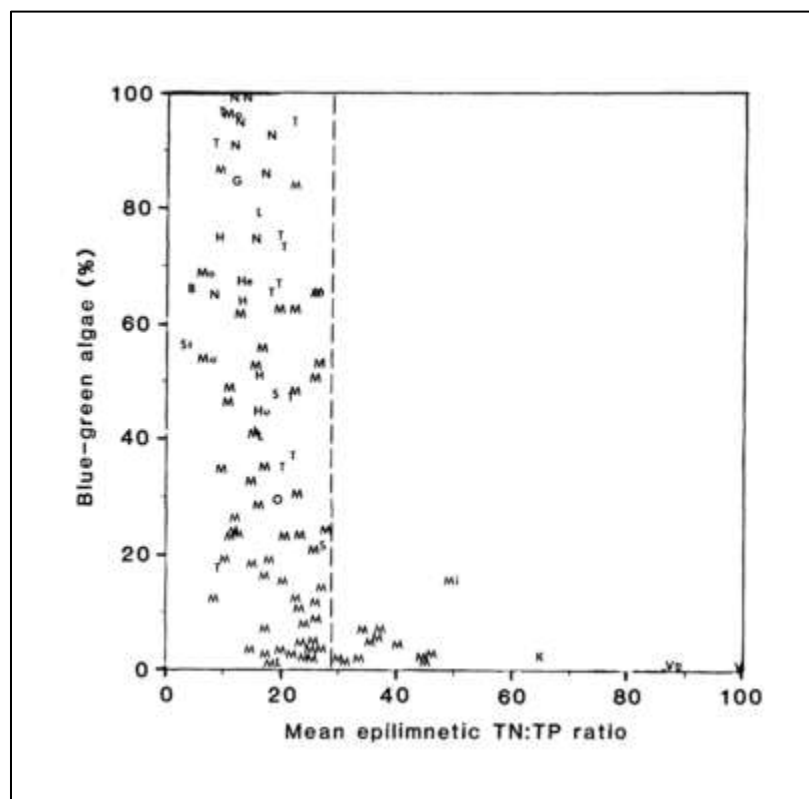


Figure 19. Smith (1983) graph analyzing 17 lakes worldwide to express cyanobacteria relative percentage with TN:TP ratio.

3.4 Microcystin and *in vivo* phycocyanin correlations

Microcystin was significantly correlated ($r = 0.33$) with cyanobacteria biovolume (Table 10). *In vivo* phycocyanin was highly correlated ($r = 0.64$) with cyanobacteria biovolume (Table 11).

Table 10. Spearman rank correlations (r_s) between Microcystin toxin and cyanobacteria biovolume.

Variable	<i>Microcystin toxin</i>		
	<i>n</i>	<i>r_s</i>	<i>p</i>
Cyanobacteria biovolume	227	0.33	<0.001

Table 11. Spearman rank correlations (r_s) between *in vivo* phycocyanin and cyanobacteria biovolume

Variable	<i>In vivo</i> phycocyanin		
	<i>n</i>	<i>r_s</i>	<i>p</i>
Cyanobacteria biovolume	365	0.64	<0.001

4.0 Discussion

4.1 Phytoplankton

Chlorophytes, dinoflagellates, and euglenophytes were not significantly affected by cyanobacteria relative percentage. This suggests that these groups are less influenced by cyanobacteria at this time of year than they are by other factors (e.g. seasonal succession, grazing, chemical and physical factors). In general, the biovolumes of chlorophytes and dinoflagellates were small and varied only slightly throughout the season. Rather, cyanobacteria and diatoms made up a majority of the total phytoplankton biovolume at any given time during the season. The pattern exhibited by the reservoirs in Nebraska is similar to a pattern seen by Sondergaard et al. (1990) in a Danish lake. Their study also revealed relatively low levels of taxa other than diatoms and cyanobacteria; and of those two major groups, greater densities of bacillariophytes occurred at times when cyanobacteria were lower, and vice versa.

Chlorophytes were significantly affected by time, which suggests that this group may be going through natural succession during the course of the year/season. Dinoflagellates were not significantly affected by time or cyanobacteria percentage. This may be due to several reasons, such as timing during the season (may be greater during other parts of the year not sampled), chemical and physical parameters, or they were never established in these reservoirs. Euglenoids were positively affected by time, which

suggests seasonal succession is occurring in this algal group. The interaction of time and cyanobacteria percentage had a negative effect on euglenoids. Although euglenoid biovolume increased through the season, at higher cyanobacteria percentages they were more negatively affected with overall less biovolume than at lower cyanobacteria percentages. Diatoms were significantly affected by time, which suggested that there was a seasonal pattern exhibited by this group during the summer with an overall pattern of increasing diatom biovolume. Cyanobacteria did have a significant negative effect on diatoms. This was expressed as lower cyanobacteria percentages correlating with higher diatom biovolumes. In general, only certain algal groups appeared to be significantly affected by cyanobacteria, such as diatoms and euglenoids. Other groups, such as chlorophytes and dinoflagellates appear to not be significantly affected by cyanobacteria.

Depending upon the cyanobacteria predictor used (relative percentage or biovolume), the phytoplankton response variable yielded different results. The divergences among the predicted lines on both sets of GAM look fairly similar to each other, such as the figures for chlorophytes and dinoflagellates. The divergence among the lines was greater for diatom biovolume when using cyanobacteria biovolume as a predictor (due to a positive significance from cyanobacteria biovolume), but then converged later in the season (due to a negative interaction effect of time and cyanobacteria biovolume). The prediction lines for euglenoid biovolume when using cyanobacteria biovolume were much closer together than cyanobacteria relative percentage. When using the relative percentage of cyanobacteria as a predictor, even if cyanobacteria were not a significant factor, the lower percentages predicted higher

biovolumes for diatoms, chlorophytes, and dinoflagellates. The opposite pattern for those three groups was observed when cyanobacteria biovolume was used as a predictor, meaning the higher cyanobacteria biovolumes predicted high biovolumes for diatoms, chlorophytes, and dinoflagellates. Even though similar patterns in predicted values were observed, differences in the degree of divergence, based on higher or lower cyanobacteria percentage or biovolume, suggest that depending upon the predictor variable very different results can be obtained using the generalized additive model.

Models were also used to determine the effects of cyanobacteria relative percentage and biovolume on the density of the respective phytoplankton groups. Diatom and euglenoid responses to cyanobacteria had no difference in the GAM output when using density or biovolume as the response variable. When using chlorophyte density as the response variable, there was a significant negative effect on density when using cyanobacteria percentage as an explanatory variable. There were no differences in chlorophyte response, between density or biovolume, when using cyanobacteria biovolume as a response variable. Dinoflagellate response to cyanobacteria did change slightly when using density instead of biovolume as a response variable. Even though the GAM output may have varied slightly and some significant effects were noted when using density in place of biovolume as a response variable, the graphical output and overall patterns were nearly identical. This suggests that, regardless of using density or abundance estimates that the phytoplankton groups react in a similar manner to cyanobacteria.

The 2011 growing season was characterized as an overly “wet” year due to heavy rainfall and flooding throughout the Midwest, including parts of Nebraska, Iowa, southern Minnesota, and the Dakotas. It has been noted that favorable conditions for cyanobacteria generally occur when days are hot, dry, and calm (Bouvy et al. 1999, Havens 2008). These conditions would lead one to expect that due to a particularly wet year that cyanobacteria should not become dominant in Nebraska reservoirs. Reichwaldt and Ghadouani (2012) reviewed the effects of rainfall on cyanobacteria and found that rainfalls following long dry periods often promoted cyanobacteria due to high pulses of nutrients to the waterbody. They also found that, while rainfalls of high intensity can break up and flush cyanobacteria blooms from a system in the short-term, in the long-term cyanobacteria will become dominant again when the water column is no longer mixing. This helps to explain why cyanobacteria dominated during a summer when it seemed likely that conditions were less than favorable for cyanobacteria growth.

A Kruskal-Wallis test (XLSTAT) was performed to compare the cyanobacteria densities from beach samples and open water samples. There were no significant differences in cyanobacteria densities between open water and beach sites ($H = 1.371$, 1 d.f., $p = 0.242$). This suggests that regardless of where a person is recreating in the lake, they will always be in constant contact with cyanobacteria during a bloom event.

4.2 Zooplankton

Cladoceran biomass was not significantly affected by cyanobacteria, but this may not be an accurate representation on how more specific cladoceran taxa (e.g. *Daphnia*, *Bosmina*, etc) were affected by cyanobacteria, because different taxa tend to be affected

differently (Arnold 1971, Fulton and Paerl 1988, Lampert 1987). Several researchers have found that cyanobacteria morphology was a contributing factor to their effect on zooplankton (Fulton and Paerl 1987, Tillmanns et al. 2008), for example, zooplankton were found to shorten filament length and even ingest filamentous cyanobacteria (Epp 1996, Tillmanns et al. 2008, Work and Havens 2003). The majority of cyanobacteria found in this study were filamentous (except in the late season in Yankee Hill reservoir), which could limit the influence of cyanobacteria on cladocerans.

Cyanobacteria did not have a significant effect on *Daphnia* biomass. Alternative food sources may have allowed for the lack of cyanobacteria inhibition, because although cyanobacteria may have made up a majority of total phytoplankton biovolume there may have been enough alternative food sources (diatoms, chlorophytes, and euglenoids) to sustain daphnids (Lampert 1987). Bednarska and Dawidowicz (2007) suggested that certain daphnid species subjected to high densities of cyanobacteria in the past may adapt to subsequent high densities of cyanobacteria and become less hindered by these situations, which could help explain the non-significant effect of cyanobacteria on daphnids in the present study. Demott et al. (2001) found that the smaller daphnid, *D. cucullata*, did better in experiments with cyanobacteria than larger daphnids, such as *D. galeata* and *D. magna*. The daphnid species that dominated Nebraska reservoirs in this study were *D. ambigua*, which are similar in size to *D. cucullata*. This may account for the significant interaction effect of time and cyanobacteria percentages on the daphnid group seen in the Nebraska reservoirs, and why cyanobacteria alone did not have a significant effect.

Bosmina biomass was significantly affected by cyanobacteria relative percentage. Cyanobacteria percentage had a positive effect on *Bosmina*, suggesting that this genus may tolerate cyanobacteria better than other cladocerans. Burns et al. (1989) found that *B. meridionalis* was able to survive and reproduce during *Anabaena* blooms in Lake Rotongaio, which supports the present findings.

The other cladoceran group (*Ceriodaphnia*, *Diaphanosoma*, *Alonella*) was significantly affected by the interaction of time and cyanobacteria percentage. Lampert (1982) found that smaller species such as, *Ceriodaphnia* and *Bosmina*, were less affected by cyanobacteria than other larger cladocerans. Fulton and Paerl (1987) found that *Diaphanosoma* clearance rates were affected very little by colonial *Microcystis*. These observations support our findings that cyanobacteria alone did not have a significant effect on other cladoceran groups and may be the reason why biomass increased later in the season.

Copepods as a group were not significantly affected by cyanobacteria. When divided into finer taxonomic grouping (cyclopoid, calanoid, and nauplii), cyclopoid and calanoid copepods were not significantly affected by cyanobacteria. This is consistent with the findings of other researchers (DeMott and Moxter 1991, Fulton and Paerl 1988, Kirk and Gilbert 1992, Lampert 1987). Nauplii had a significant positive response to cyanobacteria. DeMott (1986) found that cyclopoid nauplii were the most selective feeders, and in a feeding experiment, chose flavored spheres over cyanobacteria. This suggests that nauplii could be better at finding and choosing alternative food sources when

cyanobacteria dominate a system, and could explain why they exhibited a positive relationship with cyanobacteria in this study.

Cyanobacteria did not significantly affect rotifer biomass. Bouvy et al. (2001) observed increasing amounts of rotifers with cyanobacteria increases and subsequent decreases when cyanobacteria declined. In another case, Tillmanns et al. (2008) found rotifers had positive growth in the presence of cyanobacteria, albeit the rate at which growth occurred was lower. Fulton and Paerl (1987) found that clearance rates for rotifers were affected little when fed high densities of *Microcystis*. These observations could explain in part why rotifer biomass was not greatly affected by cyanobacteria in the present study.

Very similar results were obtained with respective zooplankton groupings, whether using cyanobacteria biovolume or relative percentage as a predictor in the GAM. In general, when there was a positive effect of cyanobacteria relative percentage on a zooplankton group, there was a positive effect of cyanobacteria biovolume. Regardless of the cyanobacteria predictor, nearly all zooplankton groups (exception of calanoid copepods) had greater predicted biomass estimates when cyanobacteria biovolumes or percentages were high. These results show that zooplankton biomass predicted from generalized additive modeling is not affected drastically by the cyanobacteria predictor, whether it is biovolume or relative percentage.

Models were also used to determine the effects of cyanobacteria relative percentage and biovolume on the density of the respective zooplankton groups. In most cases, the GAM output did not differ in the effects of the explanatory variable

(cyanobacteria relative percentage or biovolume) on the response variable (density or biomass of zooplankton groups). The graphical output for the different zooplankton groups also had very similar patterns when using density or biomass as a response variable. The response that rotifers had to cyanobacteria did differ depending on whether density or biomass was used as a response variable. For example, cyanobacteria biovolume had a significant positive effect on rotifer density, but did not have a significant effect when using rotifer biomass. This may be because rotifer biomass was extremely low, fluctuated within a narrow range, and was close to zero for most estimates. The density estimates were high, fluctuated within a larger range, and contained few zero counts. The fact that there were little to no zeroes in terms of rotifer density may help to explain why there was a significant effect of cyanobacteria on density of rotifers and not biomass.

4.3 Physical and chemical parameters

Significant positive correlations were made between cyanobacteria biovolume and total nitrogen and total phosphorus. This is consistent with the findings of several other studies (Downing et al. 2001, Graham et al. 2004, Jacoby et al. 2000). The TN:TP ratio at which cyanobacteria dominate found in this study is nearly identical to the results founded by Smith (1983). This result of TN:TP is also comparable to a study done only on natural lake systems (reservoirs excluded) by Downing et al. (2001), suggesting that in this regard, reservoirs may function similarly to natural systems. Vanni et al. (2011) found that reservoirs predominately surrounded by agriculture had the highest cyanobacteria filament densities compared to forested and mixed areas. The TN:TP ratio

was highest in their agriculture reservoir, but it was also the most variable. When the TN:TP ratio increased in late summer, the remaining phytoplankton biomass increased, which is similar to findings by Smith (1983). Bovo-Scomparin & Train (2008) found cyanobacteria to be negatively correlated with TN:TP and SRP in a lake in South America, which is also consistent with the results from this study.

Cyanobacteria biovolume was negatively correlated with Secchi disk depth, but was not significant; the fact that it is negative and appeared to have an exponential decline, is still consistent with other research observations (Graham et al. 2001, Jacoby et al. 2000, Jensen et al. 1994). Temperature was significantly and positively correlated with cyanobacteria, which is consistent with conditions favorable for cyanobacteria (Murrell & Lores 2004, Paerl 1988). The light extinction coefficient (LEC) was not significantly correlated with cyanobacteria biovolume. The higher light extinction coefficients mean that less light travels through the water column meaning shadier conditions, which is what cyanobacteria prefer. Chlorophyll α was significantly positively correlated with cyanobacteria (Downing et al. 2001, Graham et al. 2001). Since cyanobacteria do possess chlorophyll, high levels of cyanobacteria often coincide with high levels of chlorophyll α (Brakhage 2004, Downing et al. 2001, Murrell & Lores 2004, Scheffer et al. 1997).

4.4 Microcystin and *in vivo* phycocyanin

Microcystin, a toxin produced by cyanobacteria, was positively correlated with cyanobacteria biovolume. This correlation, while significant, was not robust ($r_s = 0.33$), but it is comparable to the results attained by Graham et al. (2001) who reported a $r_s =$

0.32 in their study of Midwest surface waters. Microcystin may have been highly correlated with cyanobacteria biovolume, but Amè et al. (2003) found in their study on a reservoir in Argentina that cyanobacteria abundance may not necessarily mean high levels of cyanotoxins. Similarly, Carmichael (2001) found that cyanobacteria blooms that did contain toxin producing cyanobacteria were not toxic all the time.

Many reservoirs in Nebraska are recreation sites and possess beaches for swimming. NDEQ collects beach water samples for microcystin-LR; when toxin levels are considered harmful, the beach is shut down for a period of time. In many cases, these waters are also used for jet skiing, water skiing, and water tubing. People recreating in the open water are at risk for prolonged contact with cyanobacteria toxins.

A Kruskal-Wallis (XLSTAT) was performed to compare microcystin levels between open water and beach sites. No significant differences were found in the toxin levels between open water and beach sites ($H = 0.606$, 1 d.f., $p = 0.436$), suggesting that when beaches are closed due to high levels of microcystin in the beach waters, that open water recreation/sports, such as water skiing or jet skiing, should also be limited and caution should be used by the public.

In vivo phycocyanin is a pigment only produced by cyanobacteria. The high correlation ($r_s = 0.64$) confirms that this is a satisfactory method to estimate cyanobacteria. Remote sensing of case 2 waters (turbid inland waters) has been under investigation for some time to link chlorophyll *a* and phycocyanin pigments, to biomass and concentration quantities. Gitelson et al. (1995) used outdoor ponds and a platform radiometer to successfully estimate the biomass and concentration of *Spirulina*.

Estimation of phycocyanin concentration/biomass via remote sensing has been developed from the use of satellites, such as MERIS, to be used in detection of harmful algal blooms (Hunter et al. 2010, Simis et al. 2005). One of the major drawbacks of using satellite data is the duration of time it takes for repeat measures to be made on an area because cyanobacteria can change in a matter of days (Hunter et al. 2010). Current work in Nebraska has used airborne remote sensing techniques, which can be performed more frequently than satellite data collection, to identify lakes with cyanobacteria present to incorporate into a toxic-algae alert (UNL CALMIT 2010).

5.0 Conclusions

It may be more beneficial to use finer grouping, such as *Daphnia* or *Bosmina* rather than cladocera, to define how cyanobacteria affect zooplankton taxa because cyanobacteria can have different effects on different genera (Lampert 1987). For example in this study, cyanobacteria percentage did not have significant effect on cladocerans, but had positive effects on *Bosmina*. It should also be recognized that in all of these models, predation factors are not included. Hansson et al. (2007) suggests that larger zooplankton, such as *Daphnia*, are “sandwiched” between fish predation and cyanobacteria abundance. Planktivores tend to favor larger zooplankton, which promotes smaller zooplankton (Carpenter et al. 1985, Jeppesen et al. 1997). Including that kind of information may help to further clarify the exact relationship with cyanobacteria inferred from the models. Information like this could also support alternative or additional methods to manage cyanobacteria blooms in Nebraska, such as biomanipulation. Reducing fish predation on zooplankton can help to decrease cyanobacteria in systems

(Smith & Lester 2006) and may complement reductions to nutrient loads (Gragani et al. 1999).

The high degree of non-linearity exhibited by the response variables over time and cyanobacteria biovolume/relative percentage may indicate that generalized additive modeling may be better suited to examining the interactions of phytoplankton or zooplankton with cyanobacteria. Further investigation should also be directed at a more exact relationship between cyanobacteria biovolume and *in vivo* phycocyanin. This would be a relatively easy and quick method to determine the amount of cyanobacteria in a surface water body than standard microscope intensive techniques. Cyanobacteria blooms remain a great concern in Nebraska reservoirs, thus a more complete understanding of the interaction of other phytoplankton and zooplankton groups with cyanobacteria will assist in reservoir management.

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