Climate change, the invasion of *Bythotrephes longimanus*, and recent changes in the zooplankton community of Lake Superior

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Abstract

In recent decades, average summer surface temperatures in Lake Superior have increased and the invasive predatory zooplankton, *Bythotrephes longimanus*, became established. While climate warming and *Bythotrephes* have influenced zooplankton communities in other lakes, it is unclear how either have or will influence the zooplankton community in Lake Superior. A late spring ice out in 2014 provided an opportunity to observe the response of zooplankton in Lake Superior to inter-annual variation in temperature. To evaluate this response, I compared biomass estimates, phenologies, and community compositions of the zooplankton communities in western Lake Superior during the 2014 and 2015 growing seasons. I also compared the community compositions observed in these years to published literature to determine whether the community has changed in response to climate warming or planktivory by *Bythotrephes*. I evaluated the possible role of *Bythotrephes* in zooplankton community changes using a bioenergetic model to compare the consumptive demands of *Bythotrephes* to the production rates of their potential prey. Annual peaks in zooplankton biomass were correlated with peaks in surface temperature. Peak biomass in 2014 occurred approximately 20 days later than in 2015 suggesting that continued warming could have long-term effects on the timing of peak zooplankton biomass in Lake Superior. The amount of biomass at the peak did not differ between years nor did overall community structure suggesting that zooplankton biomass and species composition in Lake Superior may be more constrained by food availability than temperature. However, long-term comparisons of zooplankton community composition indicate that densities of *Bosmina longirostris* declined and the proportional contribution of *Daphnia mendotae* to cladoceran biomass increased since the 1970s. These community changes are more consistent with the expected outcome of planktivory by *Bythotrephes* than the expected outcomes of changes in temperature, primary production, or vertebrate planktivory. The results suggest that *Bosmina* is the cladoceran species most vulnerable to suppression by *Bythotrephes* in Lake Superior, which supports the hypothesis that *Bythotrephes* has altered the cladoceran community in Lake Superior. While consumption by *Bythotrephes* did not exceed total zooplankton production in Lake Superior during 2014 or 2015, future increases in *Bythotrephes* density and temperature could cause the top-down effects of *Bythotrephes* on the zooplankton community to increase. This work helps to clarify how climate warming and *Bythotrephes* could influence the zooplankton community and energy flow pathway in Lake Superior in the future.

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Chapter I

Climate change implications for the Lake Superior zooplankton community

Introduction

In aquatic ecosystems, crustacean zooplankton cycle nutrients and convey energy from phytoplankton to higher trophic levels (Richardson 2008). The composition and biomass of zooplankton in the Laurentian Great Lakes (hereafter Great Lakes), collectively the largest freshwater ecosystem on earth, has changed in recent decades (Barbiero and Tuchman 2004a, Bunnell et al. 2012, Barbiero et al. 2014). Among these changes are sharp reductions in cladoceran biomass, sometimes upwards of 90% since the early 2000s (Barbiero et al. 2009a, Barbiero et al. 2012).

Compared to the other Great Lakes, zooplankton community changes in Lake Superior have received relatively little attention. Although crustacean zooplankton in Lake Superior were studied extensively in the late 1960s and early 1970s (Swain et al. 1970, Patalas 1972, Conway et al. 1973, Selgeby 1975, Watson and Wilson 1978), zooplankton surveys during the past 30 years are sparse and often limited to single sampling events or few sampling locations (Barbiero et al. 2001, Brown and Branstrator 2004, Johnson et al. 2004, Link et al. 2004, Yurista et al. 2009). Lake Superior is the coldest, deepest, and least productive of the Great Lakes and temperature and food resources together are dominant factors driving biomass and species composition of the crustacean zooplankton in this ecosystem (Patalas 1972, Watson and Wilson 1978, Zhou et al. 2001). A growing body of literature links climate warming to changes in the phenology, biomass, and species composition of zooplankton communities in a number of freshwater and marine ecosystems (Richardson 2008, Beaugrand et al. 2003, Winder and Schindler 2004, Carter and Schindler 2012). Though average summer surface temperatures in Lake Superior have increased by more than 2°C since the 1970s (Austin and Colman 2007), whether and how zooplankton in Lake Superior have responded is not known. This knowledge gap is critical to fill as average summer surface temperatures in Lake Superior are expected to continue to rise (Magnuson et al. 1997, Lehman 2002).

The response of crustacean zooplankton in Lake Superior to warmer surface water temperatures could take many forms. Previous studies demonstrated that the timing of life

history events (e.g., reproduction and instar advancement) for many species in Lake Superior occur a month or more later than in the warmer Great Lakes (Selgeby 1975, Watson and Wilson 1978, Sprules et al. 1990). Past work also indicates that peak zooplankton biomass in Lake Superior is correlated with peak surface water temperature (Watson and Wilson 1978, Munawar and Wilson 1978, Zhou et al. 2001, Yurista et al. 2009). These patterns point to possible changes in the timing of peak zooplankton biomass and overall quantity of biomass in response to climate warming. Such shifts might result in altered food availability for planktivorous fish at critical times of the year (Cushing 1990).

In addition to temperature, primary production has been implicated as an environmental driver of peak zooplankton biomass in Lake Superior (Munawar and Wilson 1978, Zhou et al. 2001, Yurista et al. 2009). In fact, Patalas (1972) indicated that total zooplankton biomass in Lake Superior is limited ultimately by primary production, rather than temperature. This suggests that zooplankton biomass might not be greater in warmer years or increase as a result of future climate warming unless primary production also increases. Conversely, there is some evidence that climate warming could result in reduced zooplankton biomass if it uncouples the phenologies of zooplankton and the primary producers upon which they feed (Cushing 1990, Winder and Schindler 2004).

Climate-driven changes in crustacean zooplankton biomass and phenology in Lake Superior could also be manifested through changes in the dominant species in the assemblage. In Lake Superior, zooplankton biomass is dominated by calanoid copepods and this has not changed since the 1970s (Patalas 1972, Barbiero et al. 2001, Oliver et al. 2015). Lehman (2002) predicted that increasing average summer surface temperatures in Lake Superior could allow warmer water taxa like *Bosmina longirostris* and cyclopoid species such as *Diacyclops thomasi* to become more abundant. Warm-water taxa in Lake Superior develop first in the nearshore regions in the early summer and appear offshore as surface temperatures there rise (Watson and Wilson 1978). Warmer water temperatures might accelerate the offshore expansion of these populations by enlarging the offshore regions suitable for warm-water species. However, like total zooplankton biomass, the relative abundance of cladocerans, calanoid copepods, and cyclopoid copepods is also influenced by the quantity and quality of food (McNaught 1975, Gannon and Stemberger 1978). Because species composition is influenced by temperature and food availability, it is

difficult to predict how climate warming will alter species composition without associated indices of grazing potential.

Strongly contrasting weather patterns during 2013-2015 offered an opportunity to observe the sensitivity of zooplankton phenology, biomass, and community structure in Lake Superior to inter-annual variation in temperature. The winter of 2013-2014 was unusually cold and ice persisted well into the spring while the winter of 2014-15 was milder and the extent of ice cover was considerably less. Summer surface temperatures in Lake Superior are strongly influenced by the amount of ice cover in the preceding winter (Austin and Colman 2007) and because of this, zooplankton in Lake Superior presumably experienced an earlier peak in surface temperature during 2015 than in 2014.

I conducted zooplankton surveys during the 2014 and 2015 growing seasons in the western arm of Lake Superior and evaluated relationships between water temperature, zooplankton phenology, biomass, and community structure. Long-term comparisons of zooplankton community structure were also made by comparing the 2014-2015 data against published literature. I hypothesized that colder temperatures in 2013-14 would delay the timing of peak zooplankton biomass, decrease total biomass, and change the taxonomic composition in ways that reduce the proportional contribution of warm-water species. I also hypothesized that the relative abundance of warm-water taxa in 2014-2015 would be greater than during the 1970s.

Methods

Samplings sites and dates

Zooplankton, water temperature, and fluorescence measurements were collected in the western arm of Lake Superior (Fig. 1.1, Table 1.1) from the R/V Blue Heron. In 2014, samples and data were collected on cruises during June 3-6, July 23-25, August 11-14, August 17-19, October 1-2, and October 16-19; in 2015, samples and data were collected on May 20-22, July 15-17, August 16-17, September 8-10, October 5-7, and October 16-19.

Several sampling stations were visited repeatedly. For example, I sampled stations 4 and 6 on four cruises during both years (Fig. 1.1). A number of stations were visited once during the study but were not included in Fig. 1.1. At most stations, vertical temperature and fluorescence

profiles of the water column were taken using a Sea-Bird Electronics CTD fitted with a Wet Labs WETstar Fluorometer. The CTD was attached to a rosette of Niskin bottles that were used to collect water samples for measurements of particulate organic carbon (POC) and particulate phosphorus (PP) (Data provided by R. Sterner, see Sterner (2010) for methods). Both POC and PP are indicators of food availability for zooplankton and are discussed further below. Zooplankton samples were not regularly collected at station CD1, but POC and PP were measured consistently at this site.

Zooplankton collection

Zooplankton samples were taken using a conical plankton net with a mouth opening of 1 meter diameter, 153 μm mesh, and a 4:1 aspect ratio (length to opening). Vertical tows with target depths of 60 m and 15 m were collected at each site as a means to study both total zooplankton and the shallow dwelling taxa. Recent studies have shown that more than 90% of the zooplankton biomass in Lake Superior exists at depths less than 50 m (Oliver et al. 2015, Pratt et al. 2016). Therefore, 60 m tows should collect most zooplankton taxa and biomass. Oliver et al. (2015) also demonstrated that certain species, including *Limnocalanus macrurus*, generally remain deeper than 10 m during day and night. Therefore, 15 m and 60 m tows should produce different estimates of community composition and biomass. Triplicate samples were taken at both depths starting in August of 2014. Zooplankton samples were preserved in 70% ethanol (final concentration). In 2015, nets were equipped with a RBR TWR-2050 pressure gauge to verify that nets reached target depths. The gauge was secured to the steel bridle at the mouth opening of the net and is sensitive to changes of 0.0001 dbar. Pressures were converted to depth according to Sea-Bird Electronics, Inc. (2002) as:

$$depth\ (m) = pressure\ (dbar) \times 1.0197 \qquad\qquad \text{(Eq. 1.1)}$$

Zooplankton processing

Specimens were sorted, counted, and identified under a Nikon SMZ 1500 dissecting microscope. All *Bythotrephes longimanus* and *Leptodora kindtii* were removed with forceps and counted in full. Because the sampling protocol was not designed to capture *Mysis* or *Diporiea*, both were excluded from analyses. Samples were diluted to 400 mL and subsamples were taken using a micropipette that was calibrated for 0.5 mL aliquots. Identification was done according to

Balcer et al. (1984). Individuals were identified to the species level with the exception of cyclopoid and calanoid nauplii, which were grouped together.

Replicate subsamples were taken until approximately 100 individuals from each taxon were counted. For less abundant taxa, larger subsample volumes were used and counting stopped when adequate replication of individuals per subsample was achieved or 10% of the sample volume was counted. The lengths of the first ten individuals in each taxon were measured using the microscope ocular micrometer which was calibrated using a stage micrometer. The lengths of all *B. longimanus* and *L. kindtii* individuals were measured using the same method as Branstrator (2005). For *B. longimanus*, the number barbs on the caudal spine (indicator of developmental instar) of each individual was also recorded for later bioenergetic estimates. The length of each taxon was averaged across each set of replicate samples.

Aerial (individuals m^{-2}) and volumetric (individuals m^{-3}) densities (*D*) of each taxon were calculated using equations 1.2 and 1.3, respectively:

$$D_{aerial} \quad = n_{species} \cdot \frac{V_K}{V_S \cdot a_{net}} \qquad \text{(Eq. 1.2)}$$

$$D_{volumetric} = n_{species} \cdot \frac{V_K}{V_S \cdot V_{tow}} \qquad \text{(Eq. 1.3)}$$

where $n_{species}$ is total number of individuals of a species counted in all subsamples, V_K is the dilution volume of the sample, V_S is the total volume of subsamples counted for the taxon, a_{net} is net mouth opening area, and V_{tow} is tow volume. While depth data were available for many net tows in 2015, all volumetric densities were calculated based on the assumption that the net was pulled through 15 m or 60 m of water regardless of the depth the net actually reached. For *B. longimanus* and *L. kindtii*, aerial and volumetric densities were determined by dividing the total number of individuals captured by the net area and total tow volume, respectively. Estimated densities in replicate tows were averaged for analyses.

Zooplankton biomass, phenology, and taxonomic composition

The average individual dry weight of each species in a sample was estimated using the average length of the species and previously published length-weight regressions (Table 1.2).

Average individual dry weight was multiplied by the average density of the species to estimate total biomass for that species in each tow.

Large cladocerans shrink when placed in preservatives (Yan and Pawson 1998). This is problematic because length-weight regressions for *B. longimanus* and *L. kindtii* were based on unpreserved animals (Branstrator 2005). To accurately estimate the original lengths of preserved *B. longimanus* and *L. kindtii* individuals for biomass estimates, 64 live *B. longimanus* (17, 36, and 11 individuals of instar 1, 2, and 3, respectively), and 98 live *L. kindtii* individuals were measured to the nearest tenth of a millimeter and placed in 70% ethanol for three weeks. Because of the difficulties associated with collecting and handling live zooplankton in Lake Superior, the *B. longimanus* individuals used for shrinkage assessment were collected in Island Lake Reservoir (Duluth, MN) on July 1, 2015 and *L. kindtii* were collected in Caribou Lake (Duluth, MN) on July 7, 2015. After three weeks of storage in ethanol, each individual was re-measured. To determine whether *B. longimanus* shrinkage was instar-specific, the shrinkages of each instar were compared using one-way ANOVA ($\alpha = 0.05$).

B. longimanus core body length (length of animal excluding caudal spine) and *L. kindtii* total body length shrank an average of 19.45% and 16.67%, respectively, when preserved in 70% ethanol. One-way ANOVA indicated that shrinkage in *B. longimanus* was not instar-specific ($p = 0.51$, $F_{2, 61} = 0.689$) and therefore all instars were combined for the *B. longimanus* shrinkage regression. The fresh lengths of *B. longimanus* (equation 1.4) and *L. kindtii* (equation 1.5) preserved in 70% ethanol can be predicted as:

$$Fresh\ length\ (mm)\ =\ 0.8166\ l_p\ (mm) - 0.0366, \qquad n = 64, R^2 = 0.8083 \quad \text{(Eq. 1.4)}$$

$$Fresh\ length\ (mm)\ =\ 0.8953\ l_p\ (mm) - 0.3038, \qquad n = 97, R^2 = 0.9193 \quad \text{(Eq. 1.5)}$$

where l_p is the length of the preserved individual. The lengths of *B. longimanus* and *L. kindtii* captured in Lake Superior in this study were thus corrected for shrinkage before biomass estimates were made. Because all *B. longimanus* and *L. kindtii* present in the samples were measured, biomass estimates for these species were based on the sum of all individuals in a sample.

To determine whether there was a difference in the timing of peak zooplankton biomass between years, I used generalized additive mixed models (GAMMs) to fit penalized cubic

regression splines to log-transformed zooplankton biomass with Julian day as the predicting variable. Only biomass estimates from 60 m tows were used and splines were generated in the statistics software R (version 3.2.2) with the package mgcv (Wood 2011). This type of regression spline penalizes the model fit (based on least squares) for the amount of "wiggliness" in the predicted curve (Wood and Augustin 2002, Wood 2006). In a GAMM, the penalized smoothing function is treated as a fixed effect but the "wiggliness" component is treated as a random effect (Wood 2011). The random effect reduces the influence of correlation between data points on model predictions. To evaluate differences in the timing of peak biomass at finer taxonomic scales, separate splines were made for cold-water calanoids (*L. macrurus* and *Senecella calanoides*), other calanoids (*Leptodiaptomus sicilis* and *Epischura lacustris*), cyclopoids, nauplii (all copepod nauplii), and cladocerans. Because cladocerans were often absent at individual stations, cladoceran biomass was transformed as [ln (1 + cladoceran biomass)] prior to model fitting. Confidence regions (95%) were estimated for each model fit to allow for comparisons of estimated biomass between growing seasons.

Differences in the seasonal patterns of zooplankton biomass between years were analyzed using hierarchical cluster analysis and non-metric multidimensional scaling (NMDS). Cluster analysis and NMDS are ordination methods that group observations based on a measure of similarity (Cox and Cox 2001). Two Bray-Curtis similarity matrices were generated for these analyses using the R package ecodist (Goslee and Urban 2013). A similarity matrix of monthly zooplankton community composition was generated using the square root transformed average biomass of each species in 60 m net tows during each month sampled. The other similarity matrix compared the community composition of all samples and was generated using the square root transformed biomass of each species present in each 60 m sample. The similarity matrix of average monthly data was used to generate a cluster dendrogram with a complete-linkage hierarchical cluster procedure in the R package ecodist. Following the methods in Barbiero et al. (2009a), I used a similarity measure of 75% to group similar months. The resulting clusters were evaluated for differences in monthly patterns between years.

NMDS was performed in the R package vegan (Oksanen et al. 2015) using the similarity matrix for all 60 m samples. Like cluster analysis, NMDS groups observations based on similarity, but does so in two dimensions. Species vectors were fit in the ordination space to better understand which species were associated with the different clusters of samples. Vectors point in the direction of the ordination space towards which the biomass of a species increases

most rapidly and the length of the vector is proportional to the correlation between the biomass of the species and the ordination pattern (Oksanen et al. 2015). Finally, polygons based on the groups identified in cluster analysis were overlaid in the NMDS ordination space to determine how well average monthly community composition predicted the relative position of the individual samples from each month. An analysis of similarity (ANOSIM) procedure was used to determine whether the groupings identified in cluster analysis produced statistically distinct groups of samples in NMDS.

The taxonomic composition observed in 2014-15 was compared to historical estimates of composition based on published literature to identify possible long-term community changes. Nauplii, which were often numerically important but among the smallest individuals in a sample, were problematic for these comparisons because different sampling gears and mesh sizes can bias density estimates. Nauplii were therefore omitted from total zooplankton numbers and the percent contributions of each species to total zooplankton density were adjusted accordingly.

Temperature and food

Average temperature in the top 10 m of water from each CTD cast was plotted against Julian day. Penalized cubic regression splines were fit to these data following the GAMM methods described above. Confidence regions (95%) were estimated for the model fits from both years to allow for comparisons of temperature conditions between growing seasons.

The ability of surface temperature to predict zooplankton biomass was evaluated by plotting log-transformed zooplankton biomass against degree day, which was calculated as average observed temperature in the top 10 m of water multiplied by the Julian day of sample collection. Penalized cubic regression splines were fit to biomass data for each year using the GAMM framework described above. As for Julian day, separate biomass splines were fit for cold-water calanoids, other calanoids, cyclopoids, nauplii, and cladocerans. A transformation of [ln (1+ cladoceran biomass)] was used for the cladoceran splines to account for their absence in many samples.

To determine whether there was evidence for differences in food availability for zooplankton between years, I compared measurements of fluorescence, POC, and PP over the course of each growing season. Fluorescence data were derived from CTD casts conducted during both day and night. Fluorescence in the upper 15 m of water was averaged for each

station and penalized cubic regression splines using GAMMs were fit for each year with Julian day as the predicting variable.

Measurements of POC (< 80 µm) were made for depths of 5 m at most stations. POC concentrations over the course of the two sampling seasons were also compared using GAMMs. At stations 6 and CD1 (Fig. 1.1), levels of POC and PP of size 2-80 µm were also available for samples collected at 5 m. Though data for this fraction of POC and PP were spatially limited, carbon and phosphorus in this size range are better indicators of food available to herbivorous zooplankton than fluorescence or whole POC (< 80 µm). The levels of POC and PP in this 2-80 µm size fraction were plotted against Julian day to explore possible patterns between growing seasons.

Results

Pressure gauges deployed during net tows in 2015 show that nets reached average depths of 13.7-14.0 m and 54.1-57.8 m when 15 m and 60 m of cable were paid out, respectively (Table 1.3). While net tows did not generally reach target depths, the ratios of depths reached in deep and shallow tows indicate that the deep net tows consistently reached depths four times those of shallow tows in each month sampled.

The contributions of individual taxa to total zooplankton density and biomass did not differ greatly between years (Tables 1.4-1.5). The crustacean zooplankton assemblages from both years were numerically dominated by copepods, particularly copepodid stages of *D. thomasi*, *L. sicilis*, and *L. macrurus*, as well as nauplii (Table 1.4). The biomass of calanoid copepods was an order of magnitude greater than cyclopoid copepods and cladocerans (Table 1.5). Cladocerans became common in August in 2014 but in 2015 they were already common in July. *Daphnia mendotae* was the most common cladoceran by density and biomass, both spatially and temporally, in both years. Total zooplankton density (including nauplii) ranged from 369 - 7602 individuals m^{-3} in 2014 and from 372 – 5731 individuals m^{-3} in 2015 (Table 1.4). The largest densities were generally observed during August - October.

Average offshore surface water temperatures in western Lake Superior were generally cooler during each month of 2014 compared to 2015 and Julian day predicted 76.2% and 83.6%

of the variation in temperature in 2014 and 2015, respectively (Fig. 1.2a, Table 1.6). Fluorescence and whole POC in the surface waters differed very little between years (Fig. 1.2b, c). While fluorescence and POC generally increased throughout each growing season, Julian day predicted little of the variability in fluorescence or POC in either year (Table 1.6). The quantities of POC and PP in the 2-80 µm fraction at stations 6 and CD1 were variable over the course of the season, however, there were also no consistent differences in the fractional quantities of either between years (Fig. 1.3).

Seasonal patterns in total zooplankton biomass were correlated with surface temperature in both sampling seasons (Fig. 1.4). Surface temperatures differed most between 2014 and 2015 during July, with 2015 being the warmer of the two years. Total zooplankton biomass during July of 2015 was significantly greater than in July of 2014 and peak zooplankton biomass in 2015 (day 213) occurred approximately 20 days earlier than in 2014 (day 233) (Fig. 1.5a).

L. macrurus typically accounted for more than half of the zooplankton biomass in Lake Superior (Table 1.5) and splines for cold-water stenotherm biomass (predominantly *L. macrurus* biomass) over each sampling season closely resembled the splines for total biomass (Fig. 1.5b). Julian day explained much of the variation in both total zooplankton biomass and cold-water stenotherm biomass (Table 1.7). Nauplii biomass also peaked earlier in 2015 than in 2014, and was well-predicted by Julian day in both years (Fig. 1.5e; Table 1.7). Calanoid and cladoceran biomass gradually increased during each growing season but the model fits for these taxa did not differ greatly between years and Julian day was not a strong predictor of the variability in the biomass of either group (Figs. 1.5c, f, Table 1.7). Cyclopoid biomass splines did not differ between years and the smoothing splines for cyclopoids were not significant at the 5% significance level (Fig. 1.5f, Table 1.7).

Cluster analysis and NMDS both point to a general seasonal pattern in zooplankton community structure. The cluster dendrogram comparing monthly community compositions identified four clusters at the 75% similarity level (Fig. 1.6). The three largest clusters included an early season cluster with May and June observations, a summer cluster consisting of observations from July of 2015 and August of both years, and a late season cluster consisting of September and October observations. The fourth cluster, which included only July of 2014, suggests that zooplankton community structure during 2014 and 2015 differed most during mid-summer. The same four clusters are apparent in the ordination (NMDS) of individual samples

and follow a cyclic chronological pattern through the growing season where the cluster of samples from July of 2014 fell in between the clusters of early season and later summer samples (Fig. 1.7). ANOSIM indicated that the four clusters identified in cluster analysis predicted distinct groups of individual samples in NMDS ($p < .001$, $R = 0.848$).

The overlay of species vectors in NMDS shows that the relative position of the large summer sample cluster was highly correlated with *L. macrurus* biomass. The relative position of the samples from July of 2014 along the *L. macrurus* vector indicate that *L. macrurus* biomass was lower in July of 2014 than in July of 2015. The low biomass of *L. macrurus* in July of 2014 compared to July of 2015 was largely the result of lower densities of *L. macrurus* copepodids (Fig. 1.8a). However, a one-tailed, equal variance t-test indicated *L. macrurus* copepodid individuals were also significantly smaller in July of 2014 (average length = 2.00 mm, n = 48) than in July of 2015 (average length = 2.43 mm, n = 131) ($p < 0.001$, Fig. 1.8b).

Despite differences in the timing of peak zooplankton biomass in 2014 and 2015, the amount of biomass present at the peak differed very little between years (Fig. 1.5a). When plotted against degree days, total zooplankton biomass splines from 2014 and 2015 largely overlapped (Fig. 1.9a). Degree day explained 46-66% of the variability in total zooplankton biomass (Table 1.8). The trend for cold-water stenotherm biomass was very similar to the trend in total zooplankton biomass and degree day explained more than half of the variability in cold-water stenotherm biomass in both years (Fig. 1.9b, Table 1.8). The biomasses of calanoids, cyclopoids, nauplii, and cladocerans also did not differ between years when plotted as a function of degree day but degree day explained little to none of the variation in these data (Figs. 1.9c-f; Table 1.8).

Lake Superior is historically and currently a calanoid-dominated system where *L. sicilis* is the most numerous species and *L. macrurus* is dominant in terms of zooplankton biomass (Tables 1.4-1.5, Table 1.9). One conspicuous change in the zooplankton community composition is the relative frequency of *B. longirostris* which was an order of magnitude less common in 2014-15 compared to the 1970s. The structure of the offshore herbivorous cladoceran community in Lake Superior, which includes primarily *D. mendotae*, *B. longirostris*, and *Holopedium gibberum,* has shifted since the 1970s. Most notably, *B. longirostris* contributes less to annual herbivorous cladoceran numbers than it did during the 1970s and the contribution of *D. mendotae* to cladoceran numbers was larger in 2014 and 2015 than during the 1970s (Fig. 1.10). These

changes in the cladoceran community structure were also apparent in long-term monthly comparisons (Fig. 1.11). Another change in the zooplankton community since the 1970s is the recent presence of the non-native *B. longimanus*. *B. longimanus* was observed in densities up to 3.08 individuals m^{-3} but was usually much less abundant and accounted for little of the total zooplankton biomass or numbers (Table 1.4-1.5).

Discussion

The pelagic crustacean zooplankton community of Lake Superior, the largest lake on earth by surface area (82,100 km^2), underwent substantial inter-year variation in the timing of peak biomass but less inter-year variation in overall zooplankton biomass or community composition during 2014 to 2015. Average summer surface temperatures in Lake Superior have increased by more than 2°C since the 1970s and are expected to continue to increase during this century (Austin and Colman 2007, Lehman 2002). This study provides insight into the extent that these climate-driven changes in temperature may be responsible for variation in the crustacean zooplankton community in Lake Superior. Average surface temperatures in western Lake Superior were as much as 5°C lower during the 2014 growing season compared to 2015, particularly from June to early August (Fig. 1.2). Temperature appears to be a major driver of the timing of peak zooplankton biomass in Lake Superior, but not the amount of biomass at the peak. Furthermore, neither short nor long-term compositional changes in proportional representation of the major taxa were congruent with predictions based on increasing surface temperature, suggesting a role for other factors, including top-down control by invasive species and bottom-up productivity-constrained growth.

Effects of short-term differences in temperature on the zooplankton community

While past studies have demonstrated a relationship between the timing of peak temperature and zooplankton biomass in Lake Superior (Watson and Wilson 1978, Zhou et al. 2001, Yurista et al. 2009), the present work shows that the timing of peak zooplankton biomass can vary by several weeks in consecutive years. The relationship between peak biomass and temperature differed among taxa but the dynamics of total zooplankton biomass were driven largely by *L. macrurus*. The correlation between low surface temperatures in the early part of

2014 and the delay in peak *L. macrurus* biomass is interesting because *L. macrurus* is a cold-adapted species that usually inhabits cold, deep parts of the water column (Oliver et al. 2015). However, past studies of *L. macrurus* developmental rates and habitat use in Lake Superior suggest that a causative relationship between low temperatures and a late peak in *L. macrurus* biomass is within reason. Reproduction of most copepods in Lake Superior occurs a month or more later than in Lake Michigan, which warms earlier in the year (Selgeby 1975). June is a time of rapid development for *L. macrurus* copepodids in Lake Superior (Selgeby 1975, Watson and Wilson 1978), and the early copepodid stages of *L. macrurus* tend to inhabit the epilimnion in June and July (Conway 1977). Despite *L. macrurus* being a cold-adapted species, the low surface temperatures early in the 2014 growing season could therefore have slowed the development of juveniles causing the peak in *L. macrurus* biomass to occur later in the growing season. The smaller average density and length of *L. macrurus* copepodids in July of 2014 compared to July of 2015 is consistent with delayed growth.

The later peak in nauplii biomass in 2014 was to be expected given the positive relationship between temperature and hatching rates of copepods (Patalas 1972). Despite the later peak in nauplii biomass in 2014, the biomasses of the copepodid calanoids (*L. sicilis* and *E. lacustris*) and cyclopoids (*D. thomasi*) did not differ significantly in 2014-15 and were not highly correlated with surface temperature trends. It is unclear why the difference in timing of peak nauplii biomass between years did not result in similar trends in the seasonal biomass of the various copepodid stages for species other than *L. macrurus*. One possibility is that the difference in peak nauplii biomass between years was driven by *L. macrurus* nauplii. The nets used in this study did not retain the smallest nauplii, so this study may not have been able to capture the true peaks in nauplii biomass for the smaller copepod species. Another possibility is that differences in life history between *L. macrurus* and the other dominant copepods (i.e., *D. thomasi* and *L. sicilis*) in Lake Superior explain the relatively steady amounts of biomass for cyclopoids and small calanoids in the 2014-2015 growing seasons. Unlike *L. macrurus*, *D. thomasi* and *L. sicilis* can produce multiple generations per year depending on temperature and resource availability (Selgeby 1975, Watson and Wilson 1978). This could reduce the likelihood of observing distinct peaks in the biomass of nauplii or copepodids in these species, especially when sampling events are separated by more than a month. An analysis of the progression of *L. sicilis* and *D. thomasi* through the copepodite stages in each summer would be needed to clarify the effect of surface temperatures on the phenology of these species in Lake Superior.

The presence of cladocerans in offshore areas of Lake Superior in July of 2015 but not July of 2014 is consistent with the depression of the emergence of cladocerans from resting eggs by colder temperatures (Bottrell 1975, Schwartz and Herbert 1987). However, total cladoceran biomass over the course of the 2014 and 2015 growing seasons did not differ greatly. Cladoceran biomass is generally lower in the offshore regions than the nearshore regions of Lake Superior and cladocerans do not become common in offshore areas until July and August (Watson and Wilson 1978). In this study, surface temperatures in both years began to converge on approximately 13°C by late August (Fig. 1.2a), and it is possible that cladoceran biomass in 2014 quickly reached quantities similar to those observed in 2015 at this time. However, the length of time between sampling events was sometimes greater than a month, and it is also possible that differences in cladoceran biomass between years would have been more apparent if additional data were available.

The similarities in cyclopoid and cladoceran biomasses from 2014 to 2015 are inconsistent with the hypothesis that warm-water species are more abundant in warmer growing seasons. The ability of degree day (i.e., temperatures experienced) to predict zooplankton biomass was limited only to *L. macrurus*. This could have been the result of multiple factors. For one, Julian day predicted less of the variability in the biomasses of cladocerans and smaller copepods then it did for *L. macrurus*. In addition, the biomasses of these groups were similar over the course of both growing seasons despite the inter-annual variation in temperature. Therefore, it is reasonable that degree day, when estimated as the product of Julian day and surface temperature, is not a strong predictor of biomass for taxa other than *L. macrurus*. However, the relatively strong relationship between *L. macrurus* biomass and degree day observed in both years lends further supporting evidence for a causative relationship between later peak surface temperatures and the lag in peak *L. macrurus* biomass in 2014.

Because of the lack of consistent differences in fluorescence, POC, and PP between years, it does not appear that the differences in surface temperature between 2014 and 2015 influenced the availability of food for zooplankton in the epilimnion. Like the biomasses of cladocerans and small calanoids, levels of fluorescence and whole POC in the surface water increased slowly over the growing season. Since cladocerans and small copepod species in Lake Superior are more strongly herbivorous than *L. macrurus* (Wong 1984, Nero and Sprules 1986) and because differences in surface temperatures between years did not appear to influence the

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seasonal biomass trends in these taxa, cladoceran and small copepod biomass in Lake Superior may be limited more by primary production than by temperature. If fluorescence and POC are reliable indices of food availability for zooplankton, then the relatively steady levels of fluorescence and POC in the 2014 and 2015 growing seasons would help to explain the similarities in seasonal biomass for cladocerans and small copepods between years.

While food availability in the epilimnion did not appear to differ in 2014-2015, the present study did not address whether the grazing potential of zooplankton below the epilimnion differed between years. Lake Superior supports a deep chlorophyll layer (DCL), which is a section of the water column near the thermocline with a high concentration of chlorophyll a, and *L. macrurus* exhibits strong vertical overlap with the DCL once it forms (Oliver et al. 2015). Because ice cover and the onset of thermal stratification influences the timing of DCL formation (Barbiero and Tuchman 2004b, Oliver et al. 2015), differences in temperature conditions between 2014 and 2015 might have influenced grazing potential for *L. macrurus* and thus could have influenced the rate of *L. macrurus* development. If this occurred, it would suggest that the effects of inter-annual variation in temperature on *L. macrurus* development can be indirect. Future studies will need to investigate how inter-annual variation in temperature influences the phenology and biomass of primary producers below the epilimnion.

Long-term community changes

Lehman (2002) predicted that increasing surface temperatures in Lake Superior could favor warm-water taxa like cyclopoids and small cladocerans. Some zooplankton surveys in the 1990s reported increased abundances of cyclopoids and *B. longirostris* in the offshore regions of Lake Superior after the 1970s (Johnson et al. 2004, Link et al. 2004). On the other hand, Brown and Branstrator (2004) reported reduced abundance of *B. longirostris* in August of 2001 compared to the 1970s. In the present study, there was little evidence that increased summer surface temperatures since the 1970s were accompanied by an increase in the relative abundance of warm-water taxa and the low abundances of *B. longirostris* in 2014 and 2015 are in agreement with the reduced abundance of *B. longirostris* observed by Brown and Branstrator (2004) in 2001. Because this change is inconsistent with the expected response of warm-water taxa to long-term climate warming, long-term changes in planktivory or primary production are likely to have caused the cladoceran community changes observed in this study.

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The other Great Lakes have also experienced changes in zooplankton community structure in recent decades. In Lake Michigan, cladoceran species richness and biomass declined abruptly following the establishment of *B. longimanus* in the 1980s (Lehman 1991, Lehman and Cáceres 1993). *B. longimanus* has since been implicated in changes in the cladoceran communities of Lakes Huron and Erie (Barbiero and Tuchman 2004a). In the mid-2000s, cyclopoid and cladoceran biomass in Lakes Michigan and Huron underwent additional reductions which were linked to long-term declines in primary production and, in the case of Lake Huron, occasional increases in vertebrate planktivory (Barbiero et al. 2009a, b, Barbiero et al. 2012).

Unlike Lakes Michigan and Huron, levels of primary production in Lake Superior have remained relatively unchanged in recent decades (Barbiero et al. 2012, Pratt et al. 2016). Furthermore, long-term changes in primary production are unlikely to result in reduced abundance of only *B. longirostris*. It is also unlikely that the cladoceran community changes observed in this study were due to changes in vertebrate planktivory because *B. longirostris* is not an important prey species for the dominant planktivorous fish species in Lake Superior (Isaac et al. 2012, Gamble et al. 2011a). This reduction in *B. longirostris* abundance and increase in the importance of *D. mendotae* is more similar to changes observed in the cladoceran communities of Lakes Michigan and Huron as well as a number of inland lakes following the invasion of *B. longimanus* (Lehman 1991, Yan and Pawson 1997, Barbiero and Tuchman 2004a). While *B. longimanus* was a minor component of the zooplankton community in this study, small cladocerans like *B. longirostris* are among the preferred prey for *B. longimanus* and have been suppressed by *B. longimanus* in several invaded lakes (Vanderploeg et al. 1993, Yan and Pawson 1997, Kerfoot et al. 2016). Additional analyses are needed to determine whether top-down control by *B. longimanus* explains reduced *B. longirostris* abundance.

Implications of future climate change on zooplankton in Lake Superior

Though Lake Superior is the coldest of the Great Lakes, predicting the response of its zooplankton community to further climate warming using patterns observed in the other Great Lakes is difficult. This is because the other Great Lakes have experienced larger trophic state and food web perturbations than Lake Superior during the last century (Gamble et al. 2011b, Barbiero et al. 2012). In systems other than the Great Lakes, however, climate change has resulted in range expansion of warm-water taxa and advances in the phenologies of copepods and cladocerans (Winder and Schindler 2004, Adrian et al. 2006, Richardson 2008, Carter and

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Schindler 2012). Such changes have implications for the efficiency of energy flow in the food web.

While climate warming has resulted in increased abundances of warm-water taxa in other cold-water systems (Beaugrand et al. 2003, Richardson 2008), it does not appear that warming in the offshore regions of Lake Superior has been sufficient (so far) to favor warm-water taxa like cyclopoids and cladocerans. Though additional warming in Lake Superior might cause increased importance of warm-water taxa, the abundances of these taxa are also constrained by low levels of primary production like those in Lake Superior (McNaught 1975, Gannon and Stemberger 1978). Primary production in Lake Superior is limited by phosphorus and productivity is not expected to increase due to rising temperatures alone (Sterner et al. 2004, Lehman 2002). Therefore, unless primary production increases independently from temperature, cladocerans and cyclopoids may be unable to displace calanoids in Lake Superior.

While further climate warming might not alter zooplankton community composition in Lake Superior by itself, it may lead to persistent changes in zooplankton phenology. The zooplankton surveys completed in Lake Superior in the early 1970s preceded the long-term rise in summer surface temperature in recent decades. Multiple 1970s studies reported that peak zooplankton biomass in Lake Superior occurred during September (Conway 1977, Watson and Wilson 1978). Peak zooplankton biomass in both 2014 and 2015 occurred in August, suggesting that the timing of peak zooplankton biomass may already occur earlier, on average, than it did during the 1970s.

Because peak *L. macrurus* biomass was tightly coupled with temperature and accounted for more than half of the total zooplankton biomass in Lake Superior, a long-term advancement in the timing of peak zooplankton biomass may be evidence for a forward shift in *L. macrurus* phenology since the 1970s. The coupling of *L. macrurus* phenology and temperature observed in this study also suggests that peak *L. macrurus* biomass in Lake Superior could continue to shift forward in the growing season with further climate warming. A persistent forward shift in *L. macrurus* phenology could uncouple *L. macrurus* phenology with the phenology of primary producers in the DCL which could result in a long-term reduction in *L. macrurus* biomass. Because *L. macrurus* is a key food source for commercially harvested fish like lake herring (*Coregonus artedi)* in Lake Superior, climate-driven changes in *L. macrurus* phenology and biomass have implications for energy flow to higher trophic levels (Oliver et al. 2015, Isaac et al.

2012). Future zooplankton research in Lake Superior should assess the sensitivity of *L. macrurus* phenology and biomass to above-average temperatures.

Conclusions

This study shows that temperature can influence the timing of peak zooplankton biomass in Lake Superior in the short term, but does not result in large year-to-year variation in the amount of zooplankton biomass or community structure in Lake Superior. Peak zooplankton biomass possibly occurs earlier on average than it did in the 1970s and could continue to occur earlier in the year with continued climate warming. Because zooplankton biomass in Lake Superior is driven by *L. macrurus*, an important nutrient recycler and prey item for planktivorous fish, persistent changes in zooplankton phenology have the potential to reduce energy flow in the system by uncoupling *L. macrurus* phenology with the phenologies of primary producers and planktivorous fish.

The low levels of primary production in Lake Superior appear to be a key factor driving zooplankton community composition. Continued warming is unlikely to result in an increase in the importance of warm-water taxa in Lake Superior unless primary production increases independently from temperature. The long-term changes that have occurred in the structure of the cladoceran community in Lake Superior point to changes in planktivory. These changes are more consistent with top-down control by *B. longimanus* than by planktivorous fish. Bioenergetic analyses and comparisons of overlap between *B. longimanus* and their potential cladoceran prey will be needed to evaluate this hypothesis.

Table 1.1. Location and depth of routine sampling stations (see Figure 1.1).

Station	Latitude (degrees North)	Longitude (degrees West)	Station Depth (m)
1	46.927117	-91.475283	130
2	47.331267	-91.192483	87
3	47.450517	-90.897933	241
4	47.3155	-90.547633	78
5	47.74603333	-90.22626667	155
6	47.308767	-89.851417	172
CD1	47.065	-91.431667	255

Table 1.2. Length-weight regressions used in biomass estimates for each taxon.

Taxa	Length-weight regression	Source
Bosmina longirostris	ln w (µg) = 2.71 + 2.83 ln L (mm)	Bottrell et al. 1976
Bythotrephes longimanus	w (µg) = 45.2 L (mm) -287.2	Branstrator 2005
Copepod nauplii	0.4 µg per individual	Hawkins and Evans 1979
Cyclopoid copepodite	ln w (µg) = 1.66 + 3.97 ln L (mm)	Rosen 1981
Daphnia mendotae	ln w (µg) = 1.51 + 2.56 ln L (mm)	Dumont et al. 1975
Calanoid copepodite	ln w (µg) = 1.05 + 2.46 ln L (mm)	Pace and Orcutt 1981
Leptodiaptomus sicilis	ln w (µg) = 1.05 + 2.46 ln L (mm)	Pace and Orcutt 1981
Diacyclops thomasi	ln w (µg) = 1.50 + 1.99 ln L (mm)	Persson and Ekbohm 1980
Epischura lacustris	ln w (µg) = 1.05 + 2.46 ln L (mm)	Pace and Orcutt 1981
Holopedium gibberum	ln w (µg) = 6.50 + 3.19 ln L (mm)	Persson and Ekbohm 1980
Leptodora kindtii	ln w (µg) = 0.01 + 2.042 ln L (mm)	Branstrator 2005
Limnocalanus macrurus	ln w (µg) = 0.71 + 3.01 ln L (mm) (May)	Doubek and Lehman 2011
	ln w (µg) = 0.54 + 3.78 ln L (mm) (June)	Doubek and Lehman 2011
	ln w (µg) = 2.78 + 1.85 ln L (mm) (July)	Doubek and Lehman 2011
	ln w (µg) = 0.60 + 4.40 ln L (mm) (August)	Doubek and Lehman 2011
	ln w (µg) = 2.62 + 0.62 ln L (mm) (September-October)	Keeler et al. 2015

Table 1.3. Monthly summary of average depths (m) reached during net tows when 15 m and 60 m of cable were paid out. The ratio of deep to shallow depths reached indicates the average ratio of deep to shallow depths met at each sampling station. Numbers in parentheses indicate the standard deviation and n indicates numbers of stations where depth data were available.

	Average depth (m) reached with 15 m of cable	Average depth (m) reached with 60 m of cable	Average ratio of deep to shallow depth reached	n
May	14.0 (-)	57.8 (-)	4.12 (-)	1
July	14.0 (0.75)	54.5 (3.94)	3.90 (0.33)	4
September	13.7 (0.64)	54.1 (2.72)	3.97 (0.09)	4
October	13.7 (0.74)	55.8 (4.02)	4.06(0.19)	7

Table 1.4.   Monthly volumetric densities (individuals m^{-3}) for all zooplankton taxa present in 60 m net tows at routine sampling stations (see Fig. 1.1) in 2014-15. Density estimates starting in August of 2014 represent averages of triplicate 60 m vertical net tows. Nauplii includes all copepod nauplii.

	Station	total	*Bosmina longirostris*	*Bythotrephes longimanus*	*Daphnia mendotae*	*Holopedium gibberum*	*Leptodora kindtii*	All nauplii	*Diacyclops* copepodite	*Diacyclops thomasi* adult	*Epischura lacustris*	*Leptodiaptomus* copepodite	*Leptodiaptomus sicilis* adult	*Limnocalanus macrurus*	*Senecella calanoides*
June 2014	1	543.2	0.0	0.00	0.0	0.0	0.00	170.8	13.8	70.0	0.0	110.3	134.8	43.5	0.0
	4	526.6	0.0	0.00	0.0	0.0	0.00	28.3	41.0	83.8	0.0	87.7	243.3	42.4	0.0
	6	559.9	0.0	0.00	1.4	0.0	0.00	116.4	27.3	80.7	0.0	41.4	278.4	14.2	0.0
	ave.	543.3	0.0	0.0	0.5	0.0	0.0	105.2	27.4	78.2	0.0	79.8	218.8	33.4	0.0
July 2014	1	712.0	0.0	0.00	0.0	0.0	0.00	205.4	3.4	40.7	0.0	0.0	200.3	259.7	2.4
	4	1463.6	0.0	0.00	0.0	0.0	0.00	993.1	23.0	1.2	0.0	84.9	204.9	152.8	3.6
	6	1045.3	0.0	0.00	0.0	0.0	0.00	507.2	1.7	108.6	0.0	15.3	247.9	164.7	0.0
	ave.	1073.7	0.0	0.0	0.0	0.0	0.0	568.6	9.4	50.2	0.0	33.4	217.7	192.4	2.0
August 2014	1	1090.5	179.7	0.04	28.6	0.0	0.00	96.2	46.8	63.9	3.3	243.3	123.8	303.4	1.5
	2	7601.9	0.9	0.12	251.8	2.0	0.00	645.1	1660.9	188.6	1.9	4252.6	414.0	182.0	1.8
	3	2021.8	0.0	0.00	3.7	0.0	0.00	861.6	129.7	8.4	0.0	275.7	349.4	391.5	1.9
	4	1368.0	0.0	3.08	37.3	22.1	0.04	30.6	140.9	47.5	30.6	891.3	105.3	59.4	0.0
	5	5698.6	0.0	0.00	6.6	0.0	0.00	1601.5	158.4	291.4	0.5	2651.2	714.9	272.6	1.5
	6	3934.3	0.0	0.00	0.0	0.0	0.00	1567.5	117.4	21.9	0.0	1277.5	599.8	344.5	5.7
	ave.	3619.2	30.1	0.5	54.7	4.0	0.0	800.4	375.7	103.6	6.0	1598.6	384.5	258.9	2.1
October 2014	3	1576.3	0.4	0.00	39.4	0.0	0.00	17.0	575.8	11.7	0.5	498.0	308.4	125.2	0.0
	4	2240.9	0.0	0.32	57.3	17.0	0.00	23.3	339.5	25.5	9.5	1319.9	241.9	195.2	11.3
	5	369.3	0.6	0.02	20.1	0.0	0.00	6.4	32.0	4.1	0.1	74.5	102.0	125.8	3.7
	6	3147.7	0.0	0.00	75.3	0.0	0.00	137.9	248.3	22.3	0.7	1941.7	403.2	318.3	0.0
	ave.	1833.6	0.3	0.1	48.0	4.2	0.0	46.2	298.9	15.9	2.7	958.5	263.9	191.1	3.8

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Table 1.4. (Continued)

	Station	Total density	*Bosmina longirostris*	*Bythotrephes longimanus*	*Daphnia mendotae*	*Holopedium gibberum*	*Leptodora kindtii*	All nauplii	*Diacyclops* copepodite	*Diacyclops thomasi* adult	*Epischura lacustris*	*Leptodiaptomus* copepodite	*Leptodiaptomus sicilis* adult	*Limnocalanus macrurus*	*Senecella calanoides*
May 2015	1	503.6	0.0	0.00	0.0	0.0	0.00	50.0	1.9	75.5	0.0	50.5	226.4	99.5	0.0
	2	372.3	0.0	0.00	0.0	0.0	0.00	93.0	3.9	43.1	0.0	18.0	153.8	60.1	0.2
	4	624.0	0.0	0.00	0.0	0.0	0.00	45.7	7.1	151.4	0.0	25.0	275.9	118.8	0.1
	6	509.2	0.0	0.00	0.0	0.0	0.00	47.4	9.9	109.6	0.0	29.7	216.5	95.3	0.8
	ave.	502.3	0.0	0.00	0.0	0.0	0.00	59.0	5.7	94.9	0.0	30.8	218.1	93.4	0.3
July 2015	1	2246.3	12.3	0.25	19.4	2.3	0.06	379.1	234.1	360.8	1.6	653.6	182.5	396.6	3.7
	2	4178.3	0.0	0.00	7.0	0.0	0.00	2201.3	814.9	38.7	0.0	694.6	223.4	193.9	4.6
	4	2840.4	2.3	0.00	6.5	2.6	0.00	1188.4	205.8	50.5	3.9	959.2	182.0	237.2	2.0
	6	1034.1	0.0	0.00	0.0	0.0	0.00	335.8	7.6	17.4	0.1	7.5	195.2	464.0	6.5
	ave.	2574.8	3.6	0.1	8.2	1.2	0.0	1026.1	315.6	116.8	1.4	578.7	195.8	322.9	4.2
August 2015	3	1275.0	0.0	0.00	16.4	0.0	0.00	171.2	20.3	9.3	0.0	324.0	283.9	440.9	9.1
	5	1018.3	0.0	0.00	0.1	0.0	0.00	412.5	26.7	1.9	0.0	115.1	212.4	241.0	8.6
	ave.	1146.6	0.0	0.0	8.2	0.0	0.0	291.9	23.5	5.6	0.0	219.5	248.2	341.0	8.8

Table 1.4. (Continued)

	Station	Total density	*Bosmina longirostris*	*Bythotrephes longimanus*	*Daphnia mendotae*	*Holopedium gibberum*	*Leptodora kindtii*	All nauplii	*Diacyclops* copepodite	*Diacyclops thomasi* adult	*Epischura lacustris*	*Leptodiaptomus* copepodite	*Leptodiaptomus sicilis* adult	*Limnocalanus macrurus*	*Senecella calanoides*
September 2015	1	1837.9	0.9	0.00	223.1	0.0	0.00	116.9	58.9	62.2	9.8	834.0	157.0	364.1	11.0
	2	2010.7	0.0	0.01	104.7	0.0	0.00	34.0	25.9	28.3	0.3	1472.7	196.2	145.2	3.5
	4	2518.1	0.0	0.09	209.4	12.9	0.00	75.0	468.3	182.5	22.4	1228.0	187.0	129.4	3.1
	6	2664.1	0.0	0.01	66.5	0.5	0.00	115.1	223.5	53.8	2.3	1539.2	257.9	388.1	17.3
	ave.	2257.7	0.2	0.03	150.9	3.3	0.00	85.2	194.2	81.7	8.7	1268.5	199.5	256.7	8.7
October 2015	1	2720.8	0.8	0.00	211.6	0.0	0.00	128.6	799.8	52.6	6.0	810.0	321.2	377.0	13.1
	2	5731.8	21.6	0.01	841.8	0.8	0.00	12.3	2431.4	252.1	32.7	993.1	1105.9	40.1	0.0
	3	2689.1	0.0	0.00	157.0	0.0	0.00	6.6	1058.2	42.0	15.0	277.3	936.8	189.6	6.7
	4	2832.7	0.4	0.51	316.9	28.2	0.00	62.3	308.0	195.8	19.0	1288.3	346.5	255.3	11.6
	5	900.4	0.1	0.00	15.9	0.0	0.00	21.7	175.9	2.8	2.9	92.0	303.7	276.3	9.1
	6	2834.5	0.0	0.00	152.8	0.3	0.00	55.6	286.7	67.5	1.9	1250.6	567.8	430.1	21.2
	ave.	2951.6	3.8	0.09	282.7	4.9	0.00	47.9	843.3	102.2	12.9	785.2	597.0	261.4	10.3

Table 1.5. Average percent contribution of each taxon to zooplankton biomass by month and year for 2014-15. Percentages are based on the average biomass of all 60 m tows taken in each month. All copepod nauplii are grouped with calanoid copepods. *L. kindtii* contributed no more than 0.001 percent of zooplankton biomass in any month sampled.

	2014					2015					
	June	July	Aug	Oct	average	May	July	Aug	Sept	Oct	average
Cladocerans											
Bosmina longirostris	0.00	0.00	0.05	0.00	0.01	0.00	0.01	0.00	0.00	0.02	0.01
Bythotrephes longimanus	0.00	0.00	0.15	0.07	0.06	0.00	0.03	0.00	0.04	0.08	0.04
Daphnia mendotae	0.1	0.0	0.8	9.6	2.6	0.0	0.1	0.1	7.1	14.2	5.4
Holopedium gibberum	0.0	0.0	0.1	0.2	0.1	0.0	0.0	0.0	0.1	0.3	0.1
total	0.1	0.0	1.1	9.9	2.8	0.0	0.1	0.1	7.2	14.6	5.5
Calanoids											
All nauplii	1.2	1.9	1.0	0.1	1.1	0.7	1.2	0.3	0.3	0.1	0.5
Epischura lacustris	0.0	0.0	0.2	0.3	0.1	0.0	0.0	0.0	0.6	0.7	0.3
Leptodiaptomus copepodite	2.0	0.2	3.8	12.5	4.6	1.5	1.5	0.4	14.6	8.4	6.2
Leptodiaptomus sicilis adult	46.3	9.2	7.2	19.9	20.6	41.9	3.8	4.4	11.9	28.9	12.3
Limnocalanus macrurus	39.0	85.9	84.6	49.6	64.8	40.4	90.7	93.6	55.8	34.9	68.8
Senecella calanoides	0.0	1.4	0.6	5.0	1.7	1.7	0.7	1.1	6.3	6.0	3.5
total	88.5	98.6	97.5	87.4	93.0	86.2	98.0	99.8	89.5	79.0	91.6
Cyclopoids											
Diacyclops copepodite	1.2	0.0	0.5	1.9	0.9	0.1	0.4	0.0	0.7	4.2	1.3
Diacyclops thomasi adult	10.2	1.4	0.9	0.8	3.3	13.6	1.4	0.0	2.6	2.1	1.6
total	11.4	1.4	1.5	2.7	4.3	13.8	1.9	0.1	3.3	6.4	2.9

Table 1.6. Proportion of variability (R^2) in temperature, fluorescence, and POC predicted by Julian day and p-values for GAMM model fits.

| | 2014 | | 2015 | |
	R^2	p-value	R^2	p-value
Temperature	0.762	<.001	0.836	<.001
Fluorescence	0.263	<.01	<.0001	0.475
POC	0.341	<.001	0.466	<.001

Table 1.7. Proportion of variability (R^2) in zooplankton biomass predicted by Julian day and p-values for GAMM model fits.

	2014		2015	
	R^2	p-value	R^2	p-value
Total Biomass	0.729	<.001	0.872	<.001
Cold-water stenotherms	0.777	<.001	0.723	<.001
Other calanoids	0.0511	0.141	0.647	<.001
Cyclopoids	<.001	0.717	<.001	0.558
Nauplii	0.699	<.001	0.694	<.001
Cladocerans	0.358	<.001	0.539	<.001

Table 1.8. Proportion of variability (R^2) in zooplankton biomass predicted by degree day and *p*-values for GAMM model fits.

	2014		2015	
	R^2	*p*-value	R^2	*p*-value
Total Biomass	0.457	<.001	0.663	<.001
Cold-water stenotherm	0.517	<.001	0.536	<.001
Other calanoids	0.194	<.01	0.312	<.01
Cyclopoids	<.001	0.701	<.001	0.657
Nauplii	<.001	0.484	<.001	0.66
Cladocerans	0.235	<.01	0.358	<.01

Table 1.9. Zooplankton community structure 1971-2015 classified as percent contribution of each species to total zooplankton numbers. Percentages for *D. thomasi* and *L. sicilis* include copepodite and adult densities. *L. kindtii* accounted for less than 0.001% of zooplankton numbers in 2014-2015. Nauplii were excluded from analyses to control for underestimation of nauplii densities in some studies.

	1971[a]	1971-2[b]	1973[c]	2014	2015
Cladocerans					
Bosmina longirostris	9.4	4.6	2.4	0.22	0.07
Bythotrephes longimanus				0.004	0.002
Daphnia mendotae	14.2	5.1	6.1	1.5	3.4
Holopedium gibberum	0.0	0.9	0.4	0.1	0.1
Copepods					
Diacyclops thomasi	7.1	39.1	15.2	18.7	2014
Epischura lacustris	1.2	0.2		0.1	0.2
Leptodiaptomus sicilis	57.3	49.1	63.6	62.3	55.2
Limnocalanus macrurus	10.1	0.8	11.1	16.9	20.5
Senecella calanoides	0.6	0.2	1.2	0.2	0.4

[a] Conway et al. 1973
[b] Selgeby 1975
[c] Watson and Wilson 1978

Figure 1.1
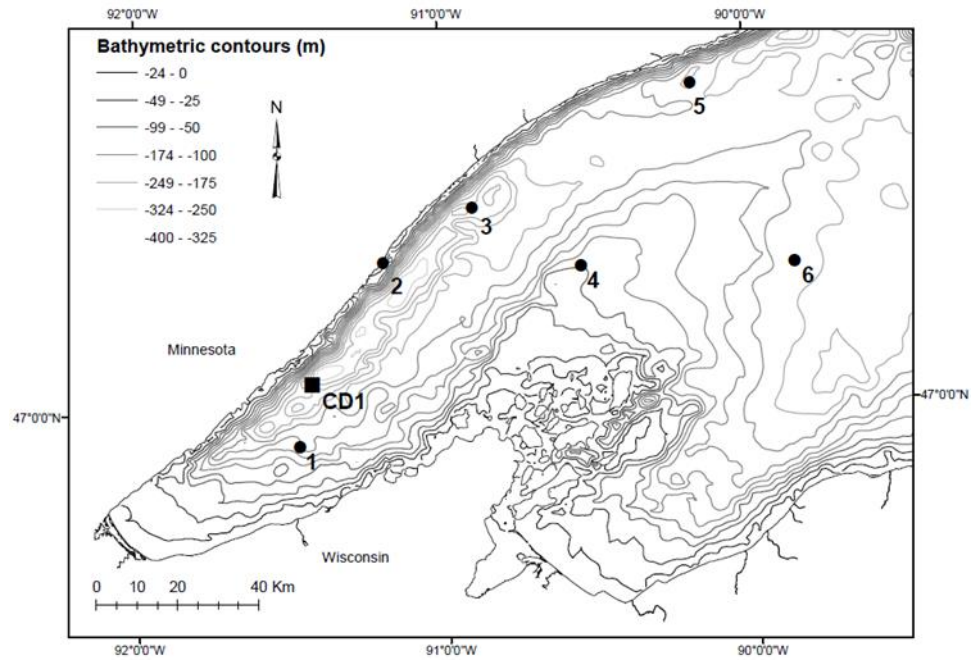
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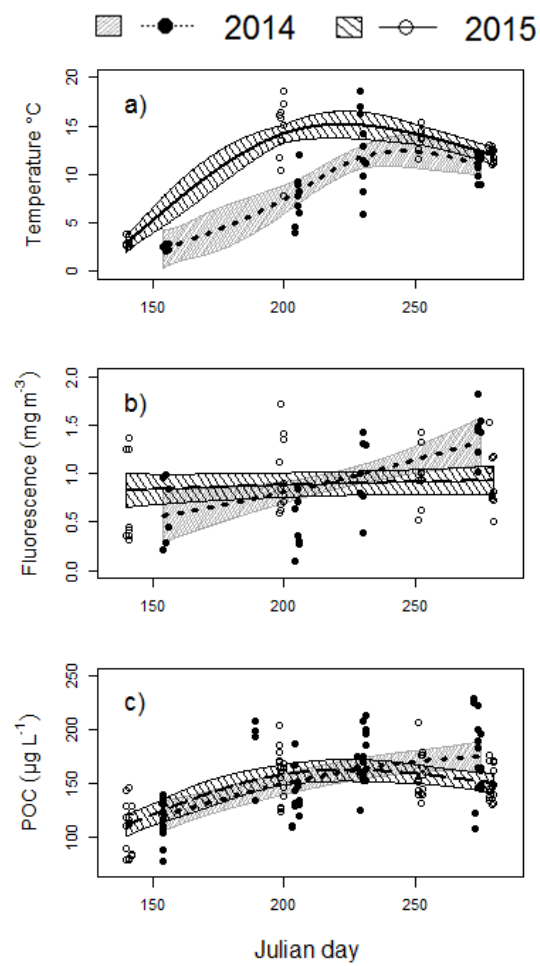
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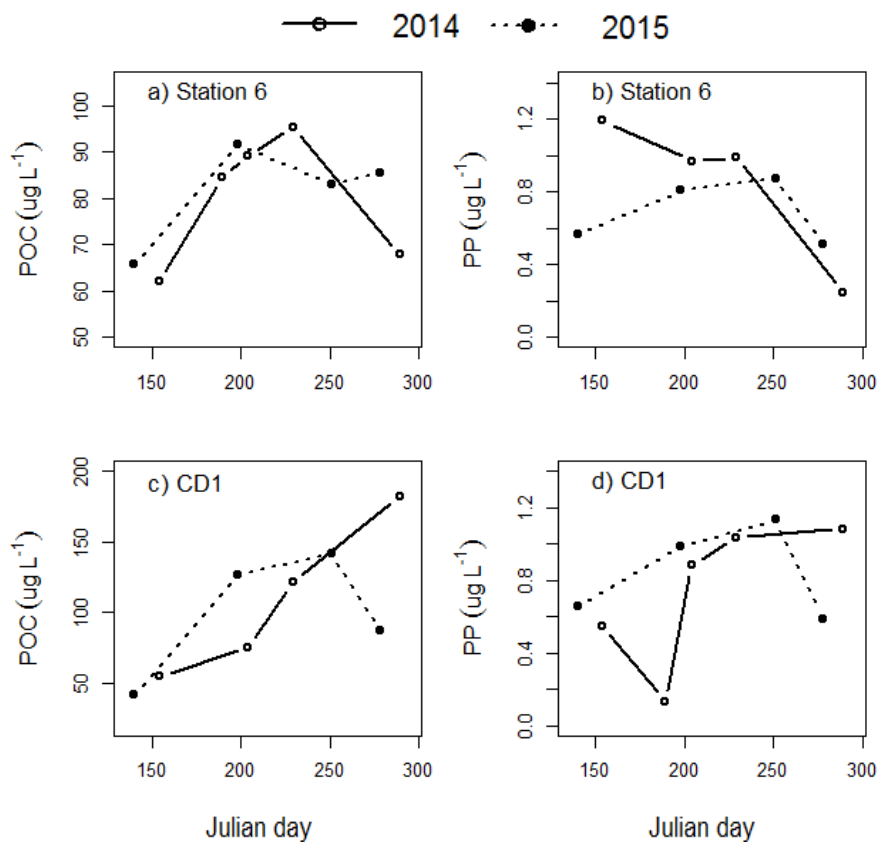
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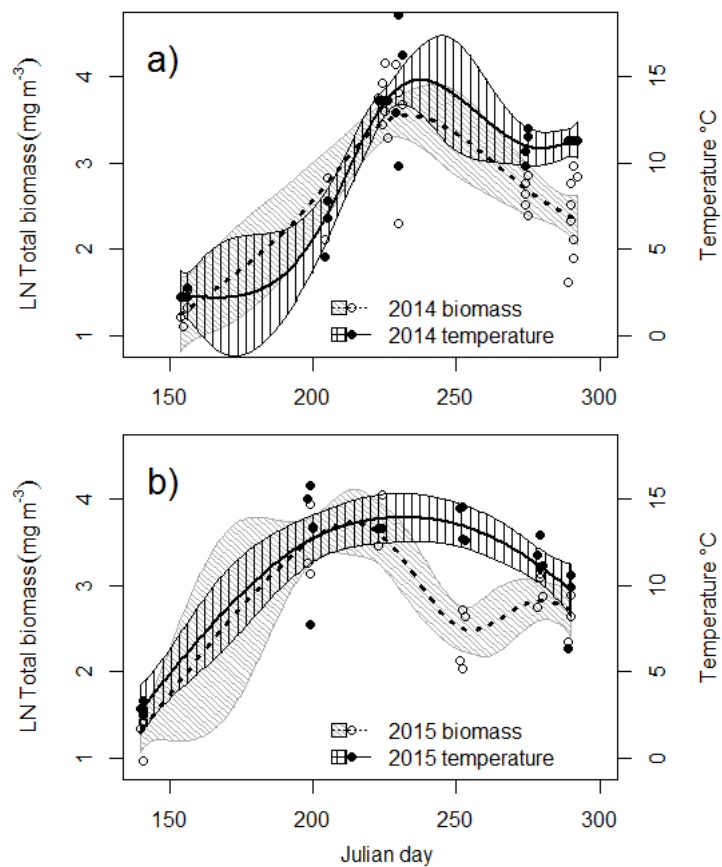
Figure 1.5

Figure 1.6

Figure 1.7

BOS = *Bosmina longirostris*
BTY = *Bythotrephes longimanus*
DPM = *Daphnia mendotae*
DCC = *Diacyclops* copepodite
DCT = *Diacyclops thomasi* adult
EPI = *Epischura lacustris*
HOL = *Holopedium gibberum*
LDC = *Leptodiaptomus* copepodite
LDS = *Leptodiaptomus sicilis* adult
LEP = *Leptodora kindtii*
LIM = *Limnocalanus macrurus*
NPL = Copepod Nauplii
SEN = *Senecella calanoides*

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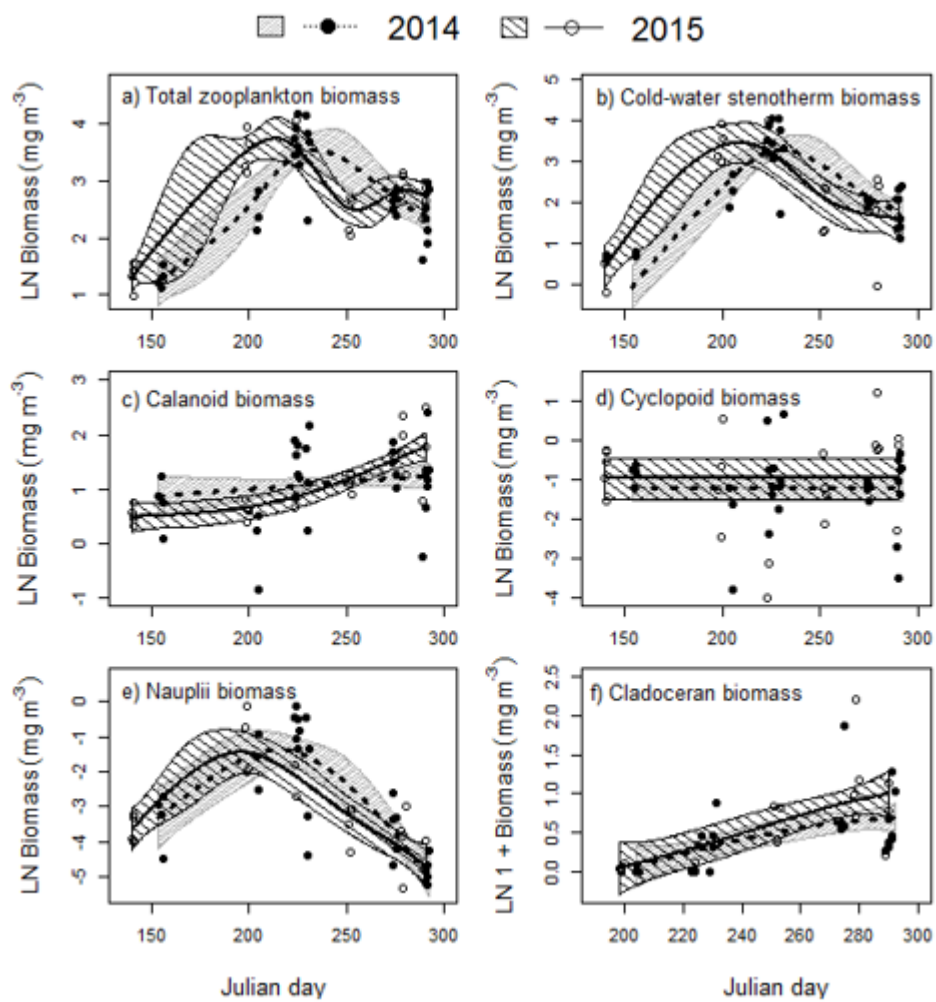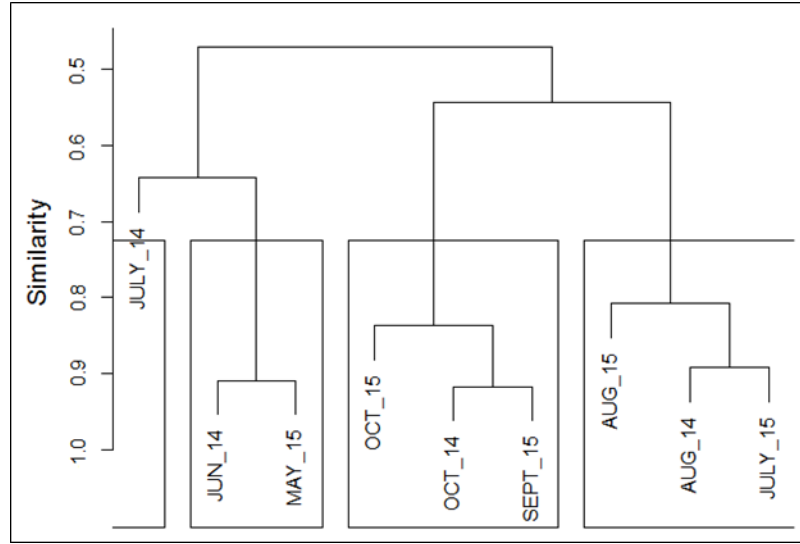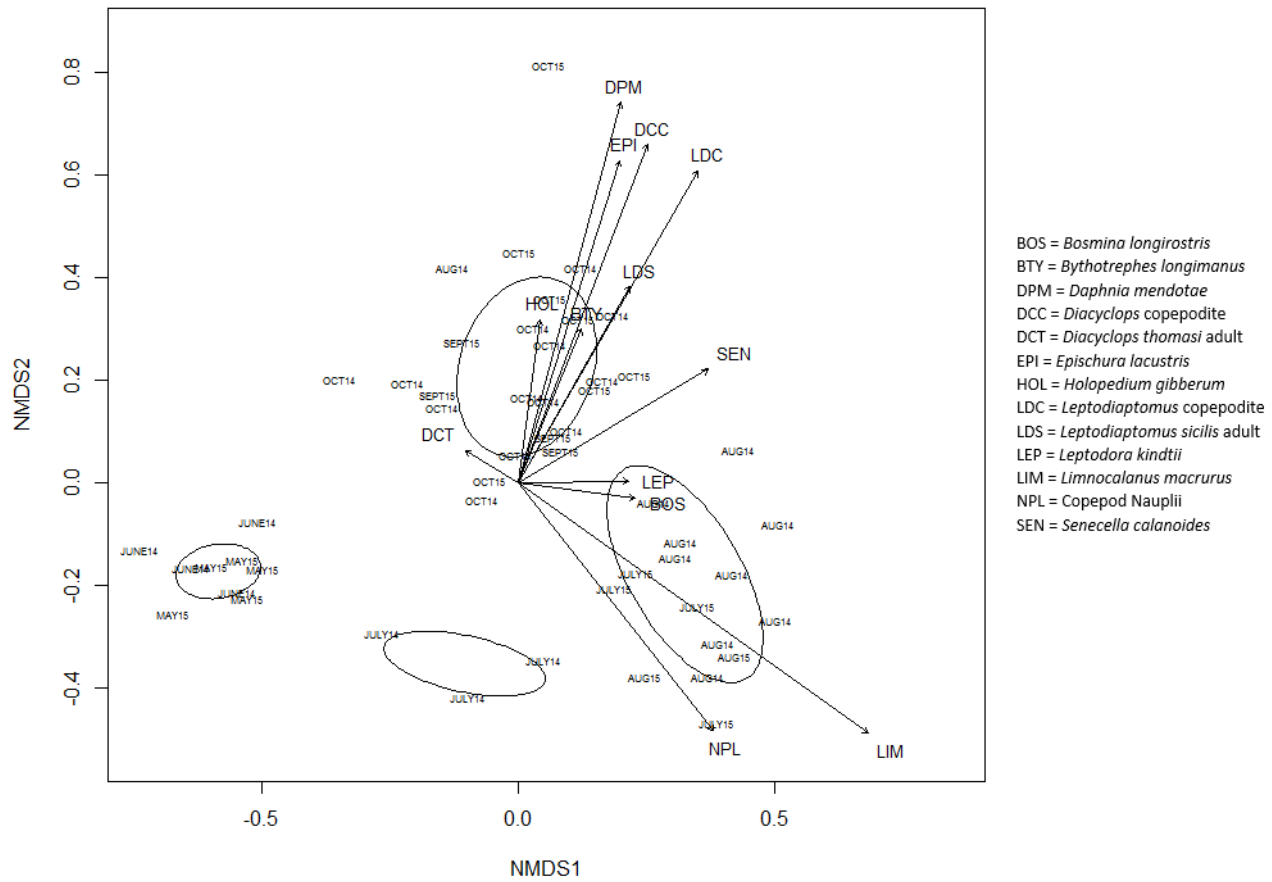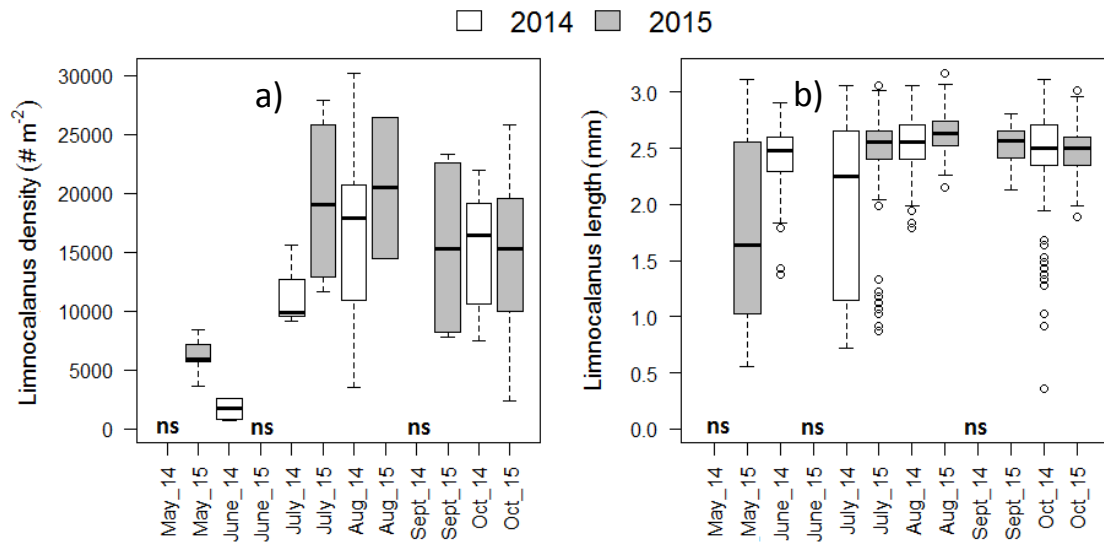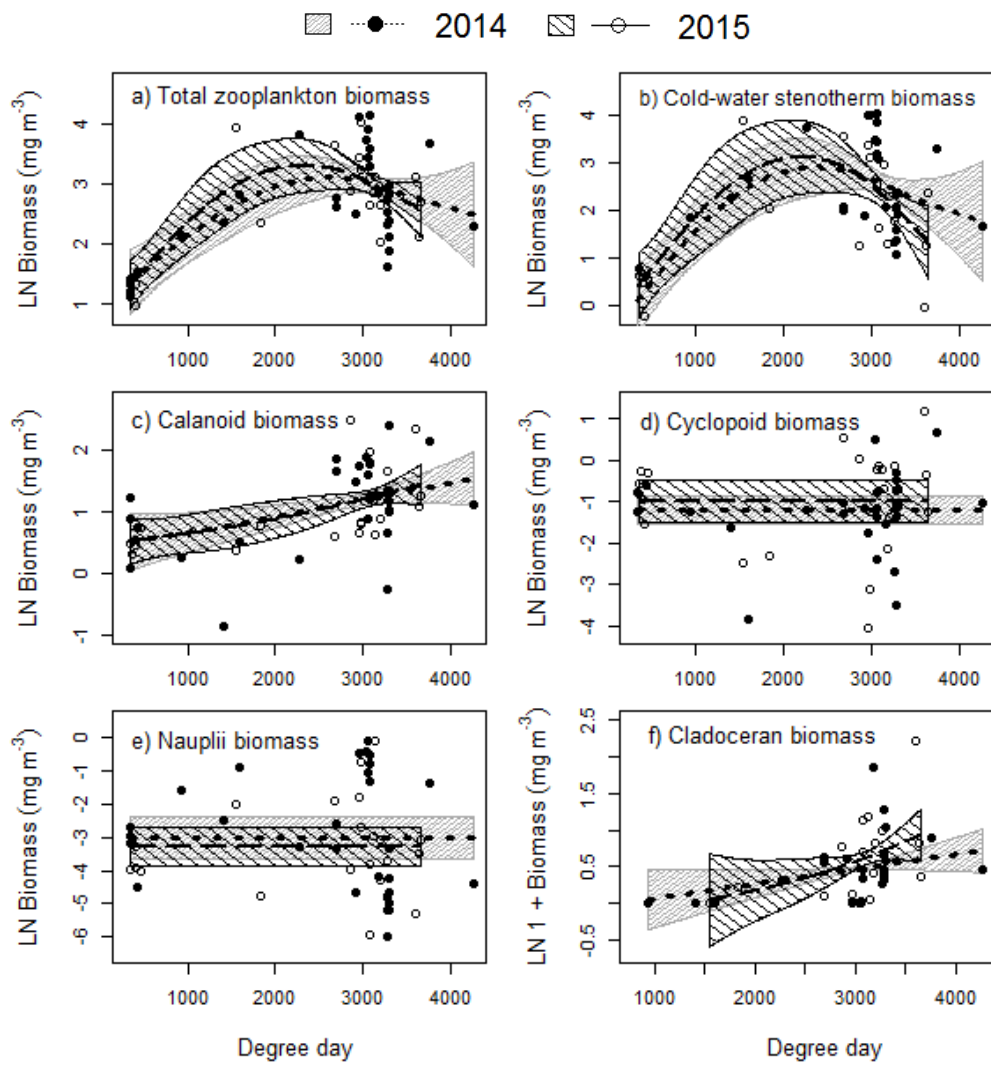
Figure 1.8

Figure 1.9

Figure 1.10
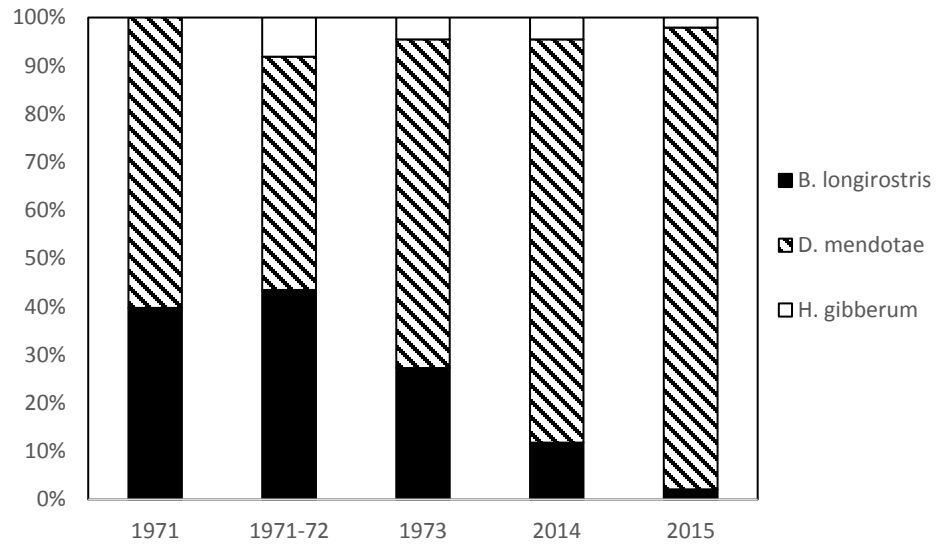
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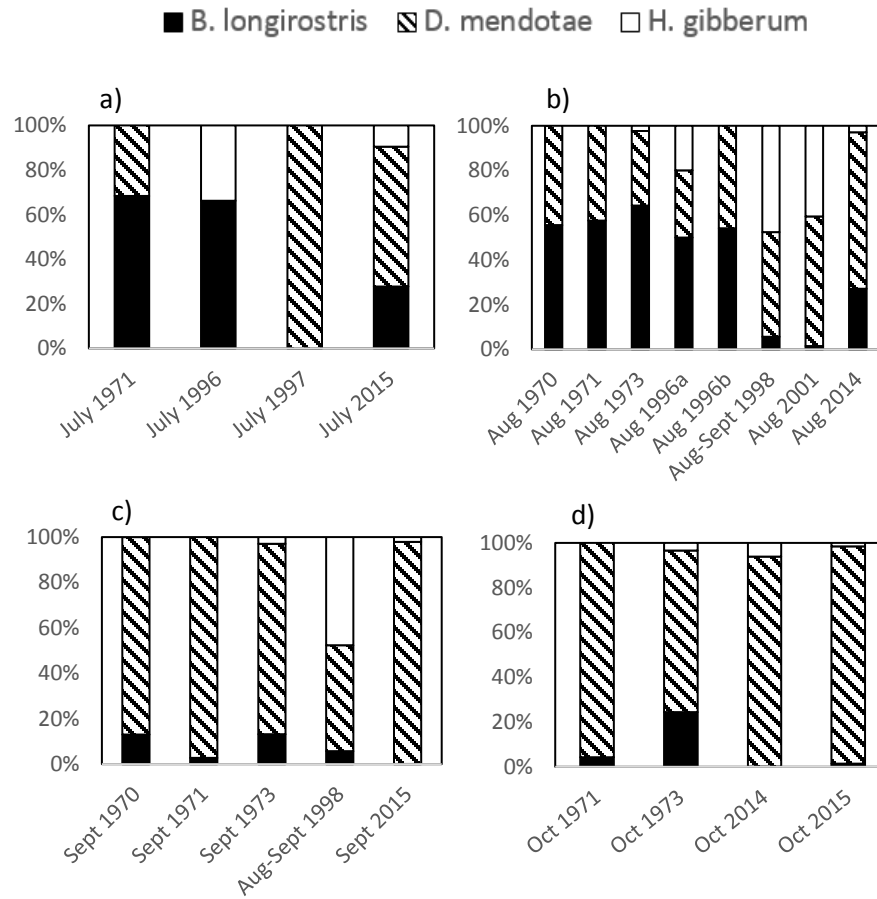
Figure 1.11

Legend: ■ B. longirostris ⊠ D. mendotae □ H. gibberum

a)

b)

c)

d)

Sample month	Source
All 1970-1971 data	Conway et al. (1973)
All 1973 data	Watson and Wilson (1978)
July 1996 Aug 1996a	R. Sterner, archived samples
Aug 1996b, July 1997	Johnson et al. (2004)
Aug-Sept 1998	Barbiero et al. (2001)
Aug 2001	Brown and Branstrator (2004)

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Chapter II

The effect of *Bythotrephes longimanus* on the Lake Superior zooplankton community

Introduction

Species introductions in aquatic ecosystems can alter the abundance and community composition of zooplankton (Brooks and Dodson 1965, Carpenter et al. 1987). In North America, introductions of *Bythotrephes longimanus,* a predatory cladoceran native to northern Europe and Asia (Lehman 1987, Burkhardt and Lehman 1994), have resulted in reduced zooplankton species richness and abundance in the Great Lakes and small inland lakes (Barbiero and Tuchman 2004a, Strecker et al. 2006, Azan et al. 2015). *Bythotrephes* prefers slow-moving cladocerans such as *Bosmina longirostris* and *Daphnia spp.* (Vanderploeg et al. 1993, Grigorovich et al. 1998, Schulz and Yurista 1999). Because they possibly lack antipredator responses to *Bythotrephes, Bosmina* may be especially susceptible to predation by *Bythotrephes* (Kerfoot et al. 2016). *Bosmina* abundance has declined following *Bythotrephes* invasion in a number of inland lakes, and multiple studies suggest that this was a direct result of consumption by *Bythotrephes* (Yan and Pawson 1997, Yan et al. 2002, Kerfoot et al. 2016).

Rapid cladoceran community changes in the Great Lakes following *Bythotrephes* invasion were observed in Lake Michigan in the late 1980s, when two of three common *Daphnia* species nearly disappeared within a year of the first detection of *Bythotrephes* (Lehman 1988, Lehman 1991, Lehman and Cáceres 1993). This reduction in *Daphnia* abundance was accompanied by an increase in the average size of remaining individuals which led Lehman (1991) to the conclusion that planktivory by *Bythotrephes,* rather than fish, was the most likely cause for these changes. *Daphnia mendotae* has remained common in Lake Michigan since *Bythotrephes* establishment, but appears to have done so by reducing the extent of its vertical overlap with *Bythotrephes* (Pangle and Peacor 2006, Pangle et al. 2007). Similar changes have now been described in the cladoceran communities of Lakes Huron and Erie (Barbiero and Tuchman 2004a, Bunnell et al. 2012).

Bythotrephes was first detected in Lake Superior in 1987 (Cullis and Johnson 1988), but the effect that it has on the zooplankton community is largely unknown. Brown and Branstrator

(2004) reported a lower abundance of *Bosmina* in August of 2001 compared to observations from the early 1970s. Though reductions in *Bosmina* abundance are consistent with planktivory by *Bythotrephes*, the authors could not clearly demonstrate such a relationship given the temporal limitations of their dataset. More recent zooplankton surveys suggest that *Bosmina* has indeed become less abundant since the 1970s while populations of the cladocerans *Daphnia mendotae* and *Holopedium gibberum* (all cladocerans hereafter referred to by genus, with *Daphnia* referring to *Daphnia mendotae* unless noted otherwise) have remained relatively stable (Pawlowski MS Thesis chapter I).

Previous studies in Lakes Michigan and Huron have compared estimates of *Bythotrephes* consumptive demands to estimates of zooplankton production to determine whether *Bythotrephes* is capable of suppressing zooplankton biomass. In both lakes, the consumptive demands of *Bythotrephes* can exceed cladoceran production and sometimes total zooplankton production in the epilimnion (Lehman and Cáceres 1993, Burkhardt and Lehman 1994, Yurista et al. 2010, Bunnell et al. 2011, Bunnell et al. 2012). These findings support the hypothesis that *Bythotrephes* could be responsible for some of the recent changes in Great Lakes cladoceran communities. While other recent studies have investigated the factors influencing *Bythotrephes* production in Lake Superior (Keeler et al. 2015), comparisons of *Bythotrephes* consumption and zooplankton production in Lake Superior have not been made.

Peak *Bythotrephes* densities in Lake Superior are generally lower than in Lakes Michigan and Huron (Barbiero et al. 2001, Brown and Branstrator 2004, Brown et al. 2012, Pothoven et al. 2012, Bunnell et al. 2014). Lake Superior is colder and less productive than the other Great Lakes (Patalas 1972) and its zooplankton community is more heavily dominated by calanoid copepods which are less favored and lower quality prey for *Bythotrephes* (Barbiero et al. 2001, Lehman and Branstrator 1995). Because of this, the consumptive demands of *Bythotrephes* in Lake Superior may still approach the rate of production of their preferred prey species even if *Bythotrephes* abundance is low. Demonstrating that consumption by *Bythotrephes* consistently approaches or exceeds production of prey species including *Bosmina*, would support a top-down hypothesis of *Bythotrephes* control on cladoceran community change in Lake Superior.

The addition of *Bythotrephes* to the Lake Superior food web is not the only important change that has occurred in this ecosystem in recent decades. Since the 1970s, average summer surface temperatures in Lake Superior have increased and populations of planktivorous fish have

fluctuated (Austin and Colman 2007, Gorman 2012, Pratt et al. 2016). These trends in climate and planktivorous fish populations are expected to continue (Lehman 2002, Bronte et al. 2003, Gorman 2012, Pratt et al. 2016). Because both temperature and vertebrate planktivory influence zooplankton community structure and zooplankton biomass, including that of *Bythotrephes* (Plante and Downing 1989, Brooks and Dodson 1965, Shuter and Ing 1997, Keeler et al. 2015), the impact of *Bythotrephes* on the Lake Superior food web is likely to change in the future.

The reduction in *Bosmina* abundance in Lake Superior since the 1970s and the lack of obvious change in the abundances *Daphnia* and *Holopedium* is consistent with cladoceran community changes attributed to *Bythotrephes* in other invaded lakes. Here I evaluate the relationship between *Bythotrephes* and these three herbivorous cladocerans and test three hypotheses regarding top-down control by *Bythotrephes*: 1) *Bythotrephes* and herbivorous cladocerans overlap vertically; 2) simultaneous densities of *Bythotrephes* and herbivorous cladoceran are negatively correlated; and 3) consumptive demands of *Bythotrephes* exceed production of the herbivorous cladoceran species. I hypothesized that evidence will support *Bosmina* as the cladoceran species most vulnerable to suppression by *Bythotrephes*. To place planktivory by *Bythotrephes* in a broader ecological context, I also compared the consumptive demands of *Bythotrephes* to total epilimnetic zooplankton production over a range of temperatures and *Bythotrephes* densities. I hypothesized that total zooplankton production in Lake Superior is currently in excess of *Bythotrephes* consumptive demands but that this could change as a result of continued climate warming and changes in the abundance of *Bythotrephes*.

Methods

Determining the vertical distributions of cladocerans

At each zooplankton collection site (see chapter I methods; Fig. 1.1), vertical net tows were taken to depths of 15 m and 60 m. I compared the estimated aerial densities (individuals m$^-$ ^{2}) of *Bythotrephes*, *Bosmina*, *Daphnia*, and *Holopedium* in the 15 m and 60 m tows taken at each of the stations using a separate paired, one-tailed t-test ($\alpha = 0.05$) for each species. These tests were done separately for 2014 and 2015 samples. Because aerial densities are not adjusted for the depth of the net tow, it can be inferred that most individuals of a species were present in the upper 15 m of water when the average density of the species in 15 m and 60 m tows are not

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significantly different. A species whose average aerial density is significantly higher in 60 m tows must be present at depths greater than 15 m in considerable numbers.

Simultaneous densities of Bythotrephes *and cladoceran prey*

To determine whether increased density of *Bythotrephes* decreases the densities of their potential cladoceran prey species, I plotted the simultaneous densities of *Bythotrephes* with *Bosmina*, *Daphnia*, and *Holopedium*. Densities derived from 15 m and 60 m tows were plotted separately to control for the effect of depth on estimated species density. Data from 2014 and 2015 were pooled for these comparisons.

Bythotrephes *consumptive demands and prey production*

I used two published models to estimate average daily *Bythotrephes* consumptive demands and average daily production of the available crustacean prey for each month sampled. Both models are based on temperature and estimated biomass. Temperature data were from CTD (Seabird Electronics) casts taken at most zooplankton sampling sites (see chapter I methods). Past work suggests that in lakes with planktivorous fish such as lake herring (*Coregonus artedi*), *Bythotrephes* generally remains in the top 15 m of water during day and night (Young and Yan 2008). For this reason, estimates of *Bythotrephes* consumptive demands and zooplankton production were based on biomass estimates from 15 m net tows only. The use of biomass data from 15 m tows also reduced uncertainty regarding vertical overlap between predator and prey compared to biomass data from 60 m tows.

Estimating Bythotrephes *consumptive demands*

Bythotrephes has three developmental instars and the consumptive demand (*C*) of each instar was estimated separately for each month sampled using the general linear model from Yurista et al. (2010):

$$C = a + b \times t(°C) + c \times t(°C)^2 + d \times wt(\mu g) + e \times wt(\mu g) \times t(°C) \qquad \text{(Eq. 2.1)}$$

where *a*, *b*, *c*, *d*, and *e* are instar-specific coefficients (available in Yurista et al. 2010), *t* is the average daily temperature in the top 15 m of water (estimated as the midpoint between the average temperature observed during sample collection and the average temperature observed

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during the previous sampling cruise), and *wt* is the median dry weight of the respective *Bythotrephes* instar. *Bythotrephes* dry weight was estimated based on Branstrator (2005) as:

$$Total\ dry\ weight\ (\mu g) = 45.2 \times total\ length\ (mm) - 287.2 \qquad (Eq.\ 2.2)$$

This regression was used for all three developmental instars of *Bythotrephes* and individual lengths were corrected for shrinkage in ethanol before dry weight estimation (see chapter I methods). Average daily consumptive demands of the *Bythotrephes* population between sampling events were estimated by multiplying the instar-specific consumption estimate for each month sampled by the average observed density of that instar and summing the resulting three estimates.

Estimating zooplankton production

Zooplankton production (*P*) was estimated based on Shuter and Ing (1997) as:

$$P = 10^{\,\alpha_{taxon} + \beta \times t(°C)} \times B \qquad (Eq.\ 2.3)$$

where *α* is a taxon-specific intercept for cladocerans, cyclopoids, and calanoids (-1.645, -1.844, and -2.294, respectively), *β* is the slope (0.04336), *B* is the biomass (dry weight) of the taxonomic group, and *t* is the average daily temperature in the top 15 m of water between sampling events.

Two calanoid species common in Lake Superior, *Limnocalanus macrurus* and *Senecella calanoides,* are large and fast swimming, and show little vertical overlap with *Bythotrephes* (Oliver et al. 2015). Because these cold-water stenotherms are not likely prey for *Bythotrephes,* calanoid production estimates were based only on the average monthly biomasses of calanoid nauplii and copepodids of *Leptodiaptomus sicilis* and *Epischura lacustris*. Following the methods of Bunnell et al. (2012), the biomasses of calanoid and cyclopoid nauplii were estimated by multiplying total nauplii biomass by the proportion of calanoid and cyclopoid copepodids present in each month.

Comparisons of Bythotrephes *consumption and zooplankton production*

For each month where *Bythotrephes* was observed, I estimated the density of *Bythotrephes* that would have been needed for *Bythotrephes* consumptive demands to overwhelm production of each of the three herbivorous cladoceran species. Each of these estimates assumed that *Bythotrephes* consumed only the prey species in question.

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Though cladocerans are their preferred prey, *Bythotrephes* also consumes nauplii and small copepods (Schulz and Yurista 1995, Schulz and Yurista 1999, Dumitru et al. 2001). I calculated the percent of cladoceran, calanoid, and cyclopoid production that would be consumed by *Bythotrephes* if *Bythotrephes* preyed only upon each of these broader groups. Assuming that *Bythotrephes* preys upon only one group or species oversimplifies actual prey selection by *Bythotrephes*. To account for this, I summed the production rates of the three broad taxonomic groups in the upper 15 m of water to estimate the proportion of total epilimnetic zooplankton production needed to meet *Bythotrephes* consumptive demands.

Modelling Bythotrephes *consumption with changes in temperature and* Bythotrephes *density*

To understand how future changes in surface water temperature and the abundance of *Bythotrephes* could influence the consumptive requirements of *Bythotrephes* and its impact on the Lake Superior zooplankton community, I repeated estimates of total epilimnetic zooplankton production and *Bythotrephes* consumption over a wide range of temperatures and *Bythotrephes* densities. The calanoid, cyclopoid, and cladoceran biomass data used in production estimates were the averages from October of 2015. Zooplankton biomass from this month represents the highest zooplankton biomass (excluding *L. macrurus* and *S. calanoides)* observed in 2015 (Pawlowski MS Thesis chapter I) and zooplankton biomass was held constant for all production estimates. The median dry weights of each *Bythotrephes* instar used for consumption estimates were also those observed in October of 2015.

To simulate climate warming, I increased the average daily surface temperature used in the production and consumption estimates from the average observed in October of 2015 (approximately 10°C) by increments of 1°C up to 25°C. A ceiling of 25°C was chosen as the maximum temperature for this simulation because *Bythotrephes* reproduction and survival declines above this temperature (Garton et al. 1990, Kim and Yan 2010, Yurista et al. 2010). Average surface temperatures in the offshore regions of Lake Superior currently do not reach 25°C, and are not likely to in the foreseeable future (Lehman 2002), but the output from this simulation will demonstrate how consumption and production compare over both realistic and unrealistic climate warming scenarios.

The *Bythotrephes* densities used for consumption estimates were varied from 1 to 131 individuals m^{-2} for each instar (i.e., total *Bythotrephes* density ranged from 3–393 individuals m^{-2}). I selected 131 individuals m^{-2} per instar as the maximum *Bythotrephes* density in this

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simulation because it approximates the maximum *Bythotrephes* densities that have been reported in Lake Superior in the past (393 individuals m^{-2}, Isaac et al. 2012). I assumed equal densities of individuals in each instar because instar densities were not provided in Isaac et al. (2012).

Estimates of total zooplankton production at each temperature were compared to the estimates of *Bythotrephes* consumption from each combination of temperature and *Bythotrephes* density. The percent of total zooplankton production required to meet the consumptive demands of *Bythotrephes* at each combination of temperature and *Bythotrephes* density was then calculated and plotted on a 3 dimensional surface plot so that interactions between temperature and *Bythotrephes* density could be observed.

Results

Vertical distributions of Bythotrephes *and cladoceran prey*

The average aerial density of *Bythotrephes* was higher in 2014 than in 2015 but the differences in *Bythotrephes* densities between 15 m and 60 m tows were not significantly different in either year (Table 2.1). Average aerial densities of *Bosmina* and *Holopedium* were also not significantly different between 15 m and 60 m tows in either year (Table 2.1). *Daphnia* aerial densities were statistically higher in 60 m tows than in 15 m tows in 2014 but were not significantly different in 2015 (Table 2.1).

Simultaneous densities of Bythotrephes *and cladoceran prey*

There was a strong inverse relationship in the densities of *Bythotrephes* and *Bosmina* in both 15 m and 60 m net tows (Fig. 2.1). High simultaneous densities of *Bythotrephes* and *Bosmina* were never observed. *Daphnia* was generally most common when *Bythotrephes* was absent or in low densities, however, large densities of *Bythotrephes* and *Daphnia* occasionally coincided (Fig. 2.1). *Daphnia* densities in 60 m tows were less negatively correlated with *Bythotrephes* densities than in 15 m tows, likely because a portion of the *Daphnia* population caught in 60 m tows does not overlap vertically with *Bythotrephes* (Fig. 2.1, Table 2.1). Overall, the densities of *Bythotrephes* and *Daphnia* were less inversely correlated than densities of *Bythotrephes* and *Bosmina*. *Holopedium* was not observed in high densities in this study.

However, the largest densities of *Holopedium* usually occurred when *Bythotrephes* were also abundant (Fig. 2.1). These data do not demonstrate a strong positive or negative relationship between *Holopedium* and *Bythotrephes* abundances.

Comparisons of Bythotrephes *consumptive demands and epilimnetic prey production*

       Bythotrephes densities varied widely by month during 2014 and 2015 and tended to be larger in July-October than in May-June (Table 2.2). August of 2014 had the highest monthly *Bythotrephes* density observed in this study with approximately 30.4 individuals m^{-2} (Table 2.2). This density was considerably larger than the average density observed during any other month sampled and was heavily influenced by one sampling station where the *Bythotrephes* density exceeded 200 individuals m^{-2}. On average, consumption by *Bythotrephes* did not exceed total epilimnetic zooplankton production estimates in any month sampled (Fig. 2.2). In August of 2014, *Bythotrephes* consumption was approximately 58% of the total available zooplankton production in the epilimnion (Fig. 2.2). However, if it is assumed that *Bythotrephes* in Lake Superior consume primarily cladocerans, the consumptive demands of *Bythotrephes* would have overwhelmed total cladoceran production in this month.

       The consumptive demands of *Bythotrephes* were compared to the production rate of each herbivorous cladoceran species individually and the *Bythotrephes* density required for consumption to outpace production of each species was determined. Estimated *Bythotrephes* consumptive demands did not exceed production of *Daphnia* in any month except during August of 2014 (Fig. 2.3). *Bythotrephes* densities in the fall of 2014 and 2015 were too low to overwhelm *Daphnia* production. The density of *Bythotrephes* required to overwhelm production rates of *Bosmina* and *Holopedium* was surpassed in every month where *Bythotrephes* was detected (Fig. 2.3).

Simulated changes in temperature and Bythotrephes *abundance*

       Projections of *Bythotrephes* consumptive demands and total epilimnetic zooplankton production in response to simulated increases in temperature and *Bythotrephes* density were made for October of 2015. Fig. 2.4 shows that when *Bythotrephes* density was increased to the maximum density reported in Isaac et al. (2012), *Bythotrephes* consumptive demands were three times higher than epilimnetic zooplankton production. On the other hand, when *Bythotrephes* densities were held constant and surface temperatures were increased, the proportion of

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zooplankton production needed to meet *Bythotrephes* consumptive requirements diminished (Fig. 2.4). However, *Bythotrephes* consumption responded more rapidly to changes in *Bythotrephes* density than to changes in temperature. As a result, the proportion of zooplankton production needed to meet the energetic requirements of *Bythotrephes* increased when *Bythotrephes* density and temperature were increased simultaneously.

Discussion

In the decades since the establishment of *Bythotrephes* in Lake Superior, *Bosmina* has become a less important member of the pelagic zooplankton community while the relative abundances of the other two common cladoceran species, *Daphnia* and *Holopedium*, have remained stable. Similar changes have occurred in the cladoceran communities of other lakes following *Bythotrephes* invasion (Lehman 1991, Yan and Pawson 1997, Kerfoot et al. 2016). I evaluated evidence for the role of predation by *Bythotrephes* in these community changes using three criteria. These criteria included analyses of both vertical and spatial distributions of *Bythotrephes*, *Bosmina*, *Daphnia*, and *Holopedium* and comparisons of the consumptive demands of *Bythotrephes* to the production rates of each herbivorous cladoceran species.

Bosmina

Both *Bosmina* and *Bythotrephes* were caught primarily in the top 15 m of water during both 2014 and 2015 (Table 1). This is consistent with the vertical distribution of *Bythotrephes* observed in other lakes with planktivores like lake herring (Young and Yan 2008). Past studies of the vertical distributions of cladocerans in Lake Michigan reported a downward shift in the average vertical position of *Bosmina* following *Bythotrephes* invasion (Pangle et al. 2007). In this way, *Bythotrephes* might indirectly reduce *Bosmina* production by causing *Bosmina* to inhabit colder water (Pangle et al. 2007). However, the data presented here indicate that *Bosmina* in Lake Superior should encounter *Bythotrephes* in the water column and that low *Bosmina* biomass is not a result of inhabiting colder water.

Despite occupying similar depths, the simultaneous densities of *Bosmina* and *Bythotrephes* imply a general lack of co-presence between these species. Peak densities of *Bosmina* were observed only when *Bythotrephes* was absent or uncommon (Fig. 2.1). This could be evidence for consumption of *Bosmina* by *Bythotrephes*. The consumptive demands of

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Bythotrephes always exceeded *Bosmina* production when *Bythotrephes* was present. While *Bosmina* production was always too low to meet the energetic requirements of *Bythotrephes*, these models demonstrate that it takes very few *Bythotrephes* eating only *Bosmina* to overwhelm average *Bosmina* production. In addition to being a preferred prey species for *Bythotrephes*, new evidence suggests that *Bosmina* may lack avoidance responses to *Bythotrephes* (Kerfoot et al. 2016). Therefore, it is possible that *Bosmina* remains an easy prey item for *Bythotrephes* in Lake Superior despite being present at low densities.

Daphnia

Large numbers of *Daphnia* were sometimes caught below 15 m, indicating that overlap between *Bythotrephes* and *Daphnia* was reduced. *Daphnia* has remained common in Lake Superior, as in several other lakes, since the establishment of *Bythotrephes* (Lehman 1991, Azan et al. 2015). As with *Bosmina*, the average vertical position of *Daphnia* in Lake Michigan shifted downward after *Bythotrephes* invasion (Lehman and Cáceres 1993, Pangle et al. 2007). This change in habitat use has been used to explain the persistence of *Daphnia mendotae* in Lake Michigan since the establishment of *Bythotrephes* despite reduced biomasses of other species of *Daphnia*. Interestingly, *Daphnia* densities in this study were only significantly greater in 60 m tows during 2014. Peak densities of *Bythotrephes* were higher in 2014 than in 2015 in both summer and fall (Table 2.1, Fig. 2.3), which could mean that *Daphnia* in Lake Superior reduce their vertical overlap with *Bythotrephes* only when *Bythotrephes* reaches some threshold density. This would help to explain why *Daphnia* has remained common in Lake Superior while *Bosmina* abundance has declined since the establishment of *Bythotrephes*. Occupying colder waters is not without consequence, though, and *Daphnia* in Lake Superior may experience reduced production as a result of migrating downward to avoid *Bythotrephes*.

Regardless of the potential consequences of occupying cooler waters, monthly production rates of *Daphnia* exceeded the consumptive demands of *Bythotrephes* in all but one month sampled. *Daphnia* also co-occurred with *Bythotrephes* more often than did *Bosmina* which is consistent with lower relative consumption rates of *Daphnia* by *Bythotrephes*. These findings support the hypothesis that *Daphnia* have remained common in Lake Superior because their behaviors reduce their vulnerability to suppression by *Bythotrephes*.

Holopedium

Like *Bosmina*, most *Holopedium* biomass occurred in the top 15 m of water, implying vertical overlap with *Bythotrephes.* In addition, the consumptive demands of *Bythotrephes* always exceeded *Holopedium* production when *Bythotrephes* was present. While these findings suggest that *Holopedium* could be suppressed by *Bythotrephes* in Lake Superior, *Holopedium* abundance was not negatively correlated with *Bythotrephes* abundance. *Holopedium*, which has not become less common in Lake Superior since the arrival of *Bythotrephes*, may be less vulnerable to predation by *Bythotrephes* because of its gelatinous coating and have occasionally become more common in other lakes after *Bythotrephes* invasion (Yan and Pawson 1997, Kerfoot et al. 2016). The relative frequency of vertical and spatial overlap between these species in the present study suggests that *Holopedium* is not a preferred prey source for *Bythotrephes* in Lake Superior. This could explain why *Holopedium* has not become less abundant in Lake Superior since the establishment of *Bythotrephes*.

The role of Bythotrephes *in cladoceran community change*

Of the common herbivorous cladocerans in Lake Superior, *Bosmina* was the only species that met all three of the criteria used in this study to evaluate vulnerability to suppression by *Bythotrephes*. Because *Bosmina* is also the only herbivorous cladoceran species to become less common in Lake Superior since the establishment of *Bythotrephes*, these findings support the hypothesis that *Bosmina* has become less common in Lake Superior as a result of top-down control by *Bythotrephes*.

It is unlikely that increasing surface water temperatures or vertebrate planktivory were responsible for the decline in this formerly abundant species. Herbivorous cladocerans are less abundant in Lake Superior than in the other Great Lakes and this is due, in part, to the low surface water temperatures in Lake Superior (Patalas 1972, Lehman 2002). While cladoceran abundance also appears to be constrained by primary productivity in Lake Superior (Pawlowski MS Thesis, Chapter I), increasing temperatures in Lake Superior should not lead to a reduction in *Bosmina* abundance. Populations of planktivorous fish like lake herring have increased in Lake Superior since the 1970s, but *Bosmina* is not effectively retained in the gill rakers of mature lake herring and *Bosmina* is not observed in the diet of this or other common pelagic planktivorous fish (e.g.,

rainbow smelt, *Coregonus spp.*) in Lake Superior (Link and Hoff 1998, Isaac et al. 2012, Gamble et al. 2011a). Juvenile fish may feed on *Bosmina*, but year classes of the various coregonines and smelt have been irregular in Lake Superior in recent decades (Bronte et al. 2003, Johnson et al. 2004, Gorman 2012, Pratt et al. 2016) while the reduction in *Bosmina* abundance appears to be persistent.

These findings suggest that *Bythotrephes* has had a measurable impact on the cladoceran community in Lake Superior. *Bythotrephes* necessarily consumes prey other than *Bosmina* and the consumptive demands of *Bythotrephes* often reached half or more of estimated cladoceran production. Seasonally, cladocerans are important sources of prey for some species of planktivorous fish in Lake Superior (Gamble et al. 2011a, Isaac et al. 2012) and *Bythotrephes* may reduce the amount of cladoceran biomass available for such planktivores, especially those that are also unable to eat *Bythotrephes*.

Implications of Bythotrephes *consumption for total zooplankton biomass*

Bythotrephes is thought to be an important competitor with planktivorous fish for food resources in some lakes (Hoffman et al. 2001, Strecker and Arnott 2008). With the exception of August of 2014, when *Bythotrephes* consumptive demands were more than half of total epilimnetic zooplankton production, total zooplankton production was much greater than the consumptive demands of *Bythotrephes*. These results suggest that *Bythotrephes* was not abundant enough during 2014-2015 to cause large reductions in overall food availability for planktivorous fish. Furthermore, the estimates of total zooplankton production in this study did not include production of *L. macrurus*, which is an abundant and important prey item for many planktivorous fish in Lake Superior (Gamble et al. 2011a, Isaac et al. 2012).

The consumptive demands of *Bythotrephes* relative to epilimnetic zooplankton production in this study were also somewhat lower than similar estimates for Lake Huron (Bunnell et al. 2012). Cladoceran species richness has declined in Lake Huron since the 1980s and the overall biomass of cladocerans and cyclopoids has been in decline since the mid-2000s (Barbiero and Tuchman 2004a, Barbiero et al. 2009a, Barbiero et al. 2012). While some of these changes were the result of reductions in primary productivity (i.e., food availability for zooplankton) and changes in vertebrate planktivory over the last several decades, *Bythotrephes* is thought to be at least partially responsible (Barbiero and Tuchman 2004a, Bunnell et al. 2012). Rates of primary production in Lake Superior have not declined in recent decades as they have in

Lake Huron (Pratt et al. 2016). If planktivory by *Bythotrephes* was an important factor in the reductions of cladoceran and cyclopoid biomass in Lake Huron since the mid-2000s, then *Bythotrephes* could potentially cause further changes in the zooplankton community in Lake Superior in the future.

Though *Bythotrephes* consumes some copepod nauplii and copepodids (Schulz and Yurista 1999, Dumitru et al. 2001), the relative abundances of the most common copepods in Lake Superior, *Leptodiaptomus sicilis* and *Diacyclops thomasi*, have not changed appreciably since the 1970s (Pawlowski MS Thesis Chapter I). However, the individual production rates of calanoids and cyclopoids in this study were occasionally met or surpassed by the consumptive demands of *Bythotrephes* (Fig. 2.2). *Bythotrephes* is thought to have suppressed populations of some small copepod species in other lakes (Yan and Pawson 1997, Barbiero and Tuchman 2004a, Kerfoot et al. 2016). In Lake Superior, limitations in cladoceran biomass could result in elevated consumption of small copepods by *Bythotrephes*. The extent of predation on copepods by *Bythotrephes* in Lake Superior could be evaluated by observing *Bythotrephes* prey selection in conditions that simulate the densities and ratios of cladocerans and copepods in the offshore regions of Lake Superior.

Interestingly, the densities of *Bythotrephes* observed in the present study were considerably lower than those reported in Brown and Branstrator (2004) and Isaac et al. (2012). It is unclear why the *Bythotrephes* densities in 2014-2015 were so much lower than in previous summers. One possibility is that the true peaks in *Bythotrephes* density occurred in between sampling events and were not observed. *Bythotrephes* densities are usually highest in late summer or fall in Lake Superior (Isaac et al. 2012). We were unable to collect zooplankton samples in September of 2014 and too few samples were collected in August of 2015 to determine *Bythotrephes* abundance in that month. Past work shows that peak *Bythotrephes* densities in an inland reservoir are short-lived (Brown et al. 2012). If peak *Bythotrephes* densities in Lake Superior are as brief as those in inland lakes, it is likely that the *Bythotrephes* densities we observed in 2014 and 2015 did not represent true peak densities for either year. Therefore, the estimates of *Bythotrephes* consumptive demands in this study are likely to be conservative.

Bythotrephes *consumption simulations and future food web implications*

While the densities of *Bythotrephes* observed in this study were not high enough to overwhelm total epilimnetic zooplankton production in any month sampled, the densities needed

to do so were within the range of *Bythotrephes* densities observed in Lake Superior in the past. Fig. 2.4 shows that increasing the density of *Bythotrephes* to the maximum quantities observed by Isaac et al. (2012) resulted in estimates of *Bythotrephes* consumption three times greater than total zooplankton production in the epilimnion. However, even the average density of *Bythotrephes* observed by Isaac et al. (2012) in fall of 2005 (135 individuals m^{-2}) would have been sufficient to overwhelm zooplankton production in October of 2015. Because it does not appear that the abundance of any species besides *Bosmina* has declined in Lake Superior since the establishment of *Bythotrephes*, peaks in *Bythotrephes* densities of the magnitude observed in Isaac et al. (2012) are likely to be brief.

Because temperature is one of the factors that influences both zooplankton production and *Bythotrephes* consumption, the consumptive demands of *Bythotrephes* relative to prey production in Lake Superior are likely to change with further climate warming. While the climate warming simulation showed that zooplankton production increased faster than *Bythotrephes* consumption at constant *Bythotrephes* densities, *Bythotrephes* consumption increased faster than total zooplankton production when *Bythotrephes* density and surface water temperatures increased simultaneously. Because *Bythotrephes* did not appear to be limited by food availability in this study, low surface temperatures might be one of the factors limiting *Bythotrephes* abundance in Lake Superior. Indeed, past studies indicate that the optimal temperatures for *Bythotrephes* growth and reproduction are in the range of 18-22°C (Yurista 1999, Kim and Yan 2010, Yurista et al. 2010), which are temperatures not consistently met in the offshore regions of Lake Superior (Austin and Colman 2007). This suggests that positive interactions between *Bythotrephes* densities and temperature, like those simulated in this study, could occur in the future. If *Bythotrephes* becomes more abundant as a result of further climate warming, the top-down effects of *Bythotrephes* on Lake Superior zooplankton will increase and make it a more important competitor with planktivorous fish.

Factors other than temperature also influence *Bythotrephes* abundance in Lake Superior and forecasting the future effects of *Bythotrephes* on the Lake Superior food web requires caution. Though increased surface temperatures could result in higher abundances of *Bythotrephes* in Lake Superior, *Bythotrephes* is also heavily consumed by planktivorous fish like lake herring (Isaac et al. 2012, Keeler et al. 2015). Keeler et al. (2015) showed that *Bythotrephes* production can be overwhelmed by vertebrate planktivory in the offshore regions of the Apostle Islands. If this occurs throughout the lake, a small positive interaction between increasing surface

water temperatures and *Bythotrephes* density may be masked by vertebrate planktivory. Because the consumptive demands of the *Bythotrephes* population are highly dependent on *Bythotrephes* density, future populations of the fish that consume *Bythotrephes* will also influence the effects of *Bythotrephes* on the Lake Superior zooplankton community in the coming decades.

Conclusions

The pattern of cladoceran community change observed in Lake Superior in recent years is inconsistent with the expected effects of long-term changes in surface temperatures, primary production, or vertebrate planktivory. The results of this study support the hypothesis that *Bythotrephes* exerts top-down control on the cladoceran community and has suppressed the population of *Bosmina* in Lake Superior. While *Bosmina* is not a particularly important source of food for planktivorous fish, *Bythotrephes* necessarily consumes other species and may reduce energy flow to higher trophic levels in Lake Superior. The future effects of *Bythotrephes* on the Lake Superior zooplankton community will depend on *Bythotrephes* abundance. Because *Bythotrephes* consumptive demands did not exceed total prey production in this study, *Bythotrephes* abundance in Lake Superior is likely limited by some combination of temperature and vertebrate planktivory. The relationships between *Bythotrephes* and these factors should be monitored in the future as should further changes in zooplankton community structure and total zooplankton biomass.

Table 2.1. Average aerial densities of *Bythotrephes*, *Bosmina*, *Daphnia*, and *Holopedium* in 15 m and 60 m tows. The 2014 comparisons were based on 30 paired observations and the 2015 comparisons were based on 22 paired observations. Differences in densities were identified with a paired, one-tailed t-test ($\alpha = 0.05$)

	2014			2015		
	# m^{-2} (15 m)	# m^{-2} (60 m)	p-value	# m^{-2} (15 m)	# m^{-2} (60 m)	p-value
Bythotrephes longimanus	11.6	9.2	0.22	1.3	2.4	0.13
Bosmina longirostris	457.4	484.5	0.19	67.6	114.7	0.12
Daphnia mendotae	2227.8	3142.6	0.04	6312.3	6709.6	0.25
Holopedium gibberum	106.3	87.1	0.12	189.2	127.2	0.12

Table 2.2. Average monthly density (individuals m^{-2}) of *Bythotrephes* observed in 15 m net tows. Standard error of the mean is indicated in parentheses. The number of stations visited during each month is indicated by n. Three replicate net tows were taken at each of the stations starting in August of 2014.

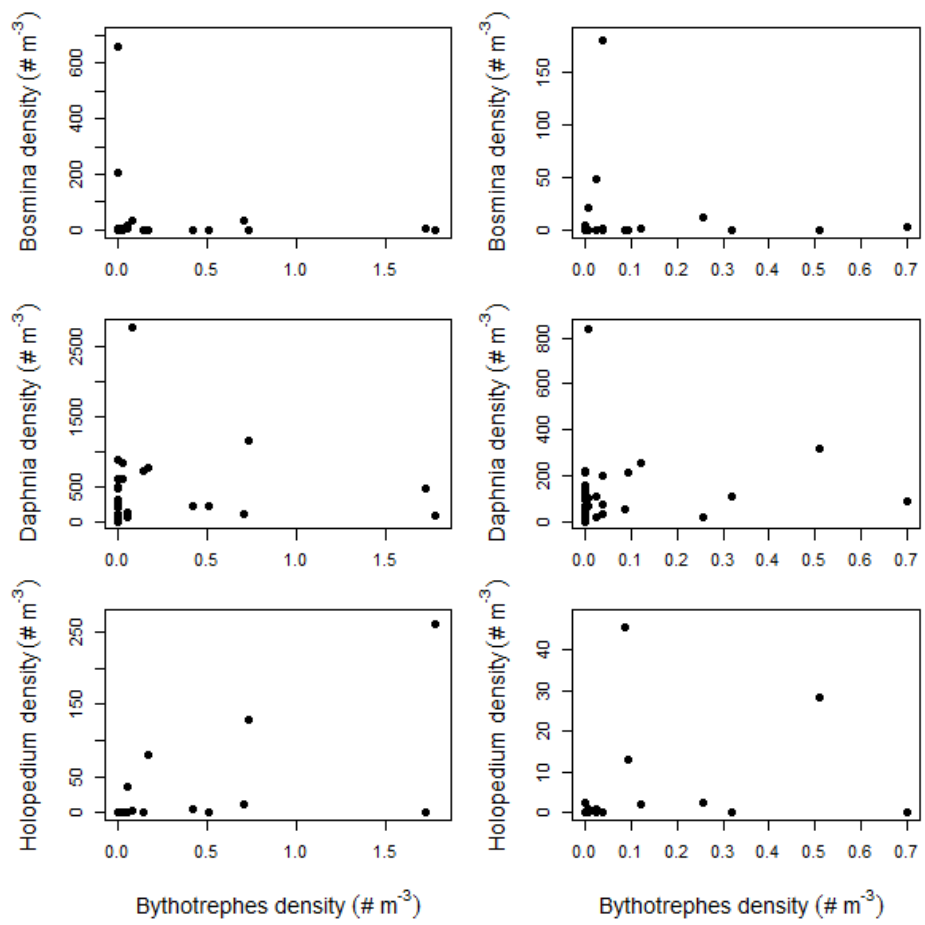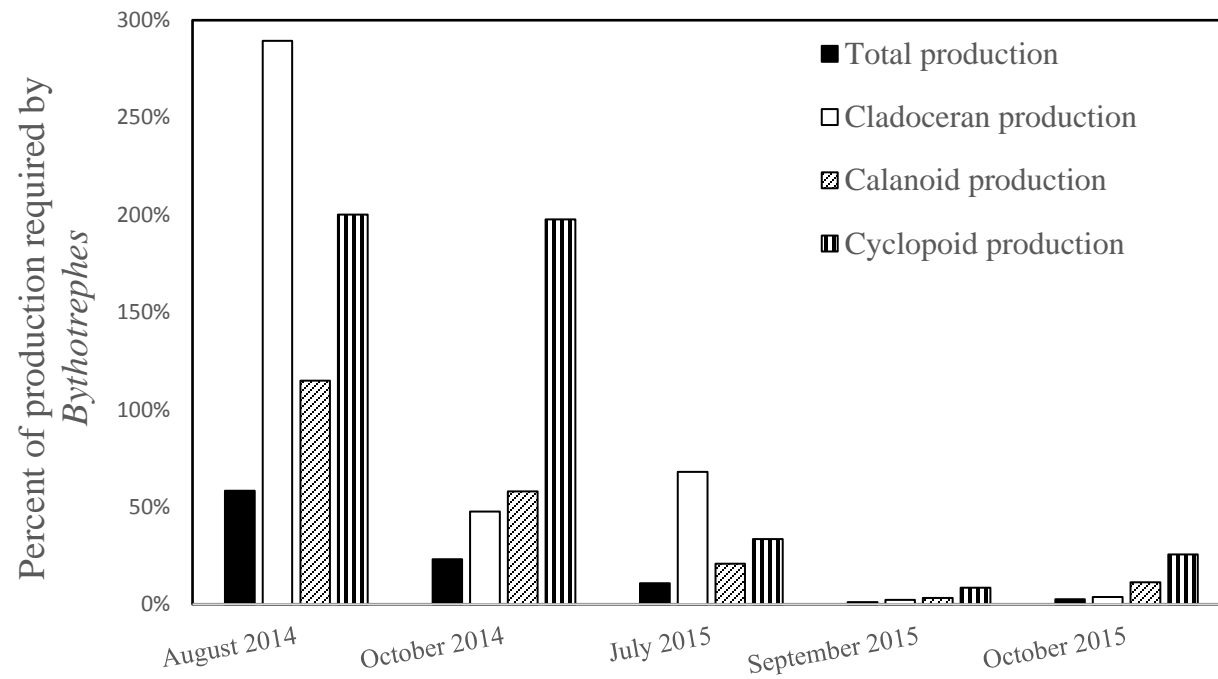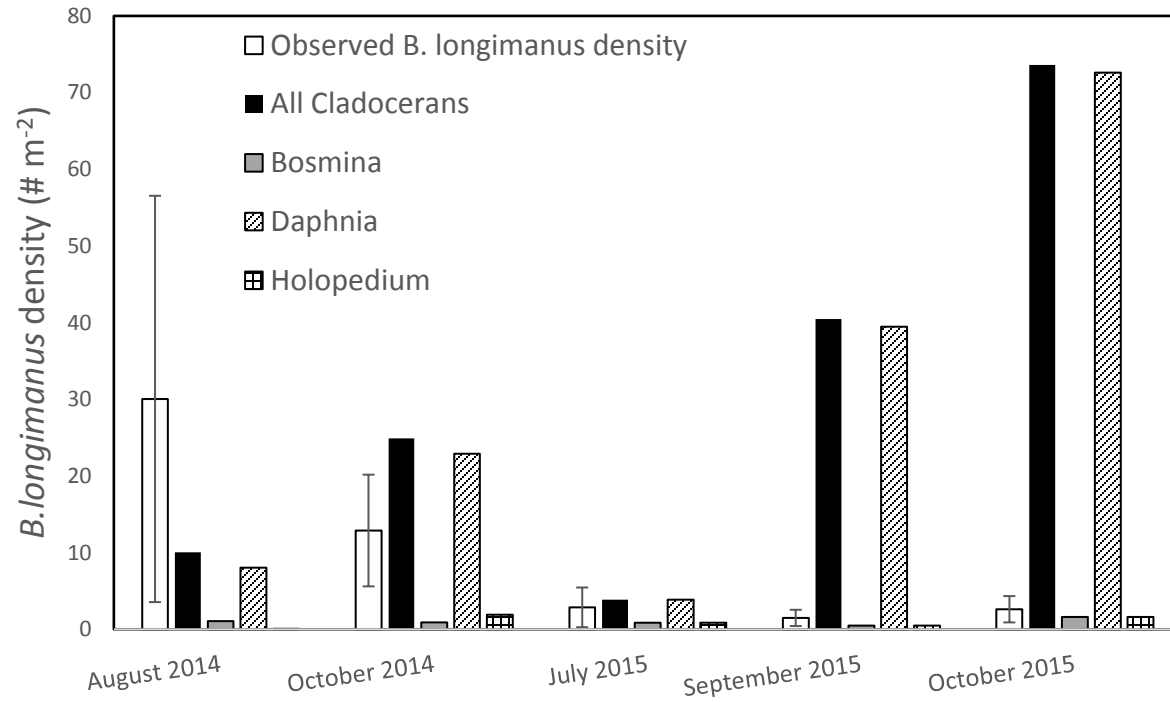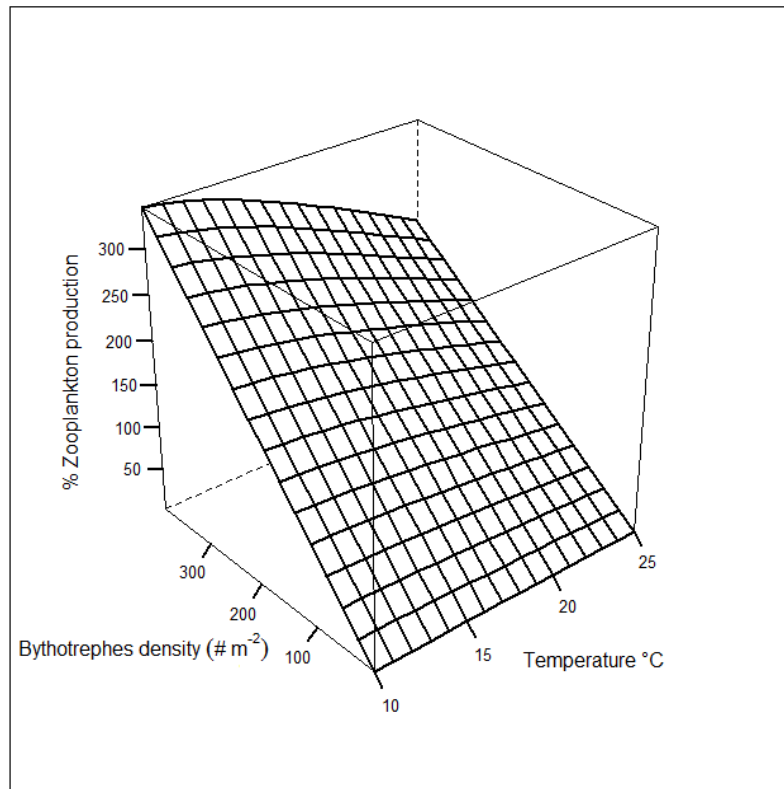|	Average *Bythotrephes* density (# m^{-2})	n
June 2014	0.0	4
July 2014	0.0	3
August 2014	30.4 (26.5)	10
October 2014	12.9 (7.3)	12
May 2014	0.0	5
July 2015	2.9 (2.6)	4
September 2015	1.5 (1.1)	4
October 2015	2.6 (1.7)	4

Figure 2.1

Figure 2.2

Figure 2.3

Figure 2.4

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