Changes in the cladoceran community of Lake Superior and the role of *Bythotrephes longimanus*

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Abstract

Introductions of *Bythotrephes longimanus* have resulted in reduced cladoceran species richness and biomass in the Laurentian Great Lakes and many inland lakes. *Bythotrephes* was first observed in Lake Superior in 1987 but its effect on the cladoceran community has been unknown. We compared the composition of the offshore cladoceran community of Western Lake Superior during 2014 and 2015 to zooplankton surveys from 1971-2001 to determine whether changes in the cladoceran community have occurred. Monthly comparisons show that the contribution of *Bosmina longirostris* to offshore cladoceran numbers was generally twice as much in the 1970s than during 2014-2015 while the relative contribution of *Daphnia mendotae* increased after the 1970s. These community changes are consistent with changes due to *Bythotrephes* observed in other lakes. To evaluate evidence for the role of *Bythotrephes* in these community changes, we used data from 2014-2015 to analyze patterns in spatial and vertical overlap between *Bythotrephes* and its cladoceran prey species (*Bosmina*, *Daphnia*, and *Holopedium*) and compared estimates of consumption by *Bythotrephes* to production of these potential prey. *Bosmina* was the species whose vertical position and rate of production made it most vulnerable to suppression by *Bythotrephes*. Of the potential cladoceran prey species, *Bosmina* densities were also the most negatively correlated with *Bythotrephes* densities. These findings support a hypothesis of top-down effects on *Bosmina* by *Bythotrephes* in Lake Superior. This work informs future zooplankton research in Lake Superior and furthers our understanding of the effects of *Bythotrephes* on the Lake Superior food web.

Keywords: *Bythotrephes*, Lake Superior, Zooplankton, Great Lakes, Invasive species, *Daphnia*

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 (particularly in cladocerans) and abundance in small inland lakes and in the Great Lakes (Barbiero and Tuchman, 2004; Strecker et al., 2006; Azan et al., 2015). *Bythotrephes* selects slow-moving cladocerans such as *Bosmina longirostris* and *Daphnia spp.* (Vanderploeg et al., 1993; Grigorovich et al., 1998; Schulz and Yurista 1999). *Bosmina* and *Daphnia* consistently become less abundant in lakes following *Bythotrephes* invasion and multiple studies suggest that this is a direct result of consumption by *Bythotrephes* (Yan and Pawson, 1997; Yan et al., 2002; Strecker et al., 2006; Kerfoot et al., 2016).

Rapid cladoceran community changes in the Great Lakes following *Bythotrephes* invasion occurred 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). *Daphnia mendotae* has remained common in Lake Michigan since *Bythotrephes* establishment, but appears to have done so partly by reducing the extent of its vertical overlap with *Bythotrephes* (Pangle and Peacor, 2006; Pangle et al., 2007). Other small cladocerans such as *Bosmina* have also become less common in Lake Michigan since the establishment of *Bythotrephes* (Makarewicz et al., 1995; Schulz and Yurista, 1999). Similar changes have been described in the cladoceran communities of Lakes Huron and Erie (Barbiero and Tuchman, 2004; Bunnell et al., 2012). In Lake Ontario, *Bosmina longirostris* and *Eubosmina spp*. abundance has declined by more than half since 2003 (Barbiero et al., 2014; Rudstam et al., 2015). These changes coincided with an order of magnitude increase in *Bythotrephes* abundance after 2003 which suggests that *Bythotrephes* has exerted top-down control on bosminids in Lake Ontario.

Bythotrephes was first detected in Lake Superior, the largest lake on Earth by surface area, in 1987 (Cullis and Johnson, 1988), but its effect on the zooplankton community is largely unknown. The Lake Superior zooplankton community is calanoid-dominated in terms of density and biomass (Patalas, 1972; Barbiero et al., 2001). Historically, the cladoceran community in Lake Superior was dominated by three species including *Bosmina longirostris*, *Daphnia mendotae,* and *Holopedium gibberum* (hereafter referred to by genus, unless otherwise noted). 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. Zooplankton surveys spanning multiple sampling seasons are needed to determine whether these or other community changes have occurred in the decades since *Bythotrephes* establishment.

Previous studies in Lakes Michigan and Huron have suggested that consumption by *Bythotrephes* can exceed production of cladocerans (Lehman and Cáceres, 1993; Bunnell et al., 2011; Bunnell et al., 2012). Consumption by *Bythotrephes* has not yet been estimated in Lake Superior. *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). However, Lake Superior is also colder and less productive than the other Great Lakes (Patalas 1972) and the consumptive demands of *Bythotrephes* in Lake Superior may still approach the rate of production of their preferred prey species. Cladoceran production is subject to a variety of losses other than by invertebrate predation. Thus, increased mortality due to *Bythotrephes* predation might be enough to reduce cladoceran abundance even if consumption by *Bythotrephes* does not exceed cladoceran production.

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, population densities of planktivorous fish have fluctuated in Lake Superior and average summer surface temperatures have increased (Austin and Colman, 2007; Gorman, 2012; Pratt et al., 2016). One key change in the planktivorous fish community since the 1970s has been an overall increase in lake herring (*Coregonus artedi*) densities. Increasing vertebrate planktivory would be expected to cause a downward shift in the average body size of zooplankton (Brooks and Dodson, 1965). Temperature is a key factor driving zooplankton production and spatial aggregation in Lake Superior and increases in surface temperature might increase zooplankton production and density (Watson and Wilson, 1978; Zhou et al., 2001). In addition, the increase in summer surface temperatures in Lake Superior since the 1970s has the potential to favor warm water taxa such as small cladocerans (Lehman, 2002). Though zooplankton abundance and production can also be influenced by changes in primary production, changes in food quality and quantity for herbivorous zooplankton are unknown over the period of *Bythotrephes* invasion. However, changes in food availability would be expected to have similar effects on the densities of all herbivorous zooplankton rather than effects on specific taxa. Therefore, while changes in temperature, vertebrate planktivory, and primary production all can cause changes in zooplankton community structure, the effects of these ecosystem changes on the cladoceran community should be distinguishable from top-down effects by *Bythotrephes*.

The purpose of this study is to determine whether long-term changes in the cladoceran community of Lake Superior have occurred since the introduction of *Bythotrephes*. Based on changes observed in the cladoceran communities of the other Great Lakes and smaller, inland lakes following *Bythotrephes* invasion, we hypothesized that small cladocerans such as *Bosmina* would be less common in offshore areas of Lake Superior than before *Bythotrephes* invasion. To test this, we compared the offshore cladoceran community observed during the 2014 and 2015 growing seasons to past zooplankton surveys. Further, we used the data from 2014 and 2015 to evaluate three lines of evidence that *Bosmina*, *Daphnia,* and *Holopedium* populations are currently negatively impacted by the presence of *Bythotrephes*. These lines of evidence were: 1) the extent of synchronous spatial overlap among the prey taxa and *Bythotrephes*, 2) patterns in vertical position of the prey taxa relative to *Bythotrephes*, and 3) the difference in temperature-driven production of the prey taxa versus temperature-driven consumptive demands of *Bythotrephes*.

Methods

Sampling sites and dates

Zooplankton and water temperature data were collected in the western arm of Lake Superior (Fig. 1) from the R/V Blue Heron. In 2014, collections occurred on June 3-6, July 23-25, August 11-14, August 17-19, October 1-2, and October 16-19; in 2015 collections occurred on May 20-22, July 15-17, September 8-10, October 5-7, and October 16-19. The number of stations visited in each month is indicated in Table 2. Stations 5, 12, and 15 were sampled in every month and station 7 was sampled in every month except July and October of 2014 (Fig. 1). The remaining stations were sampled on only one or two occasions. Zooplankton samples were collected primarily during low light conditions (between dusk and dawn) although some samples were collected during daylight hours. Additional archived zooplankton samples collected at station 6 were available from the summer of 1996 (See Table 1 for details). All stations visited were greater than 70 m in depth and the depths and coordinates of all stations are described in Appendix A.

Figure 1 here

Zooplankton collection

Zooplankton were collected 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). A metered winch was used to collect vertical tows to depths of 60 m and 15 m at each site 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., 2014; Pratt et al., 2016). At all stations visited after July of 2014, time allowed for triplicate samples to be taken at both depths to increase accuracy in zooplankton density estimates. 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 was sensitive to changes of 0.0001 dbar. Pressures were converted to depth according to Sea-Bird Electronics, Inc. (2002) as:

$$depth\ (m) = pressure\ (dbar) \times 1.0197 \qquad \text{(Eq. 1)}$$

Zooplankton processing

Specimens were sorted, counted, and identified under a Nikon SMZ 1500 dissecting microscope. Identification was done to species level according to Balcer et al. (1984). All *Bythotrephes* and *Leptodora kindtii* were removed with forceps and counted in full. Replicate subsamples were taken until approximately 100 individuals of 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 ocular micrometer. The lengths of all *Bythotrephes* and *Leptodora* were measured using the method in Branstrator (2005). For *Bythotrephes*, the number of barbs on the caudal spine (indicator of developmental instar) of each individual was also recorded for later consumption estimates. The length of each taxon was averaged for each sample (June-July 2014) or set of triplicate samples (Aug 2014-Oct 2015).

Areal (individuals m^{-2}) and volumetric (individuals m^{-3}) densities of each taxon were calculated. Volumetric density calculations were based on the amount of cable paid out (15 m or 60 m) for all stations. Estimated densities in triplicate tows were averaged for analyses.

Zooplankton biomass and taxonomic composition

The average individual dry weight of *Bosmina*, *Daphnia*, and *Holopedium* in a sample was estimated using the average length of the species and the length-weight regressions described in Bottrell et al. (1976), Persson and Ekbohm (1980), and Dumont et al. (1975), respectively. 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 *Bythotrephes* and *Leptodora* were based on unpreserved animals (Branstrator, 2005). To estimate the original lengths of preserved *Bythotrephes* and *Leptodora* individuals for biomass estimates, 64 live *Bythotrephes* (17, 36, and 11 individuals of instar 1, 2, and 3, respectively), and 98 live *Leptodora* 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, *Bythotrephes* were collected in Island Lake Reservoir (Duluth, MN) on July 1, 2015 and *Leptodora* 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 *Bythotrephes* shrinkage was instar-specific, the shrinkages of each instar were compared using one-way ANOVA ($\alpha = 0.05$).

Bythotrephes core body length (length of animal excluding caudal spine) and *Leptodora* 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 *Bythotrephes* was not instar-specific ($p = 0.51$, $F_{2, 61} = 0.689$) and therefore all instars were combined for the *Bythotrephes* shrinkage regression. The fresh lengths of *Bythotrephes (*equation 2) and *Leptodora* (equation 3) preserved in 70% ethanol can be predicted as:

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

$$Fresh\ length\ (mm) \ = \ 0.8953\ l_p\ (mm) - \ 0.3038, \qquad n = 97, R^2 = 0.9193 \quad \text{(Eq. 3)}$$

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

We compared the monthly composition of the offshore herbivorous cladoceran community (in terms of percent contribution to total numbers) observed in 2014-2015 to historical estimates of cladoceran composition starting as early as the 1970s to identify long-term changes in the cladoceran community. Changes in absolute densities of cladocerans over time could not be compared because densities were not consistently reported in the literature. To account for the relatively low number of stations visited in some months, we used a bootstrapping technique in R software to estimate the average density of each species in each month sampled. This was done by randomly selecting density estimates from the 60 m net tows from each month 1000 times with replacement and calculating average monthly densities and standard errors from the vectors of density estimates. The resulting average monthly densities of each species were used to calculate percent contribution of each species to total offshore herbivorous cladoceran numbers in each month sampled.

Data for historical comparisons came from a variety of sources and we subsetted observations from these sources that were from comparable depths (stations greater than 60 m) and regions of the lake

Table 1 here

to eliminate depth-related biases from long-term community comparisons. Sources of historical data are described in Table 1.

Determining the vertical distributions of cladocerans

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

Spatial overlap of Bythotrephes *and cladoceran prey*

To determine whether increased densities of *Bythotrephes* are associated with decreased densities of their potential cladoceran prey species, we plotted the simultaneous densities of *Bythotrephes* with *Bosmina*, *Daphnia*, and *Holopedium*. The densities of each species were displayed as the percent of the maximum observed density for each respective species to control for differences in the ranges of observed densities between taxa. Densities derived from 15 m and 60 m tows were plotted separately to control for the potential effect of depth on estimated species density. Data from 2014 and 2015 were pooled for these comparisons.

Bythotrephes *consumptive demands and prey production*

Two published models were used to estimate average daily *Bythotrephes* consumptive demands and average daily production of the available cladoceran prey for each month sampled. Both models are based on temperature and estimated biomass. Temperature data were from CTD (Seabird Electronics) casts taken at zooplankton sampling sites. Past work suggests that in lakes with planktivorous fish such as lake herring, *Bythotrephes* generally remains in the upper 15 m of water during day and night (Young and Yan, 2008). For this reason, estimates of *Bythotrephes* consumptive demands and cladoceran 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.

Because of the relatively few stations sampled in some months and the potential biases that could result from differences in the frequency of sampling at certain stations, *Bythotrephes* consumption and prey production estimates were made by bootstrapping the temperature and biomass observations from stations 5, 7, 12, and 15 (Fig. 1). These four stations were sampled every month with the exception of July of 2014 and October of 2014, when station 7 was not sampled. The bootstrapping procedures used in consumption and production estimates are described below.

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 instar-specific general linear models from Yurista et al. (2010). Model parameters include epilimnetic temperature, median individual dry weight of each instar, and the density of each instar. Because the number of *Bythotrephes* caught in some months was sometimes limited, we determined the median individual dry weight for each instar during each month using all of the individuals observed in that month. Thus, all monthly consumption estimates for an instar used the same month-specific individual dry weight for that instar.

The other model parameters, epilimnetic temperature and instar density, were bootstrapped by resampling the monthly observations 1000 times with replacement. This generated a single vector of 1000 temperature estimates for each month and a monthly density vector for each instar. Because temperature and instar densities were bootstrapped separately, the temperature and density parameters from each station were decoupled from one another. We did this to generate the largest possible range of monthly consumption estimates possible based on observed conditions. Model inputs were pulled component by component from the bootstrapped vectors of epilimnetic temperature and instar density into the instar-specific consumption equations. This generated 1000 population consumption estimates for each instar in each month. The three resulting instar consumption vectors for each month were summed to generate 1000 estimates of consumption for the entire *Bythotrephes* population which were then used in later surplus and deficit production estimates.

Estimating cladoceran production

Production (*P*) was estimated for each herbivorous cladoceran species individually based on Shuter and Ing (1997) as:

$$P = 10^{\alpha_{taxon} + \beta \times t(°C)} \times B \qquad \text{(Eq. 4)}$$

where α_{taxon} is a cladoceran-specific intercept (-1.725), β is the slope (0.044), *B* is the biomass (dry weight) of each cladoceran species, and *t* is the average daily temperature in the upper 15 m of water. We chose this model for estimating cladoceran production over other methods (i.e., the egg ratio method) because the time intervals between sampling events in this study were too long to accurately estimate the rate of population growth used in other methods. In addition, previous studies have shown that the *P*:*B* method of Shuter and Ing (1997) produces reliable estimates of production for the small herbivorous cladoceran taxa observed in Lake Superior (Stockwell and Johannsson, 1997; Carter and Schindler, 2012).

The bootstrapping protocol for cladoceran production estimates was similar to that used for *Bythotrephes* consumption. Prey biomasses in each month were bootstrapped together by resampling the biomass observation for each species at each station 1000 times with replacement. Componentwise multiplication was used to generate 1000 production estimates for each taxon in each month from the monthly biomass vectors and the same monthly temperature vectors from the *Bythotrephes* consumption estimates. The three monthly taxon-specific production estimates from each bootstrap iteration were also summed to produce 1000 estimates of total cladoceran production. These steps decoupled the prey biomass and temperature observations from each station but did not decouple biomass observations of the prey taxa at each station from each other. This was done to generate the largest range of potential production outcomes for each month and to ensure that temperature experienced was not a source of variation between taxa for monthly production estimates.

Comparing consumptive demands of Bythotrephes *to herbivorous prey production*

To determine the relative vulnerability of each cladoceran species to suppression by *Bythotrephes*, the vector of consumption estimates for the *Bythotrephes* population was subtracted, component by component, from the vectors of production estimates for *Daphnia*, *Bosmina*, and *Holopedium* for each month where *Bythotrephes* were observed. Because of the wide range of consumption and production estimates for different taxa and months, consumption and production estimates were log $(x + 1)$ transformed before subtracting the two vectors. The resulting vectors were used to produce box and whisker plots from which we could visualize the deficit or surplus production rate for each species in each month. Taxa with median monthly production rates less than *Bythotrephes* consumption were interpreted to be more vulnerable to top-down effects of *Bythotrephes* than taxa with production estimates in excess of the consumptive needs of *Bythotrephes*. The log-transformed consumptive demands of the *Bythotrephes* population were also subtracted from the vector of log-transformed total cladoceran production estimates to compare *Bythotrephes* consumptive demands to total epilimnetic cladoceran production.

Results

Pressure gauges deployed during net tows in 2015 indicated that nets reached average depths of 13.9 m (SE = 0.62) and 55.6 m (SE = 0.10) when 15 m and 60 m of cable were paid out, respectively. 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.

Cladocerans became common in August in 2014 but in 2015 they were already common in July (Table 2). *Daphnia* was the most common cladoceran by density and biomass during both years sampled. The largest average monthly densities of *Bosmina* and *Holopedium* were observed in 2014 while densities of *Daphnia* were greater in 2015 than in 2014 (Table 2). *Bythotrephes* densities varied widely by month during 2014 and 2015 and were only detected during July-October (Table 2). *Bythotrephes* was observed in densities up to 267 individuals m^{-2} but was usually much less abundant and average monthly *Bythotrephes* densities never exceeded 23 individuals m^{-2} (Table 2). Small densities of *Leptodora* were detected in August of 2014 and July of 2015 but accounted for less than 0.1 percent of cladoceran numbers and biomass.

Table 2 here

The structure of the offshore herbivorous cladoceran community in Lake Superior, which includes primarily *Daphnia*, *Bosmina*, and *Holopedium*, appears to have shifted in the past four decades. Most notably, the monthly contribution of *Bosmina* to total offshore herbivorous cladoceran numbers appeared to be smaller during the 1970s than in observations after 1996 (Fig. 2). *Bosmina* contributed 50-90% of offshore cladoceran numbers in July and August during the 1970s but only accounted for 20-30% during the summers of 2014 and 2015 (Fig. 2a, b). *Bosmina* was also a less important member of the cladoceran community in fall of 2014 and 2015 than it was during fall observations from the 1970s (Fig. 2c, d). Relatively speaking, *Daphnia* was a more important contributor to total offshore cladoceran numbers in Lake Superior in 2014 and 2015 than it was in the past. It is unclear whether absolute densities of *Daphnia* have increased since the 1970s due to differences in reporting of densities in Watson and Wilson (1978) and Conway et al. (1973). There were no obvious changes in the contribution of *Holopedium* to cladoceran numbers in 2014 or 2015 compared to the 1970s.

Figure 2 here

Vertical distributions of Bythotrephes *and cladoceran prey*

Table 3 here

The average areal 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 3). Average areal densities of *Bosmina* and *Holopedium* were also not significantly different between 15 m and 60 m tows in either year (Table 3). *Daphnia* areal densities were significantly higher in 60 m tows than in 15 m tows in 2014 (p = 0.04) but were not significantly different in 2015 (Table 3). Separating day and night samples before running t-tests produced similar results but the number of samples collected during the day were limited.

Simultaneous densities of Bythotrephes *and cladoceran prey*

Large densities of *Holopedium* often occurred when *Bythotrephes* were also abundant in both years sampled (Fig. 3). *Daphnia* was generally most common when *Bythotrephes* was absent or in low densities, however, large densities of *Bythotrephes* and *Daphnia* occasionally coincided in each year sampled (Fig. 3). High simultaneous densities of *Bythotrephes* and *Bosmina* were never observed in 2014 or 2015. The maximum *Bosmina* density occurred when *Bythotrephes* densities were about 5% of the *Bythotrephes* maximum (Fig. 3). Above 5% of the maximum *Bythotrephes* density, *Bosmina* densities never exceeded 10% of their maximum.

Figure 3 here

Comparisons of Bythotrephes *consumptive demands and epilimnetic prey production*

August of 2014 had the highest monthly *Bythotrephes* density with approximately 23 individuals m^{-2} (Table 2). This density was strongly influenced by a station where *Bythotrephes* densities were in excess of 150 individuals m^{-2}. While this density was greater than the density observed at any other stations in 2014 or 2015, it is not an unreasonable *Bythotrephes* density for Lake Superior (Brown and Branstrator, 2004; Yurista et al., 2009; Isaac et al., 2012). Estimated median *Bythotrephes* consumption was well in excess of total cladoceran production during August of 2014 (Fig. 4). Even when the large *Bythotrephes* density observed in August of 2014 was omitted from the model inputs, consumption by *Bythotrephes* still overwhelmed the production rate of each prey taxon as well as total cladoceran production although not as drastically.

Median *Bythotrephes* consumption did not exceed total cladoceran production in any month besides August of 2014. However, median *Bythotrephes* consumption in July of 2015 was greater than the median production rates of each of the three individual taxa due to relatively low densities of herbivorous cladocerans observed in this month (Table 2, Fig. 4). *Bythotrephes* consumption exceeded *Bosmina* and *Holopedium* production in every month where *Bythotrephes* was observed but did not exceed *Daphnia* production in September of 2015 or October of either year (Fig. 4).

Figure 4 here

Because of the limited dataset, there was a relatively large amount of variability in the bootstrapped surplus and deficit production estimates for some months (Fig. 4). Median to mean ratios of the surplus and deficit production estimates are summarized in Table 4. The median to mean ratio during

Table 4 here

July of 2015 was negative because mean *Bythotrephes* consumption was slightly negative while median consumption was slightly positive. The median to mean ratios did not become closer to 1 when production and consumption estimates were repeated with 10,000 bootstrapping iterations. This indicates that 1000 iterations adequately resamples the available data but that the model outputs for some months are non-normally distributed.

To evaluate the effect of decoupling biomass and temperature observations at individual stations, we estimated production and consumption for each station and bootstrapped the resulting station-specific estimates. Under this scenario, *Bosmina* production was still exceeded by *Bythotrephes* consumption in every month and *Daphnia* production was not exceeded in the September or October observations. However, assuming site dependence of biomass and temperature caused *Holopedium* production to be in excess of *Bythotrephes* production in September and October of 2015. We also evaluated the effect of using only the data from routinely sampled stations on model outputs by repeating the consumption and production estimates using data from all of the stations visited in each month. We found that the proportion of times that each prey taxon was overwhelmed by *Bythotrephes* consumptive requirements was the same as when only data from routinely sampled stations were used.

Discussion

In the decades since the establishment of *Bythotrephes* in Lake Superior, the composition of the offshore cladoceran community appears to have changed. Past studies suggest that *Bosmina* was often equally or more numerically common in Lake Superior than *Daphnia* from spring through fall (Swain et al., 1970; Patalas, 1972; Schelske and Roth, 1973; Conway et al., 1973; Selgeby 1975). *Bosmina* contributed less to cladoceran numbers in 2014 and 2015 than it did during the 1970s which is consistent with the findings of Brown and Branstrator (2004). Our results show that *Daphnia* has become a more important contributor to the cladoceran community in the offshore regions of Lake Superior and suggest that the importance of *Holopedium* has remained stable. Similar changes have occurred in the cladoceran communities of the other Great Lakes and inland lakes following *Bythotrephes* invasion (Yan and Pawson, 1997; Barbiero and Tuchman, 2004; Rudstam et al., 2015; Kerfoot et al., 2016). We analyzed patterns in simultaneous densities of predator and putative prey to evaluate evidence for consumptive effects of *Bythotrephes* on any of the cladoceran species. We also analyzed vertical distributions of *Bythotrephes*, *Bosmina*, *Daphnia*, and *Holopedium* and compared the consumptive demands of *Bythotrephes* to the production rates of each herbivorous cladoceran species to determine which were most vulnerable to top-down control.

Bosmina

Both *Bosmina* and *Bythotrephes* were caught primarily in the upper 15 m of water during both 2014 and 2015 (Table 3). 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 Lakes Michigan and Erie 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, Lakes Michigan and Erie have greater summer surface temperatures than Lake Superior and have historically had deeper thermoclines (Reavie et al., 2017). These conditions allow cladocerans in Lakes Michigan and Erie to migrate to greater depths before experiencing temperatures that cause appreciable reductions in production.

Despite occupying similar depths, the simultaneous densities of *Bosmina* and *Bythotrephes* imply a general lack of co-presence between these species. This pattern is consistent with recent observations in Lake Ontario (Barbiero et al., 2014; Rudstam et al., 2015) and could be evidence for predation on *Bosmina* by *Bythotrephes*. Finally, the consumptive demands of *Bythotrephes* in this study always exceeded *Bosmina* production when *Bythotrephes* was present. This was the case regardless of whether we used data from all stations or from only the routine stations and regardless of whether or not we assumed site-dependence of temperature and biomass data. These results demonstrate that it takes very few *Bythotrephes* eating *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 the offshore regions of Lake Superior despite being present at relatively low densities.

Daphnia

In 2014, larger *Daphnia* densities were observed in 60 m tows than 15m tows, suggesting that overlap between *Bythotrephes* and *Daphnia* may have been reduced. However, this difference was relatively small and the difference in *Daphnia* densities between deep and shallow tows was not significantly different in 2015. *Daphnia* has remained common in Lake Superior, as it has in several other lakes, since the establishment of *Bythotrephes* (Lehman, 1991; Azan et al., 2015). Like *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*. While occupying deeper depths may have allowed *Daphnia* to avoid predation, it has also resulted in reduced *Daphnia* production in Lake Michigan due to the lower water temperatures at greater depth. In Lake Superior, where surface temperatures are lower and the thermocline is generally shallower, the benefits of migrating to deeper waters may not outweigh the costs, especially at the relatively low *Bythotrephes* densities observed in 2014 and 2015.

Regardless of whether or not *Daphnia* actively reduce their vertical overlap with *Bythotrephes* in Lake Superior, monthly epilimnetic production rates of *Daphnia* exceeded the consumptive demands of *Bythotrephes* in three of the five months where *Bythotrephes* was observed. Similar trends were observed when we assumed site dependence of temperature and biomass data and when we used data from infrequently sampled locations. *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 suggest that *Daphnia* in Lake Superior is less susceptible to being overwhelmed by *Bythotrephes* and may explain why *Daphnia* has remained common in Lake Superior since the establishment of *Bythotrephes*.

Holopedium

Most *Holopedium* biomass occurred in the upper 15 m of water, implying vertical overlap with *Bythotrephes*. In addition, the monthly consumptive demands of *Bythotrephes* exceeded *Holopedium* production when *Bythotrephes* was present under most modeling scenarios. While these findings suggest that *Holopedium* could be suppressed by *Bythotrephes* in Lake Superior, *Holopedium* and *Bythotrephes*, like *Daphnia* and *Bythotrephes*, commonly occurred together. *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; Barbiero et al., 2014; 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. Taken together, these results support the hypothesis that *Holopedium* is less susceptible to predation by *Bythotrephes* and help to explain why *Holopedium* does not appear to have become less common since *Bythotrephes* establishment.

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 the possibility of suppression by *Bythotrephes*. Because *Bosmina* is also the only herbivorous cladoceran species that appears to have become less common in the offshore regions of Lake Superior since the establishment of *Bythotrephes*, these findings support the hypothesis that *Bosmina* has become a less important member of the cladoceran community 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 partially due to the low surface water temperatures in Lake Superior (Patalas, 1972; Lehman, 2002). Therefore, 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., 2011). Juvenile fish may feed on *Bosmina*, but year classes of smelt and the various coregonines 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.

It is important to note that the cladoceran community observations made in 2014 and 2015 differ from other recent zooplankton surveys in Lake Superior. For one, densities of *Bythotrephes* observed in the present study were considerably lower than those reported in other Lake Superior zooplankton surveys conducted since 2001 (Brown and Branstrator, 2004; Yurista et al., 2009; Isaac et al., 2012). It is unclear why the *Bythotrephes* densities observed in 2014-2015 were so much lower than in previous summers. Past work shows that peak *Bythotrephes* densities in an inland reservoir are short-lived (Brown et al., 2012) and it is therefore possible that the true peaks in *Bythotrephes* density did not coincide with sampling events. We were unable to collect zooplankton samples in September of 2014 and August of 2015 which is when *Bythotrephes* densities have peaked in Lake Superior in other years (Isaac et al., 2012) and because of this the estimates of *Bythotrephes* consumptive demands in this study are likely to be conservative.

Another difference between our observations and other recent surveys has to do with *Holopedium*. Brown and Branstrator (2004) reported a possible increase in *Holopedium* abundance between the 1970s and 2001. The densities of *Holopedium* we observed in 2014-2015 were considerably lower than those reported in Brown and Branstrator (2004). However, Yurista et al. (2009) and Pratt et al. (2016) also report *Holopedium* densities in 2006 and 2011 that were much higher than we observed. Therefore, while the present study does not indicate *Holopedium* is more common than it used to be, there is good evidence that *Holopedium* is often more abundant in Lake Superior than before the establishment of *Bythotrephes*. Because *Holopedium* is not thought to be a preferred prey species for *Bythotrephes*, the higher *Holopedium* densities reported in other recent studies may be evidence of a species replacement resulting from reduced competition with *Bosmina*.

Future food web implications of Bythotrephes

These findings suggest that *Bythotrephes* has had a measurable impact on the offshore cladoceran community in Lake Superior. Seasonally, cladocerans are important sources of prey for some species of planktivorous fish in Lake Superior (Gamble et al., 2011; Isaac et al., 2012). While *Bosmina* is not an important food source for planktivorous fish in Lake Superior, *Bythotrephes* necessarily consumes other species and may therefore reduce the amount of cladoceran biomass available for such planktivores, especially those that are unable to eat *Bythotrephes*. Furthermore, though the densities of *Bythotrephes* observed in this study were generally not high enough to overwhelm total cladoceran production, the densities needed to do so are within the range of *Bythotrephes* densities observed in Lake Superior in the past.

This study does not address the relationship between *Bythotrephes* and copepods in Lake Superior. While *Bythotrephes* is known to occasionally consume small copepods, cladocerans are their preferred prey (Schulz and Yurista, 1999; Dumitru et al., 2001). Because the standing stock of copepod biomass in Lake Superior greatly exceeds cladoceran biomass (Yurista et al., 2009; Barbiero et al., 2012), *Bythotrephes* is unlikely to influence the copepod community structure or biomass in Lake Superior at the densities observed in this study. In addition, zooplankton biomass has been relatively stable in Lake Superior in recent decades and the dominant copepod species in Lake Superior have not changed since the 1970s (Barbiero et al., 2001; Barbiero et al., 2012).

The consumptive demands of *Bythotrephes* relative to prey production in Lake Superior are likely to change with further climate warming because temperature is one of the factors that influences both zooplankton production and *Bythotrephes* consumption. We explored the consequences of further warming on predator consumption and prey production by projecting the production and consumption estimates made in this study over a higher range of temperatures. When both predator and prey biomass were held constant, prey production increases faster than *Bythotrephes* consumption. However, predicting the effects of continued warming in Lake Superior on this predator-prey interaction is difficult because the response of *Bythotrephes* and their prey species to further warming may not be equivalent. For example, past studies indicate that the optimal temperatures for *Bythotrephes* growth and reproduction are in the range of 18-22°C (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 low surface temperatures might be one factor limiting *Bythotrephes* abundance in Lake Superior. Warming water temperature might also favor herbivorous cladocerans over copepods in Lake Superior as it has in other cold water systems (Carter and Schindler, 2012). However, herbivorous cladoceran abundance in Lake Superior is probably also limited by food quality and quantity (Patalas 1973) and the effects of continued warming on primary production in Lake Superior are difficult to predict (Reavie et al., 2017). As such, the future top-down effects of *Bythotrephes* on Lake Superior zooplankton will depend on the extent of warming, changes in primary production, and the many possible responses of *Bythotrephes* and its potential prey species to warming.

Factors other than temperature also influence *Bythotrephes* abundance in Lake Superior and increase the uncertainty regarding the future effects of *Bythotrephes* on the Lake Superior food web. 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.

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References

Austin, J.A., Colman, S.M., 2007. Lake Superior summer water temperatures are increasing more rapidly than regional air temperatures: A positive ice-albedo feedback. Geophys. Res. Lett. 34, L06604.

Azan, S.S.E., Arnott, S.E., Yan, N.D., 2015. A review of the effects of *Bythotrephes longimanus* and calcium decline on zooplankton communities — can interactive effects be predicted? Env. Rev. 413, 395–413.

Balcer M.D., Korda N.L., Dodson S.I., 1984. Zooplankton of the Great Lakes: A guide to the identification and ecology of the common crustacean species. Madison, Wisconsin: Univ. of Wisconsin Pr.

Barbiero, R.P., Little, R.E., Tuchman, M.L., 2001. Results from the U.S. EPA's biological open water surveillance program of the Laurentian Great Lakes: III. Crustacean zooplankton. J. Great Lakes Res. 27, 167–184.

Barbiero, R.P., Tuchman, M. L., 2004. Changes in the crustacean communities of Lakes Michigan, Huron, and Erie following the invasion of the predatory cladoceran *Bythotrephes longimanus*. Can. J. Fish. Aquat. Sci. 61, 2111–2125.

Barbiero, R.P., Lesht, B.M., Warren, G.J., 2012. Convergence of trophic state and the lower food web in Lakes Huron, Michigan and Superior. J. Great Lakes Res. 38, 368–380.

Barbiero, R.P., Lesht, B.M., Warren, G.J., 2014. Recent changes in the offshore crustacean zooplankton community of Lake Ontario. J. Great Lakes Res. 40, 898–910.

Bottrell, H.H., Duncan, A., Gliwicz, Z.M., Grygierek, E., Herzig, A., Hillbicht-Ilkowska, A., Kurasawa, H., Larsson, P., Weglenska, T., 1976. A review of some problems in zooplankton production studies. Nor. J. Zool. 24, 419–456.

Branstrator, D.K., 2005. Contrasting life histories of the predatory cladocerans *Leptodora kindtii* and *Bythotrephes longimanus*. J. Plankton Res. 27, 569–585.

Bronte, C.R.., Ebener, M.P., Schreiner, D.R., Devault D.S., Petzold, M.M., Jensen, D.A., Richards, C., Lozano, S.J., 2003. Fish community change in Lake Superior, 1970 – 2000. Can. J. Fish. Aquat. Sci. 60, 1552–1574.

Brooks, J.L., Dodson, S.I., 1965. Predation, body size, and composition of plankton. Science. 150, 28–35.

Brown, M.E., Branstrator, D.K., Shannon, L.J., 2012. Population regulation of the spiny water flea (*Bythotrephes longimanus*) in a reservoir: Implications for invasion. Limnol. Oceanogr. 57, 251–271.

Brown, M.E., Branstrator, D.K., 2004. A 2001 Survey of crustacean zooplankton in the Western Arm of Lake Superior. J. Great Lakes Res. 30, 1–8.

Bunnell, D.B., Davis, B.M., Warner, D.M., Chriscinske, M.A., Roseman, E.F., 2011. Planktivory in the changing Lake Huron zooplankton community: *Bythotrephes* consumption exceeds that of Mysis and fish. Freshw. Biol. 56, 1281–1296.

Bunnell, D.B., Keeler, K.M., Puchala, E.A., Davis, B.M., Pothoven, S.A., 2012. Comparing seasonal dynamics of the Lake Huron zooplankton community between 1983-1984 and 2007 and revisiting the impact of *Bythotrephes* planktivory. J. Great Lakes Res. 38, 451–462.

Bunnell, D.B., Richard, P., Ludsin, S.A., Charles, P., 2014. Changing ecosystem dynamics in the Laurentian Great Lakes: Bottom-up and top-down regulation. Bioscience. 64, 26–39.

Burkhardt, S., Lehman, J.T., 1994. Prey consumption and predatory effects of an invertebrate predator (*Bythotrephes*: Cladocera, Cercopagidae) based on phosphorus budgets. Limnol. Oceanogr. 39, 1007–1019.

Carpenter, S.R., Kitchell, J.F., Hodgson, J.R., Cochran, P.A., Elser, J.J., Elser M.M, Lodge, D.M., Kretchmer, D., He, X., von Ende, C.N., 1987. Regulation of lake primary productivity by food web structure. Ecology. 68, 1863–1876.

Carter, J.L., Schindler, D.E., 2012. Responses of zooplankton populations to four decades of climate warming in lakes of Southwestern Alaska. Ecosystems. 15, 1010–1026.

Conway, J.B., Ruschmeyer, O.R., Olson, T.A., Odlaug, T.O., 1973. The distribution, composition, and biomass of the crustacean zooplankton population in western Lake Superior. Univ. of Minn. Water Resour. Res. Center Bull. 63.

Cullis, K.I., Johnson, G.E., 1988. First evidence of the Cladoceran *Bythotrephes cederstroemi* Schoedler in Lake Superior. J. Great Lakes Res. 14, 524–525.

Dumitru, C., Sprules, W.G., Yan, N.D., 2001. Impact of *Bythotrephes longimanus* on zooplankton assemblages of Harp Lake, Canada: An assessment based on predator consumption and prey production. Freshw. Biol. 46, 241–251.

Dumont, H., Van de Velde, I., Dumont, S., 1975. The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. Oecologia, 19, 75-97.

Gamble, A.E., Hrabik, T.R., Stockwell, J.D., Yule, D.L., 2011. Trophic connections in Lake Superior Part I: The offshore fish community. J. Great Lakes Res. 37, 541–549.

Gorman, O.T., 2012. Successional change in the Lake Superior fish community: Population trends in ciscoes, Rainbow Smelt, and Lake Trout, 1958–2008. Adv. Limnol. 63, 337-362.

Grigorovich, I.A., Pashkova, O.V., Gromova, Y.F., Van Overdijk, C.D.A., 1998. *Bythotrephes longimanus* in the Commonwealth of Independent States: variability, distribution, and ecology. Hydrobiol. 379, 183–198.

Isaac, E.J., Hrabik, T.R., Stockwell, T.R., Gamble, A.E., 2012. Prey selection by the Lake Superior fish community. J. Great Lakes Res. 38, 326–335.

Johnson, T.B., Hoff, M.H., Trebitz, A.S., Bronte, C.R., Curry, T.D., Kitchell, J.F., Lozano, S.J., Mason, D.M., Scharold, J.V., Schram, S.T., Schreiner, D.R., 2004. Spatial patterns in assemblage Structures of pelagic forage fish and zooplankton in Western Lake Superior. J. Great Lakes Res. 30, 395–406.

Keeler, K.M., Bunnell, D.B., Diana, J.S., Adams, J.V., Mychek-Londer, J.G., Warner, D.M., Yule, D.L., Vinson, M.R., 2015. Evaluating the importance of abiotic and biotic drivers on *Bythotrephes* biomass in Lakes Superior and Michigan. J. Great Lakes Res. 41, 150–160.

Kerfoot, W.C., Hobmeier, M.M., Yousef, F., Lafrancois, B.M., Maki, R.P., Hirsch, J.K., 2016. A plague of waterfleas (*Bythotrephes*): impacts on microcrustacean community structure, seasonal biomass, and secondary production in a large inland-lake complex. Biol. Invasions. 18, 1121–1145.

Kim, N., Yan, N.D., 2010. Methods for rearing the invasive zooplankter *Bythotrephes* in the laboratory. Limnol. Oceanogr. Methods. 8, 552–561.

Lehman, J.T., 1987. Palearctic predator invades North American Great Lakes. Oecologia. 74, 478–480.

Lehman, J.T., 1988. Algal biomass unaltered by food web changes in Lake Michigan. Nature 332, 537–538.

Lehman, J.T., 1991. Causes and consequences of cladoceran dynamics in Lake Michigan: Implications of species invasion by *Bythotrephes*. J. Great Lakes Res. 17, 437–445.

Lehman, J.T., Cáceres, C.E., 1993. Food-web responses to species invasion by a predatory invertebrate: *Bythotrephes* in Lake Michigan. Limnol. Oceanogr. 38, 879–891.

Lehman, J.T., 2002. Mixing patterns and plankton biomass of the St. Lawrence Great Lakes under climate change scenarios. J. Great Lakes Res. 28, 583–596.

Link, J., Hoff, M.H., 1998. Relationships of lake herring (*Coregonus artedi*) gill raker characteristics to retention probabilities of zooplankton prey. J. Freshw. Ecol. 13, 55–65.

Link, J., Selgeby, J.H., Keen, R.E., 2004. Changes in the Lake Superior crustacean zooplankton community. J. Great Lakes Res. 30, 327–339.

Makarewicz, J.C., Bertram, P., Lewis, T., Brown, E.H., 1995. A decade of predatory control of zooplankton species composition in Lake Michigan. J. Great Lakes Res. 21, 620-640.

Oliver, S.K., Branstrator, D.K., Hrabik, T.R., Guildford, S.J., Hecky, R.E., 2014. Nutrient excretion by crustacean zooplankton in the deep chlorophyll layer of Lake Superior. Can. J. Fish. Aquat. Sci. 10, 1–10.

Pangle, K.L., Peacor, S.D., 2006. Non-lethal effect of the invasive predator *Bythotrephes longimanus* on *Daphnia mendotae*. Freshw. Biol. 51, 1070–1078.

Pangle, K.L., Peacor, S.D., Johannsson, O.E., Field, E., 2007. Large nonlethal effects of an invasive invertebrate predator on zooplankton population growth rate. Ecology. 88, 402–412.

Patalas, K., 1972. Crustacean plankton and the eutrophication of St. Lawrence Great Lakes. J. Fish. Res. Board Can. 29, 1451 – 1462.

Persson, G., Ekbohm, G., 1980. Estimation of dry-weight in zooplankton populations--methods applied to crustacean populations from lakes in the Kuokkel Area, Northern Sweden. Arch. Hydrobiol. 89, 225-246.

Pothoven, S.A., Vanderploeg, H.A., Warner, D.M., Schaeffer, J.S., Ludsin, S.A., Claramunt, R.M., Nalepa, T.F., 2012. Influences on *Bythotrephes longimanus* life-history characteristics in the Great Lakes. J. Great Lakes Res. 38, 134–141.

Pratt, T.C., Gorman, O.T., Mattes, W.P., Myers, J.T., Quinlan, H.R., Schreiner, D.R., Seider, M.J., Sitar, S.P., Yule, D.L., Yurista, P.M., 2016. The state of Lake Superior in 2011 [online]. Available from: http://www.glfc.org/pubs/SpecialPubs/Sp16_01.pdf.

Reavie, E.D., Sgro, G.V., Estepp, L.R., Bramburger, A.J., Shaw Chraibi, V.L., Pillsbury, R.W., Cai, M., Stow, C.A., Dove, A., 2017. Climate warming and changes in *Cyclotella sensu lato* in the Laurentian Great Lakes. Limnol. Oceanogr. 62, 768–783.

Rudstam, L.G., Holeck, K.T., Bowen, K.L., Watkins, J.M., Weidel, B.C., Luckey, F.J., 2015. Lake Ontario zooplankton in 2003 and 2008: Community changes and vertical redistribution. Aquat. Ecosyst. Health Mgmt. 18, 43–62.

Schelske, C.L., Roth, J.C., 1973. Limnological survey of Lakes Michigan, Superior, Huron, and Erie. Univ. Mich. Great Lakes Res. Div. No. 17.

Schulz, K.L., Yurista, P.M., 1999. Implications of an invertebrate predator's (*Bythotrephes cederstroemi*) atypical effects on a pelagic zooplankton community. 1985, 179–193.

Sea-Bird Electronics, Inc., 2002. Conversion of pressure to depth. Application note number 69

Selgeby, J.H., 1975. Life histories and abundance of crustacean zooplankton in the outlet of Lake Superior, 1971–72. J. Fish. Res. Board Canada 32, 461–470.

Shuter, B.J., Ing, K.K., 1997. Factors affecting the production of zooplankton in lakes. Can. J. Fish. Aquat. Sci. 54, 359–377.

Strecker A.L., Arnott S.E., Yan N.D., Girard R., 2006. Variation in the response of crustacean zooplankton species richness and composition to the invasive predator *Bythotrephes* longimanus. Canadian Journal of Fisheries and Aquatic Sciences. 63, 2126–2136.

Swain, W.R., Olson, T.A., Odlaug, T.O., 1970. The ecology of the second trophic level in Lakes Superior, Michigan and Huron. Water Resour. Res. Cont. Bull. 26, 1-151.

Vanderploeg H.A., Liebig J.R., Omai M., 1993. *Bythotrephes* predation on Great Lakes' zooplankton measured by an in situ method: implications for zooplankton community structure. Archives fur Hydrobiol. 127, 1–8.

Watson, N.H.F., Wilson, J.B., 1978. Crustacean zooplankton of Lake Superior. J. Great Lakes Res. 4, 481–496.

Yan, N.D., Pawson, T.W., 1997. Changes in the crustacean zooplankton community of Harp Lake, Canada, following invasion by *Bythotrephes cederstroemi*. Freshw. Biol. 37, 409–425.

Yan, N.D., Pawson T.W., 1998. Seasonal variation in the size and abundance of the invading *Bythotrephes* in Harp Lake, Ontario, Canada. Hydrobiol. 361, 157-168.

Yan, N.D., Girard, R., Boudreau, S., 2002. An introduced invertebrate predator (*Bythotrephes*) reduces zooplankton species richness. Ecology letters. 5, 481–485.

Young, J.D., Yan, N.D., 2008. Modification of the diel vertical migration of *Bythotrephes longimanus* by the cold-water planktivore, *Coregonus artedi*. Freshwater Biol. 53, 981–995.

Yurista, P.M., Kelly, J.R., Miller, S.E., 2009. Lake Superior zooplankton biomass: Alternate estimates from a probability-based net survey and spatially extensive LOPC surveys. J. Great Lakes Res. 35, 337–346.

Yurista, P.M., Vanderploeg, H.A., Liebig, J.R., Cavaletto, J.F., 2010. Lake Michigan *Bythotrephes* prey consumption estimates for 1994–2003 using a temperature and size corrected bioenergetic model. J. Great Lakes Res. 36, 74–82.

Figure captions:

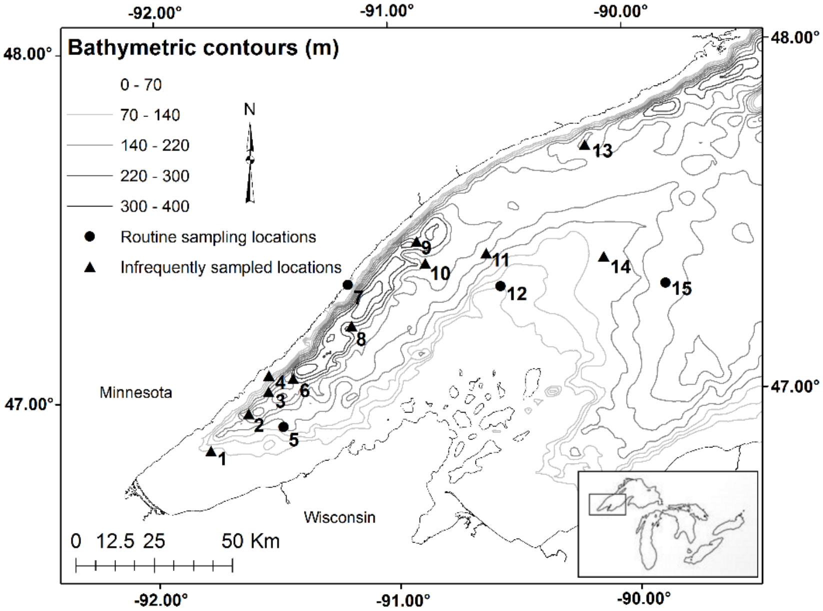

Fig. 1. Zooplankton sampling locations in 2014 and 2015. Routine sampling stations are those that were sampled during each month with the exception of station 7, which was not sampled in July or October of 2014. Infrequently sampled locations are those that were sampled on one or two occasions. Zooplankton samples were collected at station 6 once in 2015 and additional archived samples from 1996 were available for this station. Depths and coordinates for all sites are summarized in Appendix A.

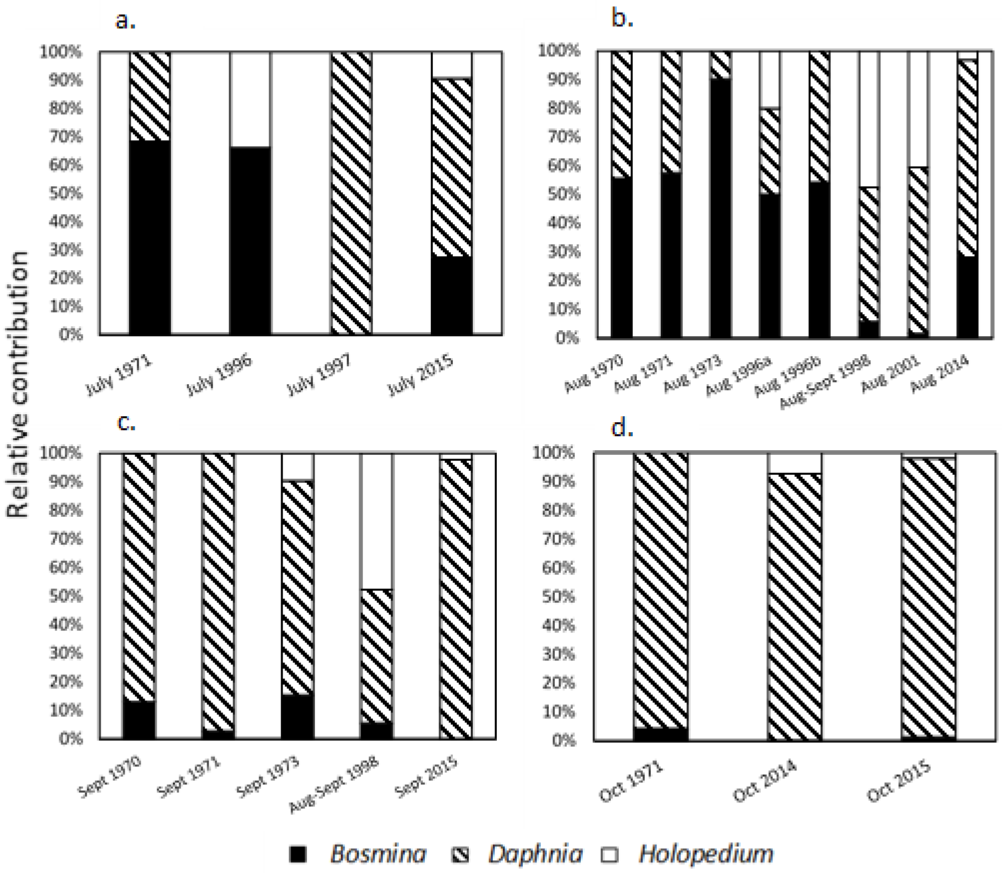

Fig 2. Comparisons of the relative contribution of *Bosmina*, *Daphnia*, and *Holopedium* to total offshore herbivorous cladoceran densities in Lake Superior during a) July; b) August; c) September; and d) October from 1971-2015. Observations 1996a and 1996b in panel b refer to different surveys (see Table 1). All 2014 and 2015 observations were from the present study and sources for previous observations are listed in Table 1.

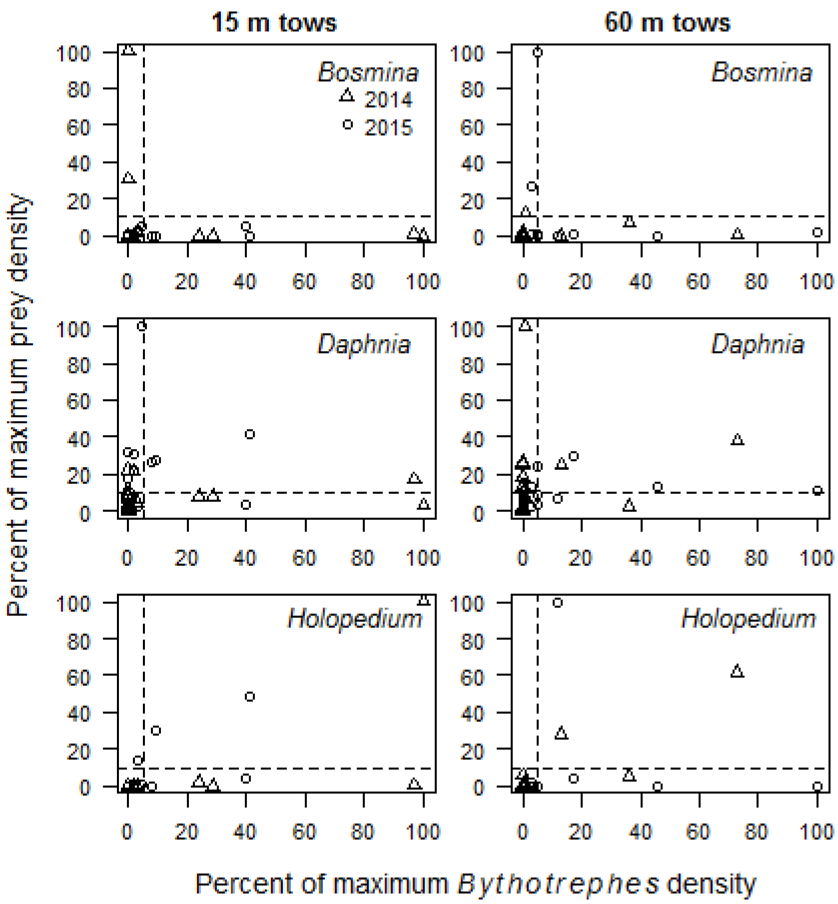

Fig. 3. Simultaneous densities of *Bythotrephes* with *Bosmina*, *Daphnia*, and *Holopedium* in 15 m net tows (left panels) and 60 m net tows (right panels) during 2014 and 2015. Densities for each species are relative to the maximum observed density for the species. The dashed horizontal and vertical lines at y = 10% and x = 5% provide a reference window to facilitate species-to-species comparisons of coexistence with *Bythotrephes*.

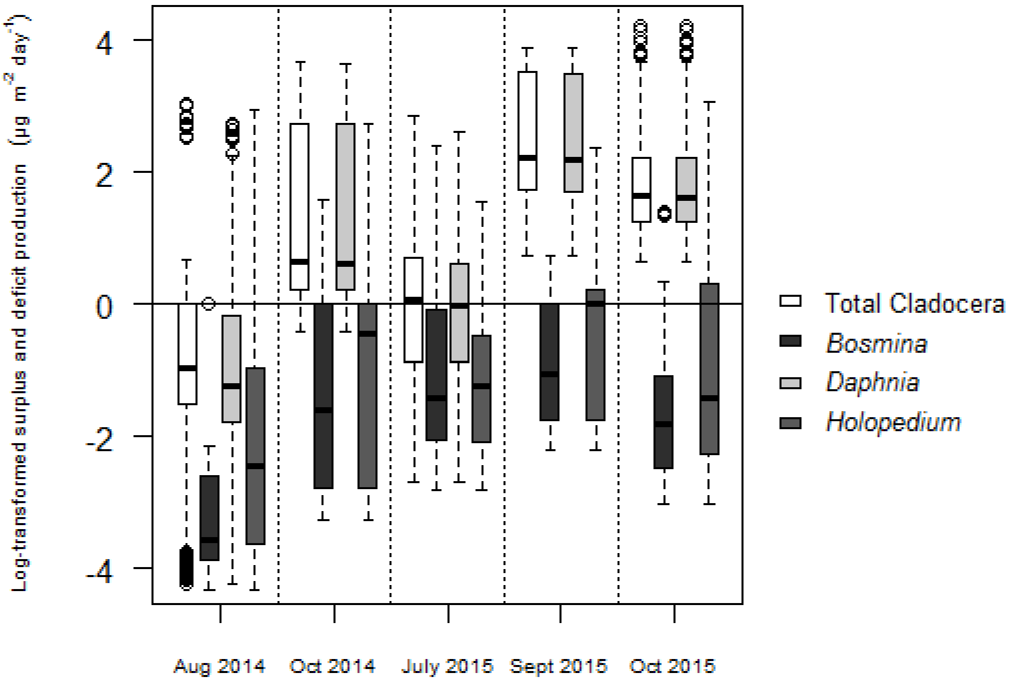

Fig. 4. Box and whisker plot of log-transformed surplus and deficit production rates (µg m^{-2} day^{-1}) for total Cladocera, *Bosmina*, *Daphnia*, and *Holopedium*. Median production values are indicated by the horizontal black lines within boxes. The lower and upper limits of the boxes represent the 25th and 75th percentiles, respectively. Whiskers represent 1.5 times the interquartile range below and above the 25th and 75th percentiles and points represent production estimates above or below the range covered by whiskers. Taxa with negative median production rates are those that would be overwhelmed by *Bythotrephes* if *Bythotrephes* fed exclusively on that taxon.

Table 1. Sources of monthly cladoceran community data for long-term comparisons. The "Data used" field describes how the data were subsetted to ensure compatibility with data from the present study. The letters a and b appended to the two August 1996 surveys differentiate columns in Fig. 2b.

Source	Months available	Data used
Conway et al., 1973	Aug-Sept 1970, July-Oct 1971	Larsmont and Stony Point stations at 2 miles from shore (155 m and 110 m deep respectively)
Watson and Wilson 1978	Aug-Sept 1973	Lake Regions 5 and 6 (Table 3 and Fig. 5 in original source)
Sterner (archived samples)	July 1996, Aug 1996a	Archived samples from R. Sterner. Collected at station 6 (Fig. 1) using 80 µm 0.5-meter diameter conical zooplankton net towed from bottom to surface. Samples processed Sept 2015.
Johnson et al., 2004	July 1997, Aug 1996b	"Open lake" region (See Fig. 2 and Table 2 in original source)
Barbiero et al., 2001	Aug-Sept 1998	All zooplankton collection done at stations of depth >90 m. Data used as is.
Brown and Branstrator 2004	Aug 2001	Stations deeper than 60 m (see Fig. 1 and Table 1 in original source).

Table 2. Average monthly density as individuals m^{-2} (standard error in parentheses) for common offshore cladocerans in Lake Superior during 2014-2015 in 60 m net tows. Averages and standard errors were estimated by bootstrapping density observations from each month. The number of stations visited during each month is indicated by n.

	Bythotrephes density (+/-SEM)	*Bosmina* density (+/-SEM)	*Daphnia* density (+/-SEM)	*Holopedium* density (+/-SEM)	n
June 2014	0	0	44.0 (2.35)	0	4
July 2014	0	0	0	0	3
Aug 2014	23.0 (1.1)	1388.8 (99.54)	3393.5 (137.71)	155.9 (12.77)	10
Oct 2014	3.6 (0.1)	6.3 (0.38)	4280.7 (85.43)	330.9 (25.43)	13
May 2015	0	0	0.5 (0.03)	0	5
July 2015	2.6 (0.07)	217.4 (9.75)	500.2 (13.49)	73.2 (2.36)	4
Sept 2015	0.4 (0.32)	14.2 (0.75)	9083.8 (124.47)	210.2 (10.65)	4
Oct 2015	1.3 (0.05)	225.0 (13.96)	16214.9 (492.0)	292.3 (19.83)	7

Table 3. Average areal densities of *Bythotrephes*, *Bosmina*, *Daphnia*, and *Holopedium* in 15 m and 60 m net tows. The 2014 comparisons were based on 30 paired observations and the 2015 comparisons were based on 20 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	11.6	9.2	0.22	1.3	2.4	0.13
Bosmina	457.4	484.5	0.19	67.6	114.7	0.12
Daphnia	2227.8	3142.6	0.04	6312.3	6709.6	0.25
Holopedium	106.3	87.1	0.12	189.2	127.2	0.12

Table 4. Monthly median to mean ratios for the surplus and deficit production estimates for total Cladocera, *Bosmina*, *Daphnia*, and *Holopedium* (see Fig. 4). Medians and means were calculated from the 1000 bootstrapped estimates of log-transformed surplus and deficit production for each month where *Bythotrephes* were observed.

	Total Cladocera	*Bosmina*	*Daphnia*	*Holopedium*
August 2014	1.04	1.21	1.13	1.28
October 2014	0.57	1.04	0.54	0.39
July 2015	-0.49	1.38	0.27	1.02
September 2015	0.88	1.34	0.88	0.00
October 2015	0.86	1.15	0.85	1.53

Appendix A. Location and depth of sampling stations shown in Fig. 1.

Site number	Latitude (degrees North)	Longitude (degrees West)	Site depth (meters)
1	46.8614	-91.7815	77
2	46.9666	-91.6200	208
3	47.0281	-91.5354	231
4	47.0753	-91.5330	102
5	46.9271	-91.4753	137
6	47.0650	-91.4317	255
7	47.3313	-91.1925	87
8	47.2129	-91.1810	276
9	47.4505	-90.8979	241
10	47.3868	-90.8633	200
11	47.4110	-90.6049	170
12	47.3155	-90.5476	77
13	47.7129	-90.1720	157
14	47.3911	-90.1089	140
15	47.3088	-89.8514	167