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

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**A B S T R A C T**

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.

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**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 (Pangie 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...
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toxan were measured to the nearest

\[ \text{Fresh length (mm)} = l_p \left(1 - F_{2, 61} = 0.689\right) \]

\[ \text{Fresh length (mm)} = 0.9898 l_p (mm) + 0.6699, n = 64, R^2 = 0.8083 \]  

\[ \text{Fresh length (mm)} = 1.0268 l_p (mm) + 0.7998, n = 97, R^2 = 0.9193 \]  

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.

Table 1

<table>
<thead>
<tr>
<th>Source</th>
<th>Months available</th>
<th>Data used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conway et al., 1973</td>
<td>Aug–Sept 1970, July–Oct 1971</td>
<td>Larssmont and Stony Point stations at 2 miles from shore (155 m and 110 m deep respectively)</td>
</tr>
<tr>
<td>Watson and Wilson, 1978</td>
<td>Aug–Sept 1973</td>
<td>Lake Regions 5 and 6 (Table 3 and Fig. 5 in original source)</td>
</tr>
<tr>
<td>Sterner (archived samples)</td>
<td>July 1996, Aug 1996a</td>
<td>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.</td>
</tr>
<tr>
<td>Johnson et al., 2004</td>
<td>July 1997, Aug 1996b</td>
<td>“Open lake” region (see Fig. 2 and Table 2 in original source)</td>
</tr>
<tr>
<td>Barbaro et al., 2001</td>
<td>Aug–Sept 1998</td>
<td>All zooplankton collection done at stations of depth $&gt;$ 90 m. Data used as is.</td>
</tr>
<tr>
<td>Brown and Branstrator, 2004</td>
<td>Aug 2001</td>
<td>Stations deeper than 60 m (see Fig. 1 and Table 1 in original source).</td>
</tr>
</tbody>
</table>

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 Electronic Supplementary Material Table S1.

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 unpressed 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 97 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 shrunk 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 (Eq. (2)) and Leptodora (Eq. (3)) preserved in 70% ethanol can be predicted as:

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.
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 subsampled observations from these sources that were from comparable depths (stations >60 m) and regions of the lake 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 >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 ($C_i$) 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:

$$P = 10^{\alpha_{\text{cladoceran}} + \beta \cdot T \text{ (°C)}} \times B$$

(4)

where $\alpha_{\text{cladoceran}}$ is a cladoceran-specific intercept (~ -1.725), $\beta$ 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 Johansson, 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 <0.1% of cladoceran densities and biomass.

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.

Vertical distributions of Bythotrephes and cladoceran prey

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 was 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.

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,

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.

<table>
<thead>
<tr>
<th></th>
<th>Bythotrephes density (± SEM)</th>
<th>Bosmina density (± SEM)</th>
<th>Daphnia density (± SEM)</th>
<th>Holopedium density (± SEM)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 2014</td>
<td>0</td>
<td>0</td>
<td>440 (2.35)</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>July 2014</td>
<td>0</td>
<td>0</td>
<td>3939.5 (137.71)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Aug 2014</td>
<td>23.0 (1.1)</td>
<td>1388.8 (99.54)</td>
<td>63.3 (0.38)</td>
<td>300 (29.43)</td>
<td>13</td>
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<tr>
<td>Oct 2014</td>
<td>3.6 (0.1)</td>
<td>217.4 (9.75)</td>
<td>63.3 (0.38)</td>
<td>300 (29.43)</td>
<td>13</td>
</tr>
<tr>
<td>May 2015</td>
<td>0</td>
<td>0</td>
<td>0.5 (0.03)</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>July 2015</td>
<td>2.5 (0.07)</td>
<td>14.2 (0.75)</td>
<td>500 (134.9)</td>
<td>210.2 (10.65)</td>
<td>4</td>
</tr>
<tr>
<td>Sept 2015</td>
<td>0.4 (0.32)</td>
<td>12.5 (0.75)</td>
<td>908 (124.7)</td>
<td>210.2 (10.65)</td>
<td>4</td>
</tr>
<tr>
<td>Oct 2015</td>
<td>1.3 (0.05)</td>
<td>225.0 (13.96)</td>
<td>16,214.9 (492.0)</td>
<td>292.3 (19.83)</td>
<td>7</td>
</tr>
</tbody>
</table>
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).

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 for total cladocera during July of 2015 was negative because mean cladoceran production was in deficit relative to Bythotrephes consumption while median cladoceran production was in excess of Bythotrephes consumption. 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

**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 (α = 0.05).

<table>
<thead>
<tr>
<th></th>
<th>2014</th>
<th>2015</th>
<th>#m⁻² (15 m)</th>
<th>#m⁻² (60 m)</th>
<th>p-Value</th>
<th>#m⁻² (15 m)</th>
<th>#m⁻² (60 m)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bythotrephes</td>
<td>11.6</td>
<td>9.2</td>
<td>0.22</td>
<td>1.3</td>
<td></td>
<td>2.4</td>
<td></td>
<td>0.13</td>
</tr>
<tr>
<td>Bosmina</td>
<td>457.4</td>
<td>484.5</td>
<td>0.19</td>
<td>67.6</td>
<td>0.04</td>
<td>114.7</td>
<td>6709.6</td>
<td>0.25</td>
</tr>
<tr>
<td>Daphnia</td>
<td>2227.8</td>
<td>3142.6</td>
<td>0.04</td>
<td>6312.3</td>
<td>0.12</td>
<td>6709.6</td>
<td>0.19</td>
<td>0.12</td>
</tr>
<tr>
<td>Holopedium</td>
<td>106.3</td>
<td>87.1</td>
<td>0.12</td>
<td>189.2</td>
<td>0.12</td>
<td>127.2</td>
<td>127.2</td>
<td>0.12</td>
</tr>
</tbody>
</table>

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 to 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.
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

### Table 4

<table>
<thead>
<tr>
<th>Month</th>
<th>Total Cladocera</th>
<th>Bosmina</th>
<th>Daphnia</th>
<th>Holopedium</th>
</tr>
</thead>
<tbody>
<tr>
<td>August 2014</td>
<td>1.04</td>
<td>1.21</td>
<td>1.13</td>
<td>1.28</td>
</tr>
<tr>
<td>October 2014</td>
<td>0.57</td>
<td>1.04</td>
<td>0.54</td>
<td>0.39</td>
</tr>
<tr>
<td>July 2015</td>
<td>−0.49</td>
<td>1.38</td>
<td>0.27</td>
<td>1.02</td>
</tr>
<tr>
<td>September 2015</td>
<td>0.88</td>
<td>1.34</td>
<td>0.88</td>
<td>0.00</td>
</tr>
<tr>
<td>October 2015</td>
<td>0.86</td>
<td>1.15</td>
<td>0.85</td>
<td>1.53</td>
</tr>
</tbody>
</table>
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 15 m 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 Dawson, 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 Branstator, 2004; Yuriita 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 Branstator (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 Branstator (2004). However, Yuriita 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; Dumitruc 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 increased 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, 1972) 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.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jglr.2017.09.011.

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References


