

Epilimnetic rotifer community responses to *Bythotrephes longimanus* invasion in Canadian Shield lakes

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Abstract

We studied whether the effects of *Bythotrephes longimanus*, a new predatory cladoceran zooplankton species to North America, can extend beyond the macrozooplankton to the rotifer community, which is known to be an important controller of the microbial loop. During summer 2003, 14 Canadian Shield lakes were sampled biweekly to evaluate the effect of *Bythotrephes* on epilimnetic rotifers. Rotifer densities, particularly those of *Conochilus unicornis*, significantly increased, with elevated *Bythotrephes* densities. *Conochilus* became the dominant rotifer species in the pelagic community of lakes with high *B. longimanus* populations. *Bythotrephes* densities were a better estimator of rotifer densities than physical and chemical lake properties. *Bythotrephes* has indirect effects beyond its preferred prey species, cladocerans. The potential for increased rotifer densities to act as a secondary source of food for *Bythotrephes* was also investigated. In a 24-h feeding experiment, *Bythotrephes* did not prey on various common rotifer species, suggesting that *Bythotrephes* ignores rotifer species as a prey even when they form the sole food source. Rotifer increases are likely either due to competitive release when crustacean zooplankton decline or predatory release when native invertebrate predator populations decline.

Nonindigenous species have been considered a major cause of extinction for native flora and fauna, reducing native biodiversity in invaded ecosystems (Wilcove et al. 1998). Lakes are perhaps the most susceptible ecosystem to invasions, due to the opening of trade shipping routes and increased between-lake boating activities, both of which act as dispersal vectors for nonindigenous species (Ricciardi and MacIsaac 2000). *Bythotrephes longimanus*, a predatory cladoceran, is one of the most recent invaders of concern in North America, likely arriving from the Baltic Sea area via ballast water into the Great Lakes in 1982 (Johannsson et al. 1991). *Bythotrephes* has since spread to an estimated 70 Canadian inland lakes, with hundreds of lakes in central Canada potentially colonizable (MacIsaac et al. 2000; Yan et al. 2002).

Bythotrephes is known to largely affect macrozooplankton

communities. *Bythotrephes* preys predominantly on medium-sized daphnids (Yan and Pawson 1997; Schulz and Yurista 1998), needing 14–20 Cladocera per day to meet its energetic requirements (Burkhardt and Lehman 1994). Because of these large feeding requirements, significant decreases of *Daphnia pulicaria* and *D. retrocurva* populations in Lake Michigan were observed following invasion (Schulz and Yurista 1998). Similarly, Harp Lake, a Canadian Shield lake, was invaded by *Bythotrephes* in 1993 and has since shown a 17% decline in zooplankton species richness, with the loss of several cladoceran species (Yan et al. 2002; Boudreau and Yan 2003). Boudreau and Yan (2003) have suggested that *Bythotrephes* with a density as low as 2 individuals m^{-3} can significantly depress cladoceran populations in Canadian Shield lakes.

Relatively few studies have examined *Bythotrephes* effects beyond cladocerans, their direct prey. There is potential, however, for *Bythotrephes*, through the reduction of cladocerans (Yan and Pawson 1997; Boudreau and Yan 2003), to cause major changes throughout the food web because cladocerans are known to have strong structural and functional effects in lake food webs (Brooks and Dodson 1965; Ives et al. 1999). Major food-web effects frequently involve cross-linkage cascades, whereby the suppression of one species by predation influences the abundance, biomass, or productivity of competing species within the same prey guild. By depressing cladoceran populations, predation by *Bythotrephes* could favor other pelagic herbivores. The focus here will be on the response of the epilimnetic rotifer community

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to *Bythotrephes* invasions. Rotifers are minute ($<250\ \mu\text{m}$) herbivores, feeding on small ($1\text{--}20\ \mu\text{m}$) phytoplankton, bacteria, and protists (Nogrady et al. 1993; Yoshida et al. 2003), and are key contributors to nutrient cycling in pelagic food webs (Makarewicz and Likens 1979; Nogrady et al. 1993). Rotifers may be responsible for up to 70% of small phytoplankton ($<10\ \mu\text{m}$) grazing in the epilimnion (Armengol et al. 2001). In addition, rotifers are an important food resource for planktivorous invertebrates (Williamson 1983) and larval fish (Guma'a 1978).

Brooks and Dodson (1965) were first to propose that a decrease in *Daphnia* populations could result in an increase in rotifer abundances through their "size efficiency hypothesis." This hypothesis and their work since (e.g., Romare et al. 1999; Sondergaard et al. 1997) suggest that the more efficiently filtering cladoceran species suppress rotifer populations through exploitative competition, unless predators are present, since planktivores generally select as prey larger zooplankton like *Daphnia* species. Further evidence suggests that rotifers also benefit from a reduction in interference competition with planktivory. Interference competition occurs when rotifers are killed, injured, or forced to drop their eggs as a result of direct physical interaction with cladocerans, including being swept into the feeding chamber of *Daphnia* (Gilbert 1985). Evidence supporting a release of rotifers from competition, owing to reduced *Daphnia* densities has come from studies on lake acidification (Yan and Geiling 1985; Svensson and Stenson 2002) and from experiments (Gilbert 1985, 1989).

A recent experiment evaluating the prey-choice selection of *Bythotrephes* indicates a preference for slow-moving prey, of a size class between 0.5 mm and 1.4 mm in length (Vanderploeg et al. 1993). It has been suggested that rotifers could act as a small alternative food source for *Bythotrephes* when food becomes scarce (Wahlstrom and Westman 1999). Thus far, the only rotifer reported to be preyed upon by *Bythotrephes* is *Asplanchna*, a large (0.6 mm) predatory rotifer (Vanderploeg et al. 1993). The characteristics of preferred prey for *Bythotrephes* suggests that another rotifer species, *Conochilus unicornis*, commonly found in Shield lakes, could be a potential prey species. *Conochilus* is unique among rotifers because it forms colonies. The colonies range from between 2 and 100 individuals, vary from 0.4 mm to 2 mm in length (Dieguez and Balseiro 1998), with dimensions that match the prey size of *Bythotrephes*. In addition, *Conochilus* colonies are slow moving and lack predatory escape movements.

The purpose of this study is to evaluate the cascading effects of *Bythotrephes* on rotifer populations by comparing epilimnetic rotifer communities in invaded and noninvaded lakes in the Canadian Shield. Increasing presence of *Bythotrephes* is predicted to result in an increase in rotifer densities across the 14 lakes included in this study. Rotifer community composition will be evaluated to determine whether there are any shifts in dominance owing to the invasion. The degree to which *Bythotrephes* density relative to physical and chemical lake properties are correlated with rotifer densities within lakes will be determined. Finally, the feasibility of rotifers as an alternate food source for *Bythotrephes* when

preferred food sources are excluded will be evaluated using a laboratory feeding experiment.

Methods

14 lake study—Study area: The Muskoka-Haliburton region of south-central Ontario, Canada, was chosen as a study area due to the high invasion rate of *Bythotrephes* in the past decade. The region also contains several noninvaded lakes allowing for comparisons between invaded and reference lakes. Ten invaded and 10 noninvaded lakes were initially chosen for the study in 2003. However, in six of the 10 noninvaded lakes, *Bythotrephes* individuals were found during summer sampling, and these lakes were therefore dropped from the study because the status and progression of the invasion was unknown. All lakes in the study were chosen using Dadswell (1974) to ensure similar characteristics (Table 1). All lakes were large, deep, and contained the glacial relict species *Mysis relicta*, suitable for *Bythotrephes*. Lakes were sampled biweekly during July and August 2003—months that typically have the highest densities of *Bythotrephes* (Yan and Pawson 1997).

Rotifer sampling: Rotifer samples were collected from the epilimnion of each lake, as most rotifers occur in the top layer of stratified lakes (Yan and Geiling 1985). Epilimnion depth was determined by recording temperature profiles of the whole water column at every sampling period. The epilimnion was defined as the top warm layer of water, bounded by the depth where a change of $>1^\circ\text{C m}^{-1}$ was observed. A vertical tube sampler (as described in Youngbluth et al. 1983) of 2.5-cm diameter was used to collect rotifers across the epilimnetic water column, in order to have every depth of the epilimnion equally represented in the sample. Thus, between 1.6 and 3.83 L of water were collected depending on the epilimnion depth (Table 1). Where volumes were less than 2 L the entire sample was used. For volumes greater than 2 L, the water was mixed in a bucket and a 2 L subsample was collected. The protocol was repeated at two sites, the deepest point of the lake and a site located 500 m away from the deep hole, and the samples from the two points were pooled together. The mixed sample was concentrated by filtering through a $30\ \mu\text{m}$ mesh and preserved in Lugol's solution (final concentration 2%), as suggested by Yoshida et al. (2003). Rotifers were identified to the genus level and counted using an Olympus CKX41 inverted microscope at $100\times$ and $1,000\times$ magnification, using random subsamples from each 4 L sample. The samples were thoroughly mixed and a wide aperture pipettor was used to draw 5 mL aliquots. Enough 5 mL subsamples were counted until a volume representing at least 500 mL of unfiltered lake water or at least 200 individuals of the most common species was examined. The five sampling periods from July and August were then averaged to obtain mean summer densities for all rotifers.

Bythotrephes sampling: *Bythotrephes* samples were collected at each visit using five vertical hauls of the entire water column, from five different locations (two of which overlapped with the rotifer collection sites), beginning 5 m

Table 1. Locations, edible chlorophyll *a* (<30 μm), and mean physical/chemical properties of the 14 study lakes. Values in brackets after mean epilimnion depth are the standard errors of the means for July and August.

| Lake | Coordinates | Elevation (m) | Surface area (km ²) | Max. depth (m) | Average epilimnion depth (m) | TP ($\mu\text{g L}^{-1}$) | TN ($\mu\text{g L}^{-1}$) | DOC (mg L ⁻¹) | Edible chl <i>a</i> ($\mu\text{g L}^{-1}$) | pH | <i>Bythotrephes</i> density class |
|----------------|-----------------|---------------|---------------------------------|----------------|------------------------------|-----------------------------|-----------------------------|---------------------------|--|------|-----------------------------------|
| Bernard | N45°22' W79°08' | 330 | 20.58 | 47.9 | 7.8 (0.6) | 9.56 | 568 | 3.4 | 2.05 | 7.13 | High |
| Buck | N45°24' W79°23' | 304 | 6.56 | 23.0 | 3.4 (0.5) | 13.30 | 558 | 10.7 | 2.38 | 6.33 | Non-Invaded |
| Doe | N45°32' W79°24' | 294 | 11.87 | 23.0 | 5.4 (0.7) | 12.00 | 428 | 7.0 | 1.88 | 7.08 | Non-Invaded |
| Fairy | N45°22' W79°08' | 284 | 7.11 | 69.5 | 5.2 (0.6) | 6.98 | 636 | 5.9 | 1.82 | 6.95 | Low |
| Harp | N45°22' W79°08' | 326 | 0.72 | 37.5 | 3.4 (0.2) | 7.22 | 537 | 6.7 | 1.17 | 6.98 | High |
| Kashagawigamog | N45°22' W79°08' | 318 | 8.18 | 39.7 | 5.4 (0.4) | 7.41 | 591 | 6.9 | 1.28 | 7.56 | Low |
| Lake of Bays | N45°22' W79°08' | 316 | 69.04 | 70.1 | 6.4 (0.7) | 3.38 | 248 | 5.1 | 1.60 | 6.97 | High |
| Mary | N45°22' W79°08' | 281 | 10.65 | 56.4 | 5.2 (0.7) | 7.21 | 551 | 6.6 | 1.78 | 6.96 | Low |
| Muskoka | N45°22' W45°22' | 225 | 122.06 | 66.5 | 7.6 (0.5) | 5.50 | 448 | 5.5 | 2.73 | 7.02 | Low |
| Peninsula | N45°20' W79°06' | 284 | 8.65 | 34.1 | 5.8 (0.5) | 9.00 | 732 | 6.1 | 1.68 | 7.14 | High |
| Pickering | N45°40' W79°17' | 312 | 5.13 | 38.0 | 4.6 (0.4) | 8.10 | 387 | 6.7 | 2.50 | 6.82 | Non-invaded |
| Sand | N45°37' W79°10' | 339 | 5.68 | 59.0 | 5.2 (0.4) | 7.10 | 507 | 6.0 | 1.31 | 6.78 | Non-invaded |
| Skeleton | N45°14' W79°26' | 281 | 21.55 | 65.0 | 7.4 (0.5) | 3.82 | 184 | 2.6 | 0.44 | 6.94 | Low |
| Vernon | N45°22' W79°08' | 284 | 15.05 | 37.0 | 4.8 (0.9) | 7.90 | 518 | 7.1 | 2.17 | 6.75 | High |

from the bottom of the lake. The vertical hauls were performed using a zooplankton net (400 μm mesh, 0.5 m diameter, 2.5 m in length). Samples were preserved in 5% formalin. Counts on entire samples and density calculations were conducted using a dissecting microscope, and enumeration procedures based on Yan and Pawson (1997). Where *Bythotrephes* abundances were higher than 32 in a sample, a Folsom plankton splitter was used to subsample until the maximum count had been reached or the entire sample counted. Before splitting, samples were well mixed to ensure that *Bythotrephes* were not clumped, and splits were verified by eye as well as with replicate counts to ensure that the process was evenly conducted. Values averaged over the five stations during the months of July and August are presented.

Crustacean zooplankton sampling: Crustacean zooplankton responses to *Bythotrephes* invasion in these lakes were investigated concurrently as part of another study on these communities. Zooplankton net hauls (110 μm mesh-sized net, 0.5 m diameter, 2.5 m in length) were taken to integrate over the hypo-, meta-, and epilimnion of each lake at the same time as sampling for rotifers and *Bythotrephes*. Zooplankton were anaesthetized with Alka-Seltzer water and preserved in 5.5% sugar formalin (final concentration). Subsamples were counted by Angela Strecker at Queen's University, Kingston, Ontario, Canada. Samples were subdivided using a magnetic mixer and a wide aperture pipette (6 mm), and counted until at least 350 animals had been identified. Because our focus is the response of the rotifer communities, we present only the data on the aggregated crustacean zooplankton density response to *Bythotrephes* invasion. The values presented are mean densities integrated across depths and averaged across the sampling period of July and August 2003.

Chlorophyll, physical and chemical data: At the deep point in each lake at every sampling date, epilimnetic water samples for chlorophyll *a* (Chl *a*) were collected in dark bottles and kept chilled until returned to the laboratory. Samples of 500 mL of epilimnetic water for total Chl *a*, and filtered water (<30 μm) for Chl *a* representative of the edible portion for zooplankton, were filtered onto GF/F filters. These were frozen and later analyzed fluorometrically using the method of Welshmeyer (1994). On each sampling occasion, also at the deep station, Secchi depth and temperature profiles were recorded. Chemical data (total P, total N, NH₄, DOC, pH, K, Si, Na, Ca, and alkalinity) for each lake were collected at the midway sampling period, between 28 July and 1 August 2003, using Ministry of the Environment (MOE) guidelines. Chemistry analysis was performed at the MOE, Dorset, Ontario, Canada, using standard methods outlined in the Protocol of Accepted Drinking-Water Testing Methods, MOE Laboratory Services Branch Report (2003).

Analyses: To evaluate the effect of *Bythotrephes* on total rotifer community density, summer mean rotifer densities were regressed against summer mean *Bythotrephes* densities across lakes. Analyses were focused further on the density relationships of the dominant rotifer genera: *Conochilus*, *Keratella*, and *Polyarthra*. For simple regressions that failed

normality or equality of variance tests (e.g., *Keratella*), densities were log transformed (Zar 1999). Stepwise multiple regressions were performed with either total rotifer or *Conochilus* densities as dependent variables to determine which combination of *Bythotrephes*, physical, or chemical lake properties were the best indicators of rotifer densities. *Conochilus* was chosen as the main genus-level variable in the regression models because of all groups, they showed the strongest response to *Bythotrephes*. Chemistry variables used for the multiple regression were pH, DOC, total P, total N, and conductivity after collinear variables were removed. Chl *a* (<30 μm) was used as a biotic variable. For the stepwise multiple regression analysis, all possible combinations of predictors were tested, and the best model (selected based on the lowest residual mean square value) was chosen, as suggested by Philippi (1993).

To determine whether community composition of rotifers changed with increased densities of *Bythotrephes*, lakes were divided into three groups: noninvaded lakes (4 lakes), low-density *Bythotrephes* lakes (5 lakes), and high-density *Bythotrephes* lakes (5 lakes). High-density *Bythotrephes* lakes were defined as lakes with observed densities of >2 individuals m^{-3} during at least 4 out of 5 sampling visits during July–August. This criterion was based on results for lakes in this area from Boudreau and Yan (2003) who reported that *Bythotrephes* had major effects on macrozooplankton communities above a threshold density of 2 individuals m^{-3} . Low-density *Bythotrephes* lakes were those with the invader, but that did not meet the threshold criterion. Mean rotifer community composition was recorded for all three *Bythotrephes* groups and measured as both percentage of community composition and as overall abundances by genus. Proportions were arcsine square root transformed (Zar 1999) for analysis purposes. Statistical differences between groups were determined using an ANOVA and an a posteriori Tukey's test.

Rotifer community diversity was assessed using three indices: richness, Shannon-Wiener diversity index (H'), and a measure of community evenness (D). Each measure was regressed against *Bythotrephes* density to determine whether rotifer diversity was influenced by *Bythotrephes* densities. For richness, specimens were identified to the lowest possible taxon. Richness was defined as the mean number of species (or lowest taxonomic identification) observed within a sample per lake visit. The Shannon-Wiener Function (H') used is calculated as follows:

$$H' = \sum (p_i)(\log_{10} p_i)$$

where p represents the proportion of total sample belonging to the i th species (Krebs 1989). The Shannon-Wiener diversity index is commonly used in rotifer community assessment (Svensson and Stenson 2002). The measure of Evenness (D) (Krebs 1989) is calculated as:

$$D = H'_{\text{observed}}/H'_{\text{max}}$$

Mean total Chl *a* and edible Chl *a* (<30 μm) for all lakes were regressed against mean *Bythotrephes* densities to determine whether the invader had a broad impact on Chl *a* levels.

Feeding experiment—*Bythotrephes* and three common rotifer species: *C. unicornis*, *K. cochlearis*, and *Ploesoma* sp.—were collected from one of the lakes within the 14 lakes study, Peninsula Lake, Ontario, Canada, on 10 August 2004. Samples were collected using 10-m vertical hauls with a zooplankton net (net: 80 μm mesh, 0.8 m diameter, 1.5 m in length, cod end: 80 μm mesh, 8 cm diameter, 15 cm in length). The specimens were placed in a cooler and transported immediately to the University of Guelph, Guelph, Ontario, Canada, where samples were sorted using a dissecting microscope, gently isolating the rotifer species and *Bythotrephes* using a wide-bored plastic pipettor. Due to difficulty in maintaining and culturing *Bythotrephes* and *Conochilus* in laboratory settings (Burkhardt and Lehman 1994; Gilbert 1988), animals were not acclimated to experimental conditions prior to the feeding experiment. However, all animals in all treatments were treated in the same way. Laboratory-cultured *D. pulex*, 1–1.5 mm in length, were used during the experiment to ensure that *Bythotrephes* would feed under these experimental settings.

A total of 100 *Bythotrephes* 3rd instar individuals were used during the feeding trial. Each *Bythotrephes* individual was placed into its own well (3 cm diameter, 1.5 cm deep) in a well tray, containing 10 mL of 30 μm filtered lake water obtained from Peninsula Lake. The selection of well size was based on the study by Modrukhai-Boltovskaya (1959, cited in Vanderploeg et al. 1993). This author successfully determined *Bythotrephes* prey choice and ingestion rates in wells of 2–4.5 cm diameter \times 1–2.5 cm deep. Other studies into plankton interactions have similarly used small vessels (e.g., 40–600 mL in Gilbert 1985, 1988). Each well was randomly assigned one individual of five prey species: (1) *D. pulex*; (2) a large *C. unicornis* colony, defined by >50 individuals; (3) a small *C. unicornis* colony, defined by those with <30 individuals; (4) *K. cochlearis*; (5) or *Ploesoma* sp. A total of 20 replicate wells were used for each prey species. The wells were covered with a clear plastic top to reduce evaporative water loss, and a thin tissue to allow only diffuse light to pass. All trays were placed in an incubator (14 h light, 10 h dark, 20°C) for 24 h. After 24 h, each well was examined under a dissecting microscope. *Bythotrephes* was recorded as dead or alive (survival rate was 100%), and each prey species was evaluated for survival. The prey species were recorded as killed by *Bythotrephes* if (1) the individual was not found in the container, or (2) the individual was partially eaten.

The survival of prey species was compared using an ANOVA with prey species as treatment levels. A Tukey's test was utilized to determine significant differences between groups ($\alpha = 0.05$).

Results

14 lake study—Total rotifer density increased significantly ($r^2 = 0.60$, $p = 0.001$, $n = 14$) with *Bythotrephes* density (Fig. 1A), reaching a maximum of 1,300 individuals L^{-1} at the highest observed densities of *Bythotrephes*. For the dominant genera, *Polyarthra* densities also showed a significant increase ($r^2 = 0.35$, $p = 0.025$, $n = 14$) (Fig. 1B), as did

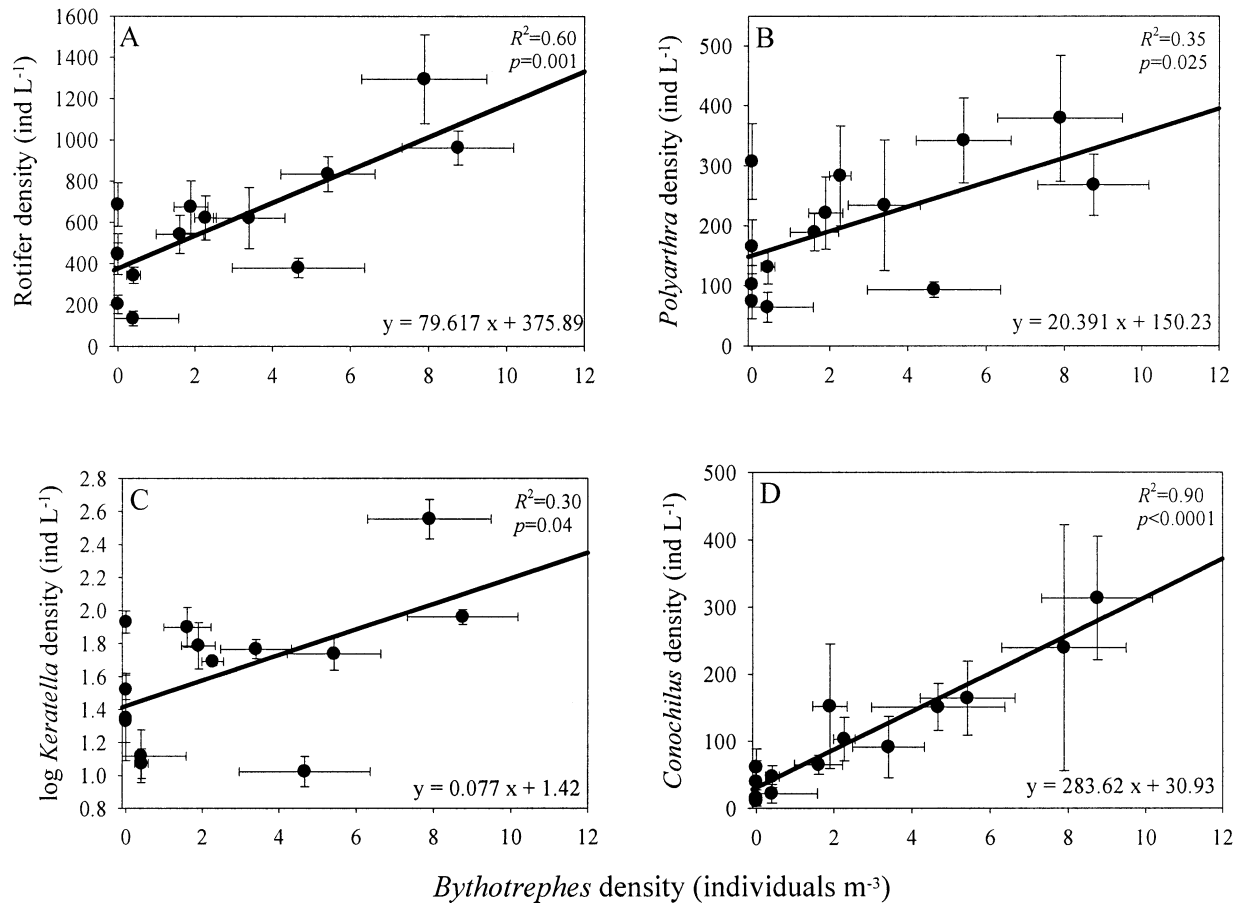


Fig. 1. Best fit linear regression model showing the positive relationship between summer mean (A) total rotifer, (B) *Polyarthra*, (C) log *Keratella*, and (D) *Conochilus* densities, and *Bythotrephes* densities for the 14 lakes. Error bars represent standard errors of the means.

Keratella ($r^2 = 0.30$, $p = 0.04$, $n = 14$) (Fig. 1C). In the case of the colonial rotifer, *Conochilus*, its densities displayed the strongest positive linear correlation with *Bythotrephes* densities ($r^2 = 0.90$, $p < 0.0001$, $n = 14$) (Fig. 1D). In examining the total increase in rotifer density by fitting a multiple regression, *Bythotrephes* was found to be the single best predictor of total rotifer densities. Adding Chl *a* to the regression improved the model by explaining an additional 17% of the variation. The best-fitting multiple regression model in explaining rotifer densities was ($R^2_{\text{adj.}} = 0.77$, $p = 0.0001$):

$$\text{rotifer density} = -23.0 + 76.9 \text{ Bythotrephes} \\ + 229.3 \text{ edible Chl } a + \varepsilon$$

Similarly, *Bythotrephes* was found to be the single best indicator of *Conochilus* densities. Unlike the total rotifer model, Chl *a* levels did not play a factor in determining *Conochilus* densities. Lake elevation, combined with *Bythotrephes* densities were determined to be the best-fitting multiple regression model for predicting *Conochilus* densities ($R^2_{\text{adj.}} = 0.93$, $p < 0.0001$):

$$\text{Conochilus density} = 216.5 + 28.4 \text{ Bythotrephes} \\ - 0.62 \text{ elevation} + \varepsilon$$

The positive effect of *Bythotrephes* on *Conochilus* was

also noted in the analysis of lakes (ANOVA) clustered into groups based on thresholds for *Bythotrephes* effects. In the rotifer community, *Conochilus* proportions were found to be significantly different between groups ($p = 0.002$, $F_{2,11} = 11.08$) significantly increasing from 6.7% in noninvaded lakes to 25.4% in high-density *Bythotrephes* lakes ($p = 0.002$) (Fig. 2). Similarly, mean *Conochilus* densities increased 5-fold, from 36 individuals L^{-1} in noninvaded lakes to 192 individuals L^{-1} ($p = 0.007$) in high-density *Bythotrephes* lakes (Fig. 3). Other dominant rotifer species (except *Ascomorpha*) showed small increases in density in high-density *Bythotrephes* lakes, however, no increases were statistically significant ($p > 0.05$) (Fig. 3). Overall, between noninvaded and high-density *Bythotrephes* lakes, mean total rotifer abundance increased from 517 individuals L^{-1} to slightly over 800 individuals L^{-1} , but the difference was not statistically significant.

While rotifer community composition showed a shift in dominance towards *Conochilus* in high-density *Bythotrephes* lakes, the diversity measures did not show any significant changes (data not shown). Rotifer species richness in relation to *Bythotrephes* density did not display any significant trend ($r^2 = 0.10$, $p = 0.26$, $n = 14$). The Shannon-Weiner diversity index (H') also showed no correlation with *Bythotrephes* densities ($r^2 = 0.007$, $p = 0.77$, $n = 14$), nor did the measure of evenness (D) ($r^2 = 0.06$, $p = 0.39$, $n = 14$).

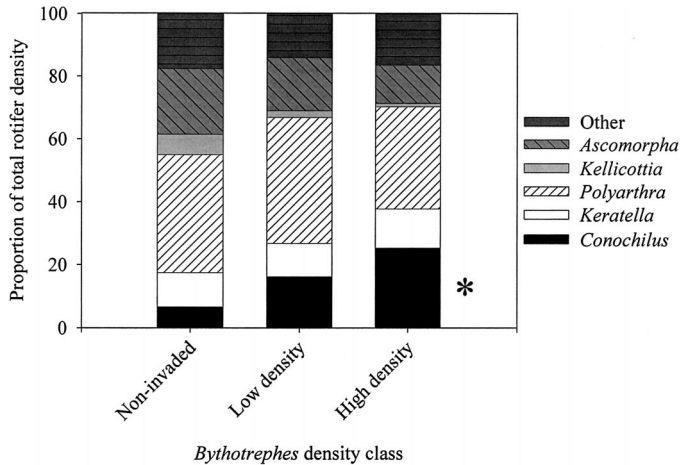


Fig. 2. Genera composition (proportion of total density) for rotifer communities in the three lake sets: noninvaded, low-density *Bythotrephes* (<2 individuals m⁻³), and high-density *Bythotrephes* (>2 individuals m⁻³) lakes. The asterisk (*) indicates a significant difference for a genus across groups as determined by ANOVA.

Total Chl *a* ($r^2 = 0.03$, $p = 0.61$, $n = 14$) and edible Chl *a* (<30 μm) ($r^2 = 0.003$, $p = 0.84$, $n = 14$) levels do not appear to be influenced by *Bythotrephes* densities across the 14 lakes (all $p > 0.05$; data not shown). Total crustacean zooplankton density declined in a nonlinear fashion with *Bythotrephes* density averaged over the summer months and integrated over depth (Fig. 4).

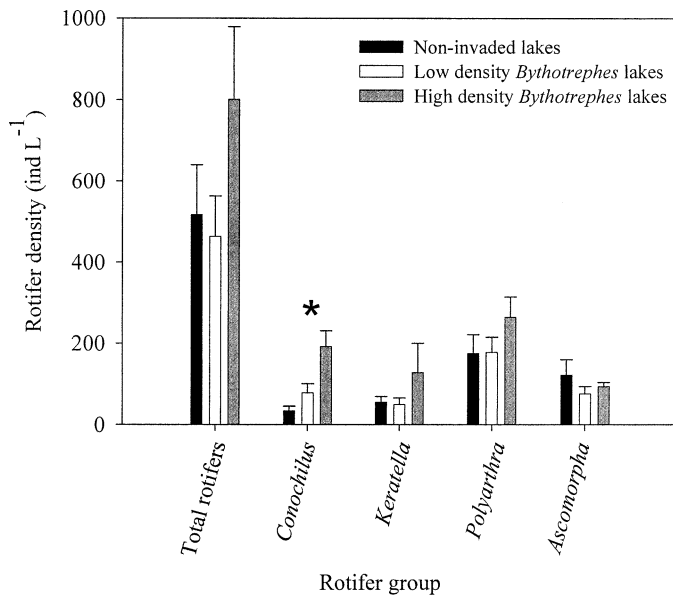


Fig. 3. Mean (± standard error) densities (L⁻¹) for total rotifers, as well as the four most dominant rotifer genera in the 14 study lakes. Densities were compared across lakes grouped as noninvaded, low *Bythotrephes* density (<2 individuals m⁻³), and high *Bythotrephes* density (>2 individuals m⁻³). The asterisk (*) indicates a significant difference by genus across lake groups as determined by ANOVA and a Tukey's test.

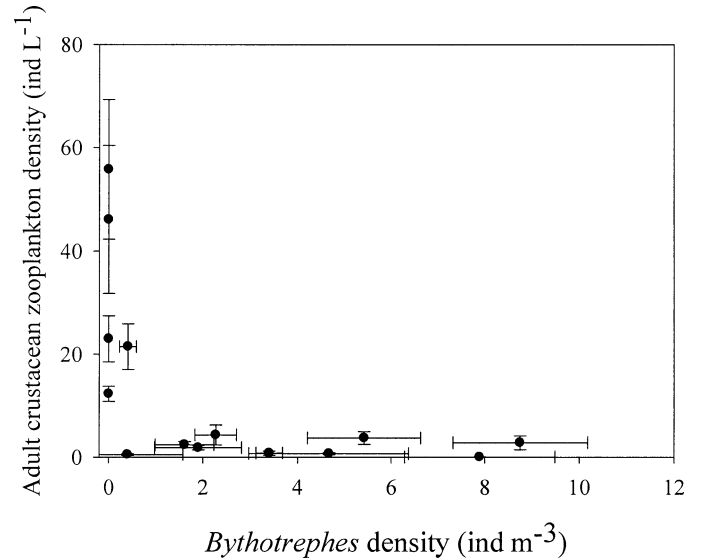


Fig. 4. Mean (± standard error) crustacean zooplankton densities (L⁻¹) over the summer months in the lakes studied in relation to the mean summer *Bythotrephes* densities.

Feeding experiment—All 100 *Bythotrephes* individuals were alive at the end of the 24-h feeding experiment. In the control trials, 14 of 20 *Daphnia* were eaten by *Bythotrephes* (Table 2). Individuals in the control treatment were consumed at significantly higher rates ($p < 0.05$) than the large *C. unicornis* colonies, small *C. unicornis* colonies, *K. cochlearis*, and *Ploesoma* sp. In the four rotifer treatments, all rotifers were still present at the end of the feeding experiment (Table 2). It should be noted that in one replicate for the large *Conochilus* colonies, the colony was found broken apart, potentially as a result of experimental stress, or from an encounter with *Bythotrephes*.

Discussion

In Canadian Shield lakes, *Bythotrephes* densities are related to large increases in total rotifer densities in the pelagic zone. Through multiple regressions, *Bythotrephes* densities were found to be a stronger determining factor of rotifer densities than the major physical and chemical parameters of the 14 lakes, explaining 60% of the variation observed. The increase in rotifer densities in invaded lakes is evidence

Table 2. Number of eaten and surviving individual prey items: *Daphnia pulex* (control), large *Conochilus* colonies, small *Conochilus* colonies, *Keratella cochlearis*, and *Ploesoma* sp. at the end of the 24-h feeding experiment with *Bythotrephes longimanus*.

| Prey species | Trials | No. eaten | Surviving |
|----------------------------------|--------|-----------|-----------|
| <i>Daphnia pulex</i> | 20 | 14 | 6 |
| Large <i>Conochilus</i> colonies | 20 | 0 | 20 |
| Small <i>Conochilus</i> colonies | 20 | 0 | 20 |
| <i>Keratella cochlearis</i> | 20 | 0 | 20 |
| <i>Ploesoma</i> sp. | 20 | 0 | 20 |

that *Bythotrephes* can have cascading effects on the food webs beyond its direct effect on macrozooplankton prey. The rotifer response observed here is among the first reported of indirect effects of *Bythotrephes* in their invaded habitats in North America.

The increase in rotifer density was mainly due to an increase in the genus *Conochilus*, a colonial rotifer. When comparing noninvaded lakes to those with high invader densities (>2 individuals m^{-3}), *Conochilus* densities increased 5-fold, so that in high-density lakes, *Conochilus* became one of the dominant rotifers within the community. In a mesocosm experiment, Wahlstrom and Westman (1999) inoculated enclosures containing lake zooplankton communities with varying *Bythotrephes* densities. While total rotifer densities were not found to be affected by *Bythotrephes*, in contradiction to the findings of this current 14 lake study, they noted a positive correlation between *Conochilus* abundance and *Bythotrephes* densities. An increase in *Conochilus* was also noted in Lake Michigan after the arrival of *Bythotrephes* (Branstrator and Lehman 1991).

Rotifers are often ignored for their potential impacts on the phytoplankton and bacteria communities, owing to their low individual clearance rates. However, rotifers have greater weight-specific feeding rates than larger zooplankton (Armengol et al. 2001). Rotifers, such as *Keratella*, have weight-specific clearance rates of $108 \mu L \mu g^{-1}$ dry wt h^{-1} , three times higher than rates for the larger cladoceran, *Daphnia pulex*, which clears at a rate of $32 \mu L \mu g^{-1}$ dry wt h^{-1} (Bogdan et al. 1980). As a result, large increases in rotifer abundance could significantly increase the filtration of small phytoplankton in the epilimnion. Clearance rates for *Conochilus*, *Keratella*, and *Polyarthra*, the dominant rotifer species in invaded lakes here, can be estimated at $7.5 \mu L \text{anim}^{-1} h^{-1}$ using an average of clearance rates found in Bogdan et al. (1980), Bogdan and Gilbert (1984), and Armengol et al. (2001). Based on the average observed increase of over 300 rotifers L^{-1} between noninvaded and high *Bythotrephes* density lakes, rotifers in high-density lakes will filter an extra $2,200 \mu L h^{-1} L^{-1}$ or $\sim 5.3\%$ of the water volume per day, for particles $<10 \mu m$ in size. We did not observe any noticeable trend in edible Chl *a* ($<30 \mu m$) levels across the 14 lakes, suggesting that this increase in total rotifer filtering rates may at least partially compensate for sharp declines in crustacean zooplankton that were observed. In addition, a lack of change in Chl *a* does not discount a possibility for changes in the nanoplankton composition of invaded lakes. Sceda and Cowell (1988) reported that a doubling of rotifer abundances produced distinct shifts in taxonomic composition and size distribution of the phytoplankton community, primarily through the consumption of nanoplankton. This suggests that the increase in rotifer abundances in invaded *Bythotrephes* lakes could have important structural and functional effects on the phytoplankton community. A detailed study of associated effects and changes in the microbial loop is warranted.

Wahlstrom and Westman (1999) noted that rotifers may act as an alternative food source for *Bythotrephes*. While *Conochilus* colonies overlap the preferred prey size of *Bythotrephes*, we found no feeding by *Bythotrephes* on *Conochilus*, during the feeding experiment. *Conochilus* may

form large colonies to deter predation (Dieguez and Balseiro 1998). However, since both large and small *Conochilus* colonies were ignored as a source of food by *Bythotrephes*, the size of the colonies was not the determining factor in preventing predation here. One possible explanation for why *Conochilus* was ignored by *Bythotrephes* is that *Conochilus* colonies are held together in a gelatinous sheath thought to be distasteful to other invertebrate predators including *Polyphemus* (Matveeva 1989). The cladoceran, *Holopedium*, is also surrounded by a gelatinous sheath, and has become one of the dominant zooplankton species in *Bythotrephes*-invaded lakes in Ontario (Yan and Pawson 1997). Solitary rotifer species were also ignored as a prey by *Bythotrephes* during the feeding experiment, probably due to their small size.

Feeding experiments can sometimes cause high stress levels for organisms, evident through high mortality rates and diminished appetites, resulting in misconstrued or false results. All 100 *Bythotrephes* individuals used here survived the feeding trial, with 70% of the *Bythotrephes* feeding in the control (*Daphnia* prey) group. The lack of mortality suggests that *Bythotrephes* were not overly stressed, while results from the control group demonstrate that most individuals willingly fed. Thus, we can discount the effect of stress as a significant factor driving the lack of predation on rotifer species by *Bythotrephes* observed.

The results we have presented here suggest large indirect effects of *Bythotrephes* invasion on rotifer communities. However, we have yet to determine mechanistically, the specific food web pathways of effects—direct and indirect—that are invoked by *Bythotrephes* and that lead to an increase in rotifers, especially *Conochilus*. There are two main hypotheses that emerge to explain the increase in rotifer densities. The first is that a competitive release of rotifers occurs due to declines in crustacean zooplankton preyed upon by *Bythotrephes*. This is the most obvious starting point, because within the 14 lakes studied in 2003, crustacean densities were reduced in invaded lakes, and several other studies have already demonstrated a decline in cladoceran abundance and richness (Yan and Pawson 1997; Boudreau and Yan 2003) in invaded lakes in this same region of Ontario. Other evidence that supports this mechanism is related to the fact that *Conochilus* showed the most pronounced response to *Bythotrephes* invasion. It is interesting to note that species that have been observed to respond with large density increases to *Bythotrephes* invasion in this, and previously mentioned studies, (*Holopedium* and *Conochilus*) are both encased in gelatinous sheaths. As a hypothesis for why these species, and *Conochilus* in particular here, are observed to increase, we propose the following. These species may normally be at an energetic disadvantage relative to their counterparts in the zooplankton because of their sheath production. Thus, if resources are liberated as their major competitors are reduced by *Bythotrephes* predation, *Conochilus* may be able to respond with rapid population growth. *Conochilus* has been shown to be relatively sensitive to abundances of larger cladoceran species like *Daphnia* through exploitative competitive for phytoplankton resources and to show large population increases when resources become available (Hampton 2005).

A second hypothesis that has been little explored in the literature but that may also play a role, is a release of direct predation pressure on rotifers, due to the suppression of other macrozooplankton predators by *Bythotrephes*. *Bythotrephes* appears to inhibit rotifer predators *Leptodora kindtii* (Branstrator and Lehman 1991; Branstrator 1995) and *Mesocyclops edax* (Barbiero and Tuchman 2004) through predation and competition, potentially resulting in a release in predation pressure and population control on rotifers. The two hypotheses for rotifer increase, competitive and/or predatory release with *Bythotrephes* establishment, must be evaluated with further observational and comparative study.

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