

The differing crustacean zooplankton communities of Canadian Shield lakes with and without the nonindigenous zooplanktivore *Bythotrephes longimanus*

Stephanie A. Boudreau and Norman D. Yan

Abstract

The nonindigenous predatory cladoceran *Bythotrephes longimanus* is spreading rapidly among Canadian Shield lakes, but only one case study of its impacts exists. In Harp Lake, the abundances of several cladoceran and one cyclopoid species fell after the invasion, and far fewer species benefited. To determine if Harp Lake provides typical results, we compared the summer crustacean zooplankton communities of 17 invaded and 13 noninvaded (reference) lakes in Ontario. The communities of the two lake groups differed. Average species richness was 30% higher in the reference (15.3 species) vs. the invaded lakes (11.8 species). Total zooplankton biomass was significantly lower in the invaded lakes, mainly because of lower abundances of all common epilimnetic cladoceran species. As these results were quite similar to those of Harp Lake, it is apparent that current summer zooplankton communities of Canadian Shield lakes with *Bythotrephes* differ substantially from noninvaded lakes.

Resume

Résumé : Bien que le cladocère prédateur non indigène *Bythotrephes longimanus* se répande rapidement dans les lacs du Bouclier canadien, il n'existe qu'une seule évaluation de son impact. Dans le lac Harp, les abondances de plusieurs espèces de cladocères et d'une espèce de cyclopoïdes ont décliné depuis l'invasion, alors qu'un petit nombre d'espèces en a tiré bénéfice. Pour voir si la situation au lac Harp est caractéristique, nous avons comparé les communautés de crustacés zooplanctoniques d'été dans 17 lacs colonisés et 13 lacs non colonisés (témoins) de l'Ontario. Les communautés des deux groupes de lacs sont différentes. La richesse moyenne en espèces est de 30 % plus élevée dans les lacs témoins (15,3 espèces) que dans les lacs affectés (11,8 espèces). La biomasse totale du zooplancton est significativement plus basse dans les lacs envahis, principalement à cause des densités réduites de toutes les espèces communes de cladocères épilimnétiques. Ces résultats sont très semblables à ceux obtenus au lac Harp et il est donc clair que les communautés actuelles de zooplancton en été des lacs envahis par *Bythotrephes* diffèrent considérablement de celles des lacs non affectés.

[Traduit par la Rédaction]

Introduction

Bythotrephes longimanus Leydig (Crustacea, Branchio-poda, Onychopoda), hereafter simply called *Bythotrephes*, is a large, predatory cladoceran species native to much of Eurasia (Grigorovich et al. 1998; Therriault et al. 2002). First discovered in the Laurentian Great Lakes in the early 1980s, *Bythotrephes* almost certainly arrived in the ballast water of transoceanic ships (Mills et al. 1993). It was first reported in inland lakes in Ontario in 1989 (Yan et al. 1992), and since then it has spread to close to 50 Canadian Shield lakes in Ontario (MacIsaac et al. 2000; Therriault et al. 2002). We have only one thorough case study of the impacts of the invader on zooplankton of Shield lakes. In 1993, *Bythotrephes* was recorded in Harp Lake, a lake with a long record of pre-invasion monitoring. Following the invasion, zooplankton species richness declined significantly, as did average abundances of many small-bodied cladoceran species and *Meso-cylops edax*. A smaller number of larger species (*Daphnia mendotae*, *Holopedium glacialis*) became more abundant (Yan and Pawson 1997; Yan et al. 2001, 2002). Although there are no other Shield lake case studies, *Bythotrephes* has also been implicated in zooplankton community changes in Lake Michigan (Lehman and Caires 1993; Makarewicz et al. 1995) and in Long Lake, Mich., U.S.A. (Hoffman et al. 2001).

Bythotrephes is found in a wide range of lakes in Europe (Grigorovich et al. 1998; Therriault et al. 2002); however, it most commonly occurs in large, deep, oligotrophic lakes (MacIsaac et al. 2000). There are many such lakes in temperate North America, particularly on the Canadian Shield. Thus, if the Harp Lake results prove to be typical, there may be widespread changes in zooplankton populations on the Shield in response to *Bythotrephes* introductions.

Received 1 January 2003. Accepted 31 August 2003.

Published on the NRC Research Press Web site at <http://cjfas.nrc.ca> on 17 November 2003. J17259

S.A. Boudreau¹ and N.D. Yan² Department of Biology, York University, 4700 Keele Street, Toronto, ON M3J 1P3, Canada.

¹Present address: 55 Brittany Avenue, Kingston, NS BOP 1R0, Canada.

²Corresponding author (e-mail: nyan@yorku.ca).

The best test of this prediction would be a comparison of pre- and post-invasion zooplankton assemblages in several lakes, but pre-invasion data sets are not commonly available. An alternative is to compare the zooplankton communities from carefully selected invaded and noninvaded Shield lakes. We do such a comparison herein. Our objective is to compare the crustacean zooplankton communities of Shield lakes that have and have not been invaded by *Bythotrephes*. As we see no reason for the Harp Lake results to be particularly atypical (Yan et al. 2002), we hypothesize that there will be large differences in the zooplankton communities of these two groups of lakes, differences similar to those that followed the introduction of *Bythotrephes* into Harp Lake.

Methods

We selected the two sets of lakes (with and without *Bythotrephes*) ensuring that means of several variables known to influence Shield lake zooplankton communities did not differ (see Boudreau (2002) for details). The 30 lakes were located in the Muskoka, Parry Sound, and Haliburton regions of south-central Ontario, Canada, the area on the Shield with the highest *Bythotrephes* incidence. Lakes with *Bythotrephes* were identified from previous sightings (Yan et al. 1992; Borbely 2001; Therriault et al. 2002). In two cases, lakes that were initially chosen as reference lakes (Kennisis and Three-Mile) unexpectedly supported *Bythotrephes* populations. Thus, although we had aimed for an equal number of invaded and reference lakes, our final data set had 17 invaded lakes and 13 reference lakes. Lake names, locations, and basic limnological characteristics are described in Boudreau (2002).

In selecting lakes, we ensured that averages of lake area ($t = 0.6, p = 0.56$), maximum depth ($t = -0.08, p = 0.94$), total phosphorus concentrations ($t = -1.6, p = 0.1$), pH ($t = 0.42, p = 0.68$), and lake alkalinity ($t = 0.037, p = 0.97$) did not differ between reference and invaded lakes. Of 14 total chemical and morphometric variables examined (Boudreau 2002), only sodium concentrations differed between the two groups of lakes. Sodium levels averaged $3.02 \text{ mg}\cdot\text{L}^{-1}$ in the invaded lakes, significantly higher (independent samples t test, $p = 0.011$) than the $1.57 \text{ mg}\cdot\text{L}^{-1}$ in the reference lakes. The primary source of elevated Na in continental temperate lakes in North America is salt applied to roads in winter as a de-icer (Rosenberry et al. 1999; Mayer et al. 1999). Differences in Na of the magnitude detected in the two groups are not known to have any impacts on zooplankton composition.

We assessed *Bythotrephes* abundance in each lake, reasoning that any impacts on zooplankton would covary with *Bythotrephes* abundance. To assess *Bythotrephes* abundance, we employed Yan and Pawson's (1998) protocol, sampling *Bythotrephes* in vertical hauls from 3 m above bottom to the surface, using a 0.75 m diameter, 2.5 m long, 285 μm mesh, Rigosha meter-equipped net at each of 10 randomly located stations in each lake. All samples were fixed and preserved in a 4% buffered sucrose-formalin solution in the field and were subsequently examined in their entirety for *Bythotrephes*. *Bythotrephes* abundances were calculated from sample volumes corrected for the measured haul filtration efficiency.

Crustacean zooplankton were sampled using a Dorset Research Centre (DRC) net (McQueen and Yan 1993), an 80 μm mesh, 0.12 m diameter, 0.75 m long, conical, metered tow net. One vertical haul was taken to the surface from 2 m off bottom at the deepest spot in the lake. Sample volumes were calculated correcting for haul filtration efficiency, which averaged 77%. Animals were counted and their video images were measured with electronic calipers using a semi-automated zooplankton counting system (Allen et al. 1994) on a Nikon SMZ-10 dissecting scope (Nikon Canada Instruments, Cooksville, Ont). Animals were identified using 20 standard and modern taxonomic references (listed in Boudreau 2002). The counting protocol was designed to identify, count, and measure the body lengths of a minimum of 300 individuals in subsamples generated with a Folsom plankton splitter (Wildco Wildlife Supply Co., Saginaw, Mich.). Splits were designed to ensure that adequate numbers of rarer taxa were enumerated. Indeed, the entire sample was usually counted to ensure identification of these rare taxa. Where length-weight regressions employed had been developed from formalin-preserved animals, weights were increased by 37% to correct for weight loss in formalin (Giguere et al. 1989). The original Ahmic Lake sample (a 23-m haul) was lost before processing. In this one case, a vertical sample through the epilimnion and metalimnion (13-m haul) was counted in place of the lost sample. We had taken these samples as backups in every case.

To reduce any confounding influence of seasonal changes in composition (lakes were sampled between July and September of 2001), the order of sampling lakes was randomized. To ensure that we did not ourselves become *Bythotrephes* transit vectors, all nets were sterilized with a 10% bleach solution between lakes, and the boat and trailer were washed with a high-pressure washer (Canadian Tire Corp. Ltd., Toronto, Ont.) then dried in the sun between sampling trips.

We were interested in testing for differences in species richness between the two groups of lakes, and it is widely recognized that richness increases with sample size. On average, significantly more animals (t test, $p = 0.004$) were counted in the reference lakes (mean of 294) than in the invaded lakes (mean of 243). However, there was no correlation between numbers counted and species richness ($r^2 = 0.01, p = 0.64$); therefore, no correction of richness for sample size was needed.

We summarized the counting data as the density (animals $\cdot\text{L}^{-1}$ or animals $\cdot\text{m}^{-3}$), biomass ($\mu\text{g}\cdot\text{L}^{-1}$ dry weight (dw)), richness (species $\cdot\text{sample}^{-1}$), mean body length (mm), and average body weight (μg dw) of each species. To test for differences between the zooplankton assemblages in the two sets of lakes, the data were first normalized using log ($x + 1$) transformation. At a community level of data integration, an independent-samples t test was used to determine if

Fig. 1. Scattergram of the frequency of occurrence of crustacean zooplankton species in the lakes with vs. without (i.e., reference lakes) *Bythotrephes*. Unidentified immatures are excluded. Species are numbered for their ranked frequency of occurrence in the reference lakes: 1, *Diacyclops bicuspidatus thomasi*; 2, *Mesocyclops edax*; 3, *Daphnia mendotae*; 4, *Holopedium glacialis* (commonly called *H. gibberum*); 5, *Bosmina freyi*; 6, *Leptodiatomus minutus*; 7, *Skistodiatomus oregonensis*; 8, *Diaphanosoma birgei*; 9, *Epischura lacustris*; 10, *Tropocyclops extensus* (commonly called *Tropocyclops prasinus mexicanus*); 11, *Daphnia dubia*; 12, *Daphnia longiremis*; 13, *Bosmina longispina*; 14, *Cyclops scutifer*; 15, *Chydorus sphaericus*; 16, *Daphnia catawba*; 17, *Bosmina tubicen*; 18, *Acanthocyclops vernalis* complex; 19, *Polyphemus pediculus*; 20, *Sida crystallina*; 21, *Ceriodaphnia lacustris*; 22, *Daphnia pulicaria*; 23, *Daphnia retrocurva*; 24, *Daphnia ambigua*; 25, *Latona setifera*; 26, *Bosmina liederii*; 27, *Limnocalanus macrurus*; 28, *Senecella calanoides*; 29, *Orthocyclops modestus*; 30, *Bosmina coregoni*; 31, *Leptodiatomus ashlandi*; 32, *Leptodiatomus sicilis*.

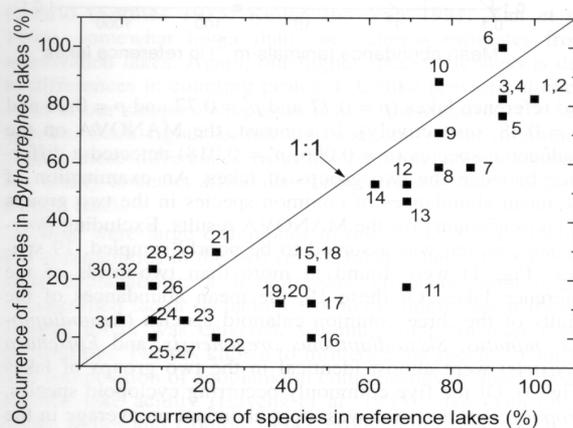
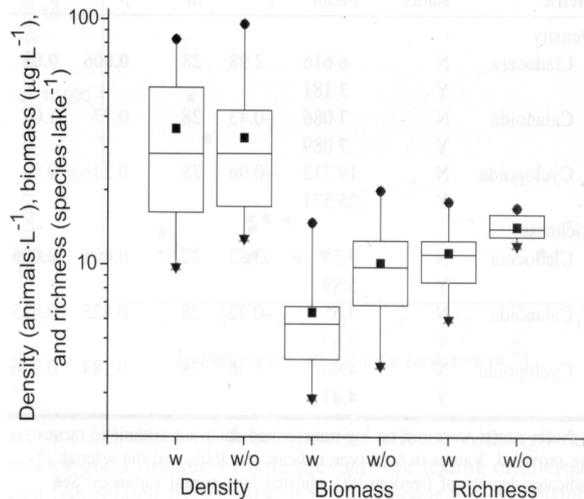


Fig. 2. Box-and-whisker plots of total crustacean zooplankton density (animals·L⁻¹), biomass (µg dry weight·L⁻¹), and species richness (species·sample⁻¹) in lakes with (w) and without (w/o) *Bythotrephes*. The box boundaries and mid-rib represent the quartiles and median. The mean (■) is within the box. Whiskers represent 99% confidence limits.



the total zooplankton density, richness, biomass, or mean animal size differed between the two sets of lakes. The more conservative, unequal variance assumption was used in the test if a Levene's test detected unequal variances. The test was repeated for total density and richness of Cladocera, Calanoida, and Cyclopoida. To detect changes in species composition between the two lake sets, multiple analysis of variance (MANOVA) was used on the abundances of individual species. Species found in less than 10% of the lakes (*Daphnia ambigua*, *Latona setifera*, *Leptodiatomus ashlandi*, *Limnocalanus macrurus*, and an unidentified Harpacticoida species) were excluded from the MANOVAs, but immature copepods were included. *Polyphemus pediculus* was also excluded, as it is notoriously patchy and its abundances are not presumed to be well estimated at a single mid-lake station. The well-known problem of increased probability of type 1 error (incorrect declaration of a significant difference) arises with the use of multiple tests. This probability is calculated as $1 - (1 - \alpha)^n$, where n is the number of tests. The standard solution, the Bonferroni adjustment of α , may create its own problems (Perneger 1998), potentially overprotecting for type 1 error rates at the expense of creating type 2 errors (failing to declare a difference that is real). In our study, we erected null hypotheses at three different hierarchical levels: the entire crustacean zooplankton community, the ordinal or subordinal taxonomic level (Cladocera, Calanoida, Cyclopoida), and the species level. Community-level differences were assessed with total abundance, biomass, richness, and mean animal body size, whereas differences at the species level were assessed by MANOVAs on species within Cladocera, Calanoida, and Cyclopoida. As we planned on rejecting the nulls of no difference between the two lake groups at a prespecified hierarchical level if any component test at that level was significant, adjustments for multiple tests were appropriate. Because of the overly conservative nature of Bonferroni adjustments, we adopted the Legendre and Legendre (1998) alternative. We computed adjusted p values (p') for each test where $p' = p/(k - i + 1)$, with k being the number of p values and i being the rank of the particular p value from lowest to highest. No corrections for tied p values were necessary.

We tested three principal null hypotheses, i.e., that there were no differences in zooplankton assemblages between the two groups of lakes at the community level ($k = 4$ for richness, biomass, abundance, and body size), at the ordinal or subordinal levels ($k = 6$ for richness and abundance of Cladocera, Calanoida, and Cyclopoida), and at the species level ($k = 3$ for the MANOVA on cladoceran, cyclopoid, and calanoid species). We rejected these nulls when any one component $p' < 0.05$. We recognize that adjustments for multiple tests are contentious in ecology, and many ecologists wish to preserve the option of evaluating each test. For this reason, we report both p and p' values.

Results

We observed 32 crustacean zooplankton species in the 30 lakes, and there were clear and significant differences in the communities of the two groups of lakes. These differences were not evident in overall species rankings, as frequencies of occurrence of species were highly correlated ($r = 0.86$, $n = 32$; Fig. 1) in the two lake groups. However, the frequencies of occurrence of all but two of the 20 species found in >35% of the reference lakes were reduced in the lakes with *Bythotrephes* (Fig. 1). As a result, species richness was 30% higher ($t = 3.380$, $p = 0.003$, $p' = 0.012$; Fig. 2) in the reference lakes (mean of 15.3 species-sample⁻¹) than in the invaded lakes (mean of 11.8 species-

Table 1. Summary of the t tests used to determine if cladoceran, calanoid, and cyclopoid density (animals·m⁻³) and richness (species·lake⁻¹) differed between lakes with (Y) and without (N) *Bythotrephes*.

Metric	Invaded status	Mean	t	df	p	p'
Density						
Cladocera	N	6 616	2.98	28	0.006	0.03
	Y	3 181				
Calanoida	N	7 086	-0.43	28	0.77	1.0
	Y	7 089				
Cyclopoida	N	19 712	-0.96	28	0.24	0.72
	Y	25 571				
Richness						
Cladocera	N	9.55	3.83	22.3*	0.001	0.006
	Y	5.89				
Calanoida	N	3.55	-0.22	28	0.825	0.825
	Y	3.63				
Cyclopoida	N	4.90	1.36	28	0.184	0.736
	Y	4.47				

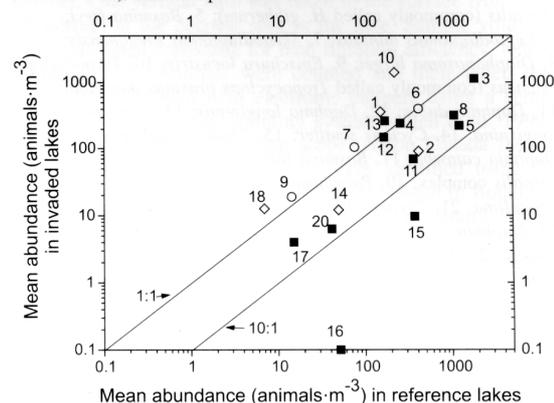
Note: t tests were run on log-transformed data, but arithmetic means are provided. Values in bold type indicate $p < 0.05$, and the asterisk (*) indicates degrees of freedom (df) adjusted for unequal variances. See Methods section for p' calculation method.

sample⁻¹). Total zoo-plankton density did not differ between the two groups of lakes ($t = 0.251$, $p = 0.8$, $p' = 0.8$; Fig. 2), with arithmetic means of 35.8 animals·L⁻¹ in invaded lakes and a similar 32.9 animals·L⁻¹ in reference lakes. In contrast, total zoo-plankton biomass averaged 5.41 µg dw·L⁻¹ in the invaded lakes, significantly lower after log transformation ($t = 2.753$, $p = 0.01$, $p' = 0.03$) than the mean of 9.17 µg·L⁻¹ recorded in the reference lakes (Fig. 2). This difference in biomass, without a corresponding difference in abundance, suggested that mean animal weight was larger in the reference lakes than in the invaded lakes; however, mean animal weight did not differ between the two groups of lakes after p values were adjusted for the four tests run at the community level ($t = 2.17$, $p = 0.039$, $p' = 0.078$). In the reference lakes, the mean animal weight was 0.35 µg dw in comparison with 0.21 µg in the lakes with *Bythotrephes*.

The communities of the two groups of lakes also differed significantly at the ordinal or subordinal levels. Total calanoid and cyclopoid density and richness did not differ, after log transformation, between the two groups of lakes, but the differences were significant for the Cladocera (Table 1). Cladoceran density and richness were, respectively, 50% and 36% lower in the invaded lakes.

The differences in total cladoceran standing stocks between the two groups of lakes could have been produced by many different changes in underlying cladoceran species composition. Further, the lack of differences in the copepod summary metrics could have masked significant, but counterbalanced, changes in copepod species composition. Hence, MANOVAs were performed to detect any differences in species composition between the two groups of lakes, and once again, the predominant differences detected were for Cladocera. The MANOVA on the calanoid and cyclopoid species abundances indicated no difference between invaded and reference lakes ($p = 0.77$ and $p = 0.77$ and $p = 0.24$ and $p' = 0.48$, respectively). In contrast, the MANOVA on the cladoceran species ($p = 0.006$, $p = 0.018$) detected a difference between the two groups of lakes. An examination of the mean abundances of common species in the two groups of lakes accounts for the MANOVA results. Excluding *Polyphemus*, which was assumed to be poorly sampled, 19 species (Fig. 1) were found in more than two-thirds of the reference lakes. Of these 19, the mean abundances of the adults of the three common calanoid species (*Leptodiaptomus minutus*, *Skistodiaptomus oregonensis*, and *Epischura lacustris*) were almost identical in the two groups of lakes (Fig. 3). Of the five commonly occurring cyclopoid species, *Tropocyclops extensus* was more abundant on average in the invaded lakes, whereas *Mesocyclops edax*, in particular, was much less abundant in invaded lakes. A more consistent pattern emerged for the Cladocera, especially among the epi-limnetic taxa. Of 11 common species, mean abundances of the hypolimnetic *Daphnia longiremis* and *Bosmina longispina* (numbers 12 and 13 in Fig. 3) were quite similar in the invaded and reference lakes, as were abundances of the gelatin-encapsulated *Holopedium* (number 4 in Fig. 3). Mean abundances of the other cladoceran taxa were lower in the invaded lakes. *Daphnia catawba* and *Chydorus sphaericus* were more than 10 times lower in the invaded lakes, whereas mean abundances of *Bosmina tubicen*, *Sida cry-stallina*, *Daphnia dubia*, *Bosmina freyi*, and *Diaphanosoma birgei* were, respectively, 4, 7, 5, 5, and 3 times lower in the invaded lakes (Fig. 3). This consistent pattern among the epilimnetic and unprotected cladoceran species, large helmets or body size do not protect Cladocera from *Bythotrephes* (Schulz and Yurista, 1999), accounts for the MANOVA results.

Fig. 3. Scatterplot of the invaded vs. reference lake mean abundances of the 19 species (all but *Polyphemus*) most commonly occurring in the reference lakes. Species are coded as in Fig. 1. Open symbols are copepods, i.e., Calanoida (○) and Cyclopoida (◇). Solid squares (■) are Cladocera. 1:1 and 10:1 (reference:invaded ratio) lines are provided for reference.



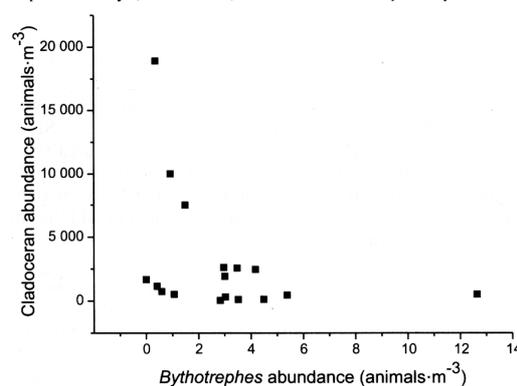
Discussion

There have been many mid-summer surveys of zooplankton in Canadian Shield lakes (e.g., Sprules 1975; Keller and Yan 1991; Yan et al. 1996), and our results from the non-invaded lakes differ from several of these past studies in two important ways. The zooplankton biomasses that we observed are, in general, lower than other published data for Canadian Shield lakes (e.g., Yan and Strus 1980) and the species richness is higher than what has been previously recorded in nonacidic lakes (Keller and Yan 1991; Yan et al. 1996, 2002). In both cases, methodological differences between studies are the cause. The low biomass is attributable to differences between studies in sampling methods. We collected zooplankton in a single, vertical haul, whereas Yan et al. (1996) and Yan and Pawson (1997) collected volume-weighted composite samples, which inevitably yielded higher biomass estimates because they included a greater representation of surface waters, with their higher zooplankton densities. These differences in biomass estimation methods do not interfere with our ability to detect differences in relative biomass between invaded and reference lakes. Several previous studies have detected an average of 10 species of crustacean zooplankton per count in nonacidic lakes in Ontario (Sprules 1975; Keller and Yan 1991; Yan et al. 1996), somewhat lower than our richness estimates from noninvaded lakes. Again, our higher species richness is due to differences in counting protocol. Unlike previous studies, most of our samples were examined in their entirety for rare species. Such rigour is not characteristic of the literature. Again, these differences from the literature do not obscure our ability to detect differences between the invaded and reference lakes.

There were large differences in the crustacean zooplankton communities in the two sets of lakes. There are two possible explanations: a *Bythotrephes* effect or some other uncontrolled factor or factors. To be confident that a *Bythotrephes* effect was detected, we must first consider other possibilities. Factors known to influence the species richness and composition of zooplankton communities in Shield lakes include lake acidity (Havens et al. 1993), size (Yan et al. 1996; Keller and Conlon 1994), productivity (Dodson et al. 2000) or its covariate total phosphorus (Yan 1986), elevation and location (Keller and Pitblado 1989; Patalas 1990), water hardness (Keller and Pitblado 1989), fish predation (Magnan 1988; Yan et al. 2001), time of sampling, and annual climatic differences (Rusak et al. 2002). We can eliminate most of these alternative possibilities, all but differences in fish communities.

By design, there was no difference in when the two groups of lakes were sampled. The mean day of year at sampling did not differ between the two groups ($t = 0.278$, $p = 0.78$, $df = 28$), and all lakes were sampled in the same year. By design, there were no differences in the mean pH, total phosphorus concentration, or size of the study lakes (see Methods for details). As was previously mentioned, samples were collected in the summer at a single deep spot in each lake as is the conventional norm in synoptic zooplankton surveys (e.g., Sprules 1975; Patalas 1990; Keller and Conlon 1994). Hence, the differences in zooplankton communities that we observed between the two groups of lakes were not an artifact of differences in times of sampling or of water quality and lake size. We have very little fisheries data for the study lakes; however, the changes in cladoceran densities, i.e., the reductions in abundance of many small species, are not indicative of direct fisheries effects. Although our lack of good fish survey data prevents our testing of alternative fish-related hypotheses, it would appear that the differences detected between the two groups of lakes are not attributable to most logical uncontrolled factors in the study design.

Fig. 4. Scatterplot of cladoceran density (animals·m⁻³) vs. *Bythotrephes* density (animals·m⁻³) in the lakes with *Bythotrephes*.



The differences that we detected between the lakes with and without *Bythotrephes* in our survey largely concur with temporal trends observed in the small number of previous studies on lakes invaded by *Bythotrephes*. Cladoceran species appear to be the most vulnerable to the invasion, as they are a suspected preferred food item (Grigorovich et al. 1998; Schulz and Yurista 1999). Consistent with our results, cladoceran biomass declined in Lake Michigan (Lehman and Caceres 1993) and in Harp Lake (Yan et al. 2001) after the introduction of *Bythotrephes*. Also consistent with our results, cladoceran species richness declined in Harp Lake (Yan et al. 2002) and probably in Lake Michigan (Makarewicz et al. 1995) after invasion. Further, the epilimnetic cladoceran species apparently least impacted in our survey, *Holopedium* and *Daphnia mendotae*, are also the least-impacted Cladocera in Harp Lake (Yan et al. 2001).

Mesocyclops edax was the cyclopoid taxon with largest intergroup difference in density in our study, and it is the only copepod for which density declined significantly in Harp Lake (Yan et al. 2001). Reasons for the apparent particular sensitivity of *M. edax* among Cyclopoida to *Bythotrephes* clearly warrant study.

Of course, it is not possible to use our data to prove that *Bythotrephes* was responsible for the differences in zooplankton communities observed between the two groups of lakes. Pre-invasion data from these lakes do not exist, nor can we model the actual impacts of *Bythotrephes* predation on zooplankton without far more than single mid-summer samples. What we do know is that the results are very consistent with the one, long-term, intensive study that is available, i.e., the study of Harp Lake (Yan and Pawson 1997; Yan et al. 2001; Dumitru et al. 2001). In addition, correlations between cladoceran abundance and *Bythotrephes* abundance are consistent with a causal *Bythotrephes* link (Fig. 4). A nonlinear, but negative, relationship between cladoceran density and *Bythotrephes* abundance in the invaded lakes is consistent with the hypothesized *Bythotrephes* effect and suggests an effect threshold of about 3-5 *Bythotrephes*·m⁻³. A regression of log cladoceran species richness vs. *B. longi-manus* abundance is also consistent with the *Bythotrephes* effect, i.e., it is negative and marginally significant ($r^2 = 0.22$, $p = 0.056$). Against expectation (Yan 1986), cladoceran density (log-transformed) was not correlated with total phosphorus ($r^2 = 0.01$, $p = 0.66$) in this data set. Similarly, cladoceran richness was not related to lake area ($r^2 = 0.07$, $p = 0.30$). Hence, there was no need to build multiple regression models to predict cladoceran abundance or richness from lakewater phosphorus and lake area in addition to *Bythotrephes* abundance.

The evidence is building that *Bythotrephes* is having an effect on the zooplankton communities of inland Ontario lakes, particularly on the cladoceran fauna. Although ours was a small synoptic study, it certainly suggests that the Harp Lake results are not unique. We may be at the early stages of broad-scale reductions in zooplankton biodiversity and cladoceran abundances in small and mid-size Canadian Shield lakes, i.e., lakes similar to those that we sampled, in response to the introduction and spread of *Bythotrephes*, although only additional monitoring will tell the tale. This could have impacts on both the predators and prey of zooplankton. Water clarity may change in response to changes in cladoceran herbivory should such changes occur (Carpenter et al. 1985), and small zooplanktivorous fish may be forced to compete more heavily for their prey with the invertebrate predator, *Bythotrephes* (Lehman and Branstrator 1995). Coupled with the Harp Lake trends, the results of our survey indicate that it is time to look for any such broader food-web impacts of *Bythotrephes* introductions into Shield lakes.

Acknowledgements

Support for this research was provided by the EJLB Foundation, the Canada Trust Friends of the Environment Foundation, and a Natural Sciences and Engineering Research Council of Canada (NSERC) research grant to N.D.Y. We thank staff of the Dorset Environmental Science Centre, Dorset, Ont., particularly R. Girard, for technical support and for water quality data. We also thank J. Cullen for constructing the "Woody Winder", used to haul the *Bythotrephes* net, and A. Boudreau and L. Whitty for technical support.

References

- Allen, G., Yan, N.D., and Geiling, W.T. 1994. ZEBRA2 — Zooplankton Enumeration and Biomass Routines for APIOS: a semi-automated sample processing system for zooplankton ecologists. Ontario Ministry of Environment and Energy.
- Borbely, J. 2001. Modelling the spread of the spiny waterflea (*Bythotrephes longimanus*) in inland lakes in Ontario using gravity models and GIS. M.Sc. thesis, University of Windsor, Windsor, Ont. Natl. Libr. Can., Can. Theses Amicus No. 25968094.
- Boudreau, S. 2002. *Bythotrephes longimanus*, tracking the spread of a nonindigenous species and evaluating its impact on the crustacean zooplankton communities of Ontario inland lakes. M.Sc. thesis, York University, Toronto, Ont.
- Carpenter, S.R., Kitchell, J.F., and Hodgson, J.R. 1985. Cascading trophic interactions and lake productivity. *BioScience*, 35: 634-639.
- Dodson, S.I., Arnott, S.E., and Cottingham, K. 2000. The relationship in lake communities between primary productivity and species richness. *Ecology*, 81: 2662-2679.
- Dumitru, C., Sprules, W.G., and 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.
- Giguere, L.A., St. Pierre, J.F., Bernier, B., Vezina, A., and Rondeau, J.G. 1989. Can we estimate the true weight of zooplankton samples after chemical preservation? *Can. J. Fish. Aquat. Sci.* 46: 522-527.
- Grigorovich, I.A., Pashkova, O.V., Gromova, Y.F., and van Overdijk, C.D.A. 1998. *Bythotrephes longimanus* in the Commonwealth of Independent States: variability, distribution and ecology. *Hydro-biologia*, 379: 183-198.
- Havens, K.E., Yan, N.D., and Keller, W. 1993. Lake acidification: effects on crustacean zooplankton populations. *Environ. Sci. Technol.* 27: 1621-1624.
- Hoffman, J.C., Smith, M.E., and Lehman, J.T. 2001. Perch or plankton: top-down control of *Daphnia* by yellow perch (*Perca flavescens*) or *Bythotrephes cederstroemi* in an inland lake? *Freshw. Biol.* 46: 759-775.
- Keller, W., and Conlon, M. 1994. Crustacean zooplankton communities and lake morphometry in Precambrian Shield lakes. *Can. J. Fish. Aquat. Sci.* 51: 2424-2434.
- Keller, W., and Pitblado, R. 1989. The distribution of crustacean zooplankton in Northern Ontario, Canada. *J. Biogeogr.* 16: 249-259.
- Keller, W., and Yan, N.D. 1991. Recovery of crustacean zooplankton species richness in Sudbury area lakes following water quality improvements. *Can. J. Fish. Aquat. Sci.* 48: 1635-1644.

- Legendre, P., and Legendre, L. 1998. Numerical ecology. 2nd ed. Developments in environmental modelling 20. Elsevier Science Ltd., Amsterdam.
- Lehman, J.T., and Branstrator, D.K. 1995. A model for growth, development, and diet selection by the invertebrate predator *Bythotrephes cederstroemi*. J. Great Lakes Res. 21: 610-619.
- Lehman, J.T., and Caceres, C.E. 1993. Food-web responses to species invasion by a predatory invertebrate: *Bythotrephes* in Lake Michigan. Limnol. Oceanogr. 38: 879-891.
- Maclsaac, H.J., Ketelaars, H.A.M., Grigorovich, I.A., Ramcharan, C.W., and Yan, N.D. 2000. Modeling *Bythotrephes longimanus* invasions in the Great Lakes basin based on its European distribution. Arch. Hydrobiol. 149: 1-21.
- Maclsaac, H.J., Grigorovich, I.A., and Ricciardi, A. 2002. Reassessment of species invasions concepts: the Great Lakes basin as a model. Biol. Invasions, 3: 405-416.
- Magnan, P. 1988. Interactions between brook charr, *Salvelinus fontinalis*, and nonsalmonid species: ecological shift, morphological shift, and their impact on zooplankton communities. Can. J. Fish. Aquat. Sci. 45: 999-1009.
- Makarewicz, J.C., Bertram, P., Lewis, T., and Brown, E.H., Jr. 1995. A decade of predatory control of zooplankton species composition of Lake Michigan. J. Great Lakes Res. 21: 620-640.
- Mayer, T., Snodgrass, W.J., and Morin, D. 1999. Spatial characterization of the occurrence of road salt and their environmental correlations as chlorides in Canadian surface waters and benthic sediments. Water Qual. Res. J. Can. 34: 545-574.
- McQueen, D.J., and Yan, N.D. 1993. Metering filtration efficiency of fresh water zooplankton hauls: reminders from the past. J. Plankton Res. 15: 57-65.
- Mills, E.L., Leach, J.H., Carlton, J.T., and Secor, C.L. 1993. Exotic species in the Great Lakes: a history of biotic crises and anthropogenic introductions. J. Great Lakes Res. 19: 1-54.
- Patalas, K. 1990. Diversity of the zooplankton communities in Canadian lakes as a function of climate. Verh. Int. Verein. Limnol. 24: 360-368.
- Perneger, T.V. 1998. What is wrong with Bonferroni adjustments. Br. Med. J. 136: 1236-1238.
- Rosenberry, D.O., Buckaveckas, P.A., Buso, D.C., Likens, G.E., Shapiro, N.M., and Winter, T.C. 1999. Movement of road salt to a small New Hampshire lake. Water Air Soil Pollut. 109: 179-206.
- Rusak, J.A., Yan, N.D., Somers, K.M., Cottingham, K.L., Micheli, F., Carpenter, S.R., Frost, T.M., Paterson, M.J., and McQueen, D.J. 2002. Temporal, spatial, and taxonomic patterns of crustacean zooplankton variability in unmanipulated north-temperate lakes. Limnol. Oceanogr. 47: 613-625.
- Schulz, K.L., and Yurista, P.M. 1999. Implications of an invertebrate predator's (*Bythotrephes cederstroemi*) atypical effects on a pelagic zooplankton community. Hydrobiologia, 380: 179-193.
- Sprules, W.G. 1975. Factors affecting the structure of limnetic crustacean zooplankton communities in central Ontario lakes. Verh. Int. Verein. Limnol. 19: 535-643.
- Therriault, T.W., Grigorovich, I.A., Cristescu, M.E., Ketelaars, H.A.M., Viljanen, M., Heath, D.D., and Maclsaac, H.J. 2002. Taxonomic resolution of the genus *Bythotrephes* Leydig using molecular markers and re-evaluation of its global distribution. Divers. Distrib. 8: 67-84.
- Yan, N.D. 1986. Empirical prediction of crustacean zooplankton biomass in nutrient poor Canadian Shield lakes. Can. J. Fish. Aquat. Sci. 43: 788-796.
- Yan, N.D., and 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., and Pawson, T.W. 1998. Seasonal variation in the size and abundance of the invading *Bythotrephes* in Harp Lake, Ontario, Canada. Hydrobiologia, 361: 157-168.
- Yan, N.D., and Strus, R. 1980. Crustacean zooplankton communities of acidic, metal contaminated lakes near Sudbury, Ontario. Can. J. Fish. Aquat. Sci. 37: 2282-2293.
- Yan, N.D., Dunlop, W.I., Pawson, T.W., and MacKay, L.E. 1992. *Bythotrephes cederstroemi* (Schoedler) in Muskoka lakes: first records of the European invader in inland lakes in Canada. Can. J. Fish. Aquat. Sci. 49: 422-426.
- Yan, N.D., Keller, W., Somers, K.M., Pawson, T.W., and Girard, R.E. 1996. Recovery of crustacean zooplankton communities from acid and metal contamination: comparing manipulated and reference lakes. Can. J. Fish. Aquat. Sci. 53: 1301-1327.
- Yan, N.D., Blukacz, A., Sprules, W.G., Kindy, P.K., Hackett, D., Girard, R.E., and Clark, B.J. 2001. Changes in zooplankton and the phenology of the spiny water flea, *Bythotrephes*, following its invasion of Harp Lake, Ontario, Canada. Can. J. Fish. Aquat. Sci. 58: 2341-2350.
- Yan, N.D., Girard, R., and Boudreau, S. 2002. An introduced predator (*Bythotrephes*) reduces zooplankton species richness. Ecol. Lett. 5: 481-485.