Zooplankton community response to experimental acidification in boreal shield lakes with different ecological histories

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Abstract: Community responses to acidification and recovery of boreal lakes are poorly understood, particularly after several years of recovered lake-water pH (pH ≥ 6.0). We tested if zooplankton communities in two circumneutral lakes with different acidification histories were adapted to historical lake-water pH with a reciprocal transplant field enclosure experiment. A second objective was to assess the influence of local environments on zooplankton survival and abundance. Differences in acid tolerance could be detected in some zooplankton among the two lakes with different acidification histories — zooplankton from acid-recovering Carlyle Lake, recovered to pH ≥ 6.0 for 6 years were more speciose and maintained higher total abundance at pH 4.8 than the community from a buffered lake. The zooplankton community in this historically acidified lake was comprised of two dominant species with acid-adaptable tolerances, *Holopedium gibberum* and *Leptodiaptomus minutus*. High establishment of transplanted zooplankton in our experiment has important implications for the recovery of zooplankton communities because it suggests that local conditions are suitable for most species in acid-recovering lakes with pH ≥ 6.0 and that other factors such as dispersal limitation and biotic interactions may be impeding recovery.

Résumé : Dans les lacs boréaux, les réactions de la communauté à l’acidification et sa récupération restent mal comprises, particulièrement lorsque le pH du lac a été rétabli (pH ≥ 6,0) depuis plusieurs années. Une expérience d’enclos en nature avec transfert réciproque nous a servi à vérifier si les communautés zooplanctoniques dans deux lacs à pH près de la neutralité, mais avec des histoires d’acidification différentes, se sont adaptées au pH historique des lacs. Un second objectif est d’évaluer l’influence des environnements locaux sur la survie et l’abondance du zooplancton. Il existe dans ces deux lacs à histoire d’acidification distincte des différences décelables de tolérance à l’acidité chez certains organismes du zooplancton : le zooplancton du lac Carlyle, qui a retrouvé un pH ≥ 6,0 depuis 6 ans, est plus riche en espèces et maintient des densités totales plus grandes à un pH de 4,8 qu’une communauté provenant d’un lac tamponné. La communauté zooplanctonique dans ce lac qui a subi une acidification dans le passé comprend deux espèces dominantes, *Holopedium gibberum* et *Leptodiaptomus minutus*, qui possèdent des tolérances adaptables à l’acidité. La forte colonisation du zooplancton transplanté dans notre expérience a des implications importantes sur le rétablissement des communautés zooplanctoniques : elle indique, en effet, que les conditions locales sont adéquates pour la plupart des espèces dans les lacs en récupération à pH ≥ 6,0 et ce que sont peut-être d’autres facteurs, tels que les contraintes de la dispersion et les interactions biotiques, qui nuisent à la récupération.

[Intaduit par la Rédaction]

Introduction

Acid rain has been a serious environmental problem in North America and Europe for several decades (Schindler 1988) and is emerging as a growing threat in other parts of the world where there is rapid industrialization, e.g., China (Larssen et al. 2006). Acid deposition has damaging effects on freshwater ecosystems, causing loss of fish and invertebrate populations (Minns et al. 1990), reduced biological diversity (Beamish and Harvey 1972; Confer et al. 1983), loss of ecosystem function, particularly at higher trophic levels (Fischer et al. 2001a; Vinebrooke et al. 2003), and increased sensitivity to the impacts of multiple stressors (Frost et al. 1999). Evolutionary implications of lake acidification have been less studied but include shifts in acid tolerances in copepods (Fischer et al. 2001b; Derry and Arnott 2007), as well as community-level responses in crustacean zooplankton (Fischer et al. 2001b).

Legislation was introduced in North America and Europe that reduced acidic atmospheric deposition of sulphur during the 1980s and 1990s, and subsequently, many lakes damaged by acid precipitation are starting to recover chemically (Stoddard et al. 1999; Jeffries et al. 2000). Research has shown time lags in the re-establishment of predisturbance aquatic communities following chemical lake-water recovery (Keller et al. 2002; Holt and Yan 2003; Frost et al. 2006). Local (pH, community resistance, egg bank recruitment, invertebrate predation) and regional (dispersal) factors may...
limit the re-establishment of zooplankton species diversity in acid-recovering lakes (Arnott et al. 2001, 2006; Binks et al. 2005). A relatively unexplored factor in community recovery following disturbance is the adaptive shift in stress tolerance in response to environmental change.

Rapid local changes in stress tolerance can evolve in anthropogenically disturbed habitats (Reznik and Ghahramani 2001) and have potential ecological consequences for populations, communities, and ecosystems (Hairston et al. 2005). Studies in freshwater and marine systems detected heavy metal tolerance in invertebrates (Klerks and Weis 1987), resilience of corals to bleaching (Baker et al. 2004), altered behavior of copepods in response to low dissolved oxygen (Decker et al. 2003), and increased tolerance of zooplankton to cyanobacterial toxins following lake eutrophication (Hairston et al. 1999, 2001). Variation in acid tolerance has been detected among populations of frogs (Räsänen et al. 2003), fish (Rahel 1983), amphipods (France and Stokes 1987), and zooplankton (Price and Swift 1985; Fischer et al. 2001b). Individual populations often exist within a framework of complex community interactions. Few studies (but see Fischer et al. 2001b) have examined how historical exposure to an environmental stressor can influence community response to that stressor, particularly after it has been removed for several years.

In this study, we expand on the work of Fischer et al. (2001b) by investigating adaptive responses of crustacean zooplankton to large-scale regional acidification. We conducted a reciprocal transplant field enclosure experiment to test if the zooplankton community present in an acid-recovering boreal shield lake showed evidence of enhanced acid tolerance to historical acidification following 6 years of recovered (pH ≥ 6.0) lake-water pH. A secondary objective was to assess the influence of the local environment on survival and abundance of zooplankton that had been transferred from one lake to the other to determine if subtle differences in local water chemistry and food conditions between the buffered lake and the acid-recovering lake were limiting the recovery of species in acid-recovering lakes with pH ≥ 6.0.

Materials and methods

Our experiment was conducted in Killarney Provincial Park, Ontario, Canada, an area exposed to regional atmospheric acidification during the 20th century (Beamish and Harvey 1972; Sprules 1975). However, variation in local geology (Debicki 1982) resulted in lakes with different acidification histories (Dixit et al. 2002). We conducted our mesocosm experiments in two circumneutral lakes (pH ≥ 6.0) with different acidification histories: Carlyle and Ishmael lakes. We chose to use pH 6.0 as a threshold for circumneutral pH because it is a widely used measure for biological recovery of acid-sensitive species (Keller et al. 2002). In contrast, Ishmael Lake is located in a watershed that contains outcrops of sandstone and limestone (Debicki 1982) and, as a result, has buffered lake water that never acidified below pH 6.0 (Dixit et al. 2002). Carlyle and Ishmael lakes were selected because they had similar physicochemical properties (Table 1) and zooplankton communities (Holt and Yan 2003) so that we could make community-level comparisons.

This 2 × 2 × 2 factorial design experiment (n = 3 replicates) was comprised of three main factors: incubation lake (IL; acid-recovering Carlyle Lake versus buffered Ishmael Lake), zooplankton source (ZS; Carlyle Lake zooplankton versus Ishmael Lake zooplankton), and pH (circumneutral pH ≥ 6.3 versus pH 4.8). With this experimental design, we were able to distinguish between effects caused by environmental variation between sites and differences among populations between study lakes. Twelve 3925 L polyethylene enclosures (1 m diameter × 5 m deep) were suspended from wooden frames in each of Carlyle and Ishmael lakes during the week of 25 June 2004. These enclosures extended through the epilimnion and were deep enough to have refuges for the zooplankton to avoid potential ultraviolet B (UVB) effects (1% UVB attenuation depth was approximately 3 m in both lakes).

The enclosures were filled with epilimnetic water from the lake in which they were anchored, which was filtered through 53 µm mesh to remove crustacean zooplankton but allow most algal species to pass through. After enclosures were filled with water, six enclosures in each lake were left at ambient pH (pH ~ 6.3 in Carlyle Lake and pH ~ 7.0 in Ishmael Lake) and the other six were acidified with 1 mol·L⁻¹ sulfuric acid to pH 4.8.

During the next day, we reciprocally stocked zooplankton in the enclosures: half received zooplankton from the lakes in which the enclosures were suspended and the other half was stocked with zooplankton from the other lake. Zooplankton were collected by vertical tows with 35 cm diameter, 53 µm mesh nets and were stocked at ambient densities of the lake from which they came. There was no evidence of loss of certain taxa from getting caught on the surface film during the transfer process. Even if some zooplankton species had been preferentially lost over others, all animals were handled similarly and our comparisons were primarily done for populations within species and for aggregate community measures rather than between-species comparisons. Zooplankton for each treatment were collected by two teams, one at each lake. Half of the collected zooplankton was transported to the other lake, while the other half of the zooplankton remained in coolers at the side of the lake from which they were collected. All zooplankton were added to the enclosures at the same time, approximately 6 h after collection.

Zooplankton and water samples were collected from each enclosure once per week for 4 weeks. Week 0 samples were collected 1 to 2 days after zooplankton were added. Zooplankton were sampled by taking two vertical hauls from 5 m to the surface with a 15 cm diameter, 53 µm mesh net (4.5% of enclosure volume). Zooplankton from both hauls were pooled and preserved in a buffered 4% sugar–formalin solution for later enumeration. Water samples were collected...
from 5 m to the surface in the enclosures with a weighted 2.5 cm inner diameter Tygon tube. These samples were stored in dark Nalgene bottles that were kept cool with ice packs in coolers until returning to the laboratory. pH and conductivity were measured in the laboratory using a WTW inoLab 740 pH/conductivity meter (WTW Inc., 6E Gill Street, Woburn, MA 01801, USA) on the day that samples were collected. To maintain the pH treatments, sulfuric acid for the dominant taxa, no more than 50 copepodids per order were included in the tally to 250 individuals, even though more may have been enumerated. Zooplankton were identified and counted using a Leica MZ16 dissecting microscope (Leica Microsystems (Canada) Inc., Suite 400, 111 Granton Drive, Richmond Hill, ON L4B 1L5, Canada). Taxonomic keys that were used included Hebert (1995) and Dodson and Frey (2001) for cladocerans, Smith and Fernando (1978) for copepods, Taylor et al. (2002) for Bosminidae, and Smith (2001) for general zooplankton identification. The taxa *Bosmina* spp. was identified only to subgenera because of taxonomic difficulties in distinguishing species. *Daphnia* species were pooled because of low abundances. Calanoid and cyclopoid nauplii were combined.

### Statistical analyses

Shannon–Wiener indices, species richness, and total abundance were calculated for zooplankton on each sample day in each treatment (Magurran 2004). Shannon–Wiener indices of entropy were transformed to measures of diversity (Jost 2006). Because initial differences (week 0) were detected by factorial analyses of variance (ANOVA) for all species abundances, community indices, and chlorophyll a concentrations, we used repeated-measures analyses of variance (RM-ANOVA) to test for main effects (incubation lake, zooplankton source, and pH) on weeks 0 to 4. Individual species abundance data were $\log_{10}(x + 1)$ transformed and algal concentrations (total and edible chlorophyll a), species richness, and total abundance were $\log_{10}$ transformed to improve homogeneity of variances and normality. These results were presented despite the fact that most normality and equality of variance assumptions were violated because RM-ANOVA is robust to these violations when sample sizes are equal (Quinn and Keough 2002). Shannon–Wiener diversity and conductivity were not transformed because Levene’s test and inspection of box plots indicated that ANOVA assumptions were met. Because we detected treatment differences on week 0, we considered time × treatment interactions using the Greenhouse–Geisser adjustment for departure from sphericity when necessary (Quinn and Keough 2002). RM-ANOVA were followed by planned contrasts for significant time × treatment interactions to test for differences among sets of means that followed linear or quadratic trends (Quinn and Keough 2002). Sequential Bonferroni corrections were applied to sets of contrasts to control for family-wise error associated with multiple hypothesis testing. All RM-ANOVA, factorial ANOVA, trend analyses, and Bonferroni corrections were performed using Statistica 6.0 software (Statsoft Inc., 2300 East 14th Street, Tulsa, OK 74104). Post hoc power for RM-ANOVA with this experimental design was calculated with G-power software (Erdfelder et al. 1996).

To infer the influence of treatments on zooplankton community composition, we conducted principal components analysis (PCA) on species abundances that were averaged.
over the last 3 weeks of the experiment (CANOCO 4.5 for Windows, with scaling that focuses on interspecies correlations; ter Braak and Šmilauer 1998). These averaged abundances were square root transformed to reduce the effect of high densities of a few taxa in the PCA. Although multivariate normality in the PCA could not be guaranteed, most species abundances followed univariate normal distributions. Factorial ANOVAs (Statistica 6.0) were performed on sample scores from the first and second PCA axes, respectively, to test for main and interaction treatment effects on the underlying community composition of zooplankton in the reciprocal transplant experiment. All assumptions of normality and equal variance were met for these factorial ANOVAs.

The results that we presented are focused on time interactions with main effects (incubation lake, zooplankton source, and pH) and on time interactions of zooplankton source with pH because we were primarily interested in testing if zooplankton response to pH was dependant on the acidification history of the source lake from which they were collected. This experiment also tested if there was an effect of incubation lake × pH because we were primarily interested in testing if zooplankton response to pH was dependant on the acidification history of the source lake from which they were collected. pH values (mean ± standard deviation) in circumneutral enclosures were 6.33 ± 0.12 in Carlyle Lake and 7.02 ± 0.13 in Ishmael Lake. Acidified enclosures in both lakes were pH 4.8 ± 0.15. Conductivity was slightly higher in Ishmael Lake than in Carlyle Lake (Table 1), and acid additions resulted in elevated conductivity in acidified treatments (33.4 ± 3.4 µS·cm⁻¹ in Carlyle Lake and 39.3 ± 2.4 µS·cm⁻¹ in Ishmael Lake) compared with circumneutral treatments (25.1 ± 4.4 µS·cm⁻¹ in Carlyle Lake and 29.0 ± 1.3 µS·cm⁻¹ in Ishmael Lake).

Chlorophyll a (chl a) concentrations were higher in enclosures with Carlyle Lake water than in mesocosms with Ishmael Lake water (comparison of linear trends for total chl a, Pillai’s trace = 0.35, $F_{[1,16]} = 8.78$, $P < 0.01$; comparison of linear trends for edible chl a, Pillai’s trace = 0.36, $F_{[1,16]} = 9.0$, $P < 0.01$) (Table 3; Fig. 1). Algal concentrations were dependant on the zooplankton population source (Table 3) because chl a concentrations were higher in enclosures containing zooplankton from Ishmael Lake than from Carlyle Lake (comparison of linear trends: total, Pillai’s trace = 0.50, $F_{[1,16]} = 15.9$, $P < 0.01$; edible, Pillai’s trace = 0.57, $F_{[1,16]} = 20.8$, $P < 0.01$; Fig. 1). We did not detect differences in linear trends that would indicate an overall acid effect on chl a concentrations throughout the experiment (Table 3; Fig. 1).

Community indices

There was a loss of species (comparison of linear trends, Pillai’s trace = 0.67, $F_{[1,16]} = 32.9$, $P < 0.01$) in acidic enclosures, as indicated by a significant time × pH interaction (Table 3; Fig. 2). However, the response of species richness to pH treatment depended on the population source (Table 3, time × ZS × pH) as enclosures with Carlyle-source zooplankton maintained more species than enclosures with Ishmael-source zooplankton at acidic pH (comparison of linear trends, Pillai’s trace = 0.47, $F_{[1,16]} = 14.3$, $P < 0.01$; Fig. 2). In addition, total abundance of zooplankton from Carlyle Lake was less impacted than zooplankton from Ishmael Lake in acidic enclosures (comparison of linear

**Table 2.** Mean ± standard error of number of zooplankton per litre detected in week 0 among circumneutral treatments with “home” lake water: Ishmael zooplankton in circumneutral Ishmael Lake water and Carlyle zooplankton in circumneutral Carlyle Lake water.

<table>
<thead>
<tr>
<th>Species/Genus</th>
<th>Ishmael Lake</th>
<th>Carlyle Lake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthocyclops vernalis</td>
<td>Detected in week 1</td>
<td>Detected in week 1</td>
</tr>
<tr>
<td>Bosmina spp.*</td>
<td>1.91±0.48</td>
<td>0.72±0.58</td>
</tr>
<tr>
<td>Daphnia spp.</td>
<td>0.052±0.02</td>
<td>0.01±0.01</td>
</tr>
<tr>
<td>Diacyclops bicuspidatus thomasi*</td>
<td>0.04±0.01</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>Diaphanosoma birgei*</td>
<td>0.02±0.00</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>Epischura lacustris</td>
<td>0.02±0.01</td>
<td>0.02±0.00</td>
</tr>
<tr>
<td>Holopedium gibberum*</td>
<td>0.14±0.03</td>
<td>0.64±0.14</td>
</tr>
<tr>
<td>Leptodiaptomus minutus*</td>
<td>1.59±0.38</td>
<td>1.10±0.30</td>
</tr>
<tr>
<td>Leptodiaptomus sicidus</td>
<td>0.13±0.01</td>
<td>0.01±0.01</td>
</tr>
<tr>
<td>Mesocyclops edax*</td>
<td>0.04±0.01</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>Sida crystallina</td>
<td>0.002±0.00</td>
<td>0</td>
</tr>
<tr>
<td>Skistodiaptomus oregonomensis</td>
<td>0.18±0.03</td>
<td>0</td>
</tr>
<tr>
<td>Tropocyclops extensus</td>
<td>0.004±0.00</td>
<td>0.002±0.00</td>
</tr>
<tr>
<td>Calanoid copepods</td>
<td>1.54±0.26</td>
<td>0.37±0.07</td>
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<tr>
<td>Cyclopoid copepods</td>
<td>0.21±0.04</td>
<td>0.42±0.16</td>
</tr>
<tr>
<td>Nauplii</td>
<td>0.79±0.17</td>
<td>1.97±0.79</td>
</tr>
</tbody>
</table>

**Note:** Asterisks indicate taxa that were historically present in Carlyle Lake in 1972 during acidification (Sprules 1975).
Table 3. Repeated-measures analysis of variance (RM-ANOVA) of time interactions for algal biomass (total chlorophyll $a$ (Chl $a$), edible Chl $a$), zooplankton community responses (Shannon–Weiner diversity (S-W diversity), species richness, total abundance), and zooplankton population responses ($Bosmina$ spp. (Bos), $Diacyclops$ bicuspidatus thomasi (D. bicus), $Diaphanosoma$ birgei (D. birg.), $Holopedium$ gibberum (H. gibb.), $Leptodiaptomus$ minutus (L. min.), $Mesocyclops$ edax (M. edax), $Epischura$ lacustris (E. lac.), $Leptodiaptomus$ sicilis (L. sic.), and $Tropocyclops$ extensus (T. ext.).

<table>
<thead>
<tr>
<th></th>
<th>Algal biomass</th>
<th>Community responses</th>
<th>Population responses</th>
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<tbody>
<tr>
<td>df effect</td>
<td>4</td>
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</tr>
<tr>
<td>df error</td>
<td>64</td>
<td>64</td>
<td>35.3</td>
</tr>
<tr>
<td>$F$</td>
<td>10.00</td>
<td>10.00</td>
<td>6.70</td>
</tr>
<tr>
<td>$P$</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Time × zooplankton source (ZS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P$</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>—</td>
</tr>
<tr>
<td>Time × pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F$</td>
<td>19.70</td>
<td>19.70</td>
<td>—</td>
</tr>
<tr>
<td>$P$</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Time × IL × ZS</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$F$</td>
<td>2.70</td>
<td>2.70</td>
<td>—</td>
</tr>
<tr>
<td>$P$</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Time × IL × pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F$</td>
<td>9.10</td>
<td>9.10</td>
<td>—</td>
</tr>
<tr>
<td>$P$</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>—</td>
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<tr>
<td>Time × ZS × pH</td>
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</tr>
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</tr>
<tr>
<td>$P$</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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</tr>
<tr>
<td>Time × IL × ZS × pH</td>
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</tr>
<tr>
<td>$F$</td>
<td>—</td>
<td>—</td>
<td>3.85</td>
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<tr>
<td>$P$</td>
<td>0.03</td>
<td>—</td>
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</table>

Note: $Acanthocyclops$ vernalis and $Daphnia$ spp. were not included in the table because no significant results were detected for these species. Bolded $P$ values are significant at $P < 0.05$ and nonsignificant results are indicated by "—". $P$ values and degrees of freedom (df) for time interactions were corrected with the Greenhouse–Geisser adjustment for any departures from sphericity.
Species richness and total abundance were influenced by local environmental conditions (Table 3; Fig. 2). Species richness (comparison of linear trends, Pillai’s trace = 0.30, $F_{[1,16]} = 6.86$, $P = 0.02$) was higher in enclosures with Ishmael Lake water compared with Carlyle Lake water. Although we found a significant time × incubation lake effect for Shannon–Wiener diversity (Table 3), slopes of linear trends between the two incubation lakes were not different. Total zooplankton abundance was greater in enclosures in Carlyle Lake than in Ishmael Lake (comparison of linear trends, Pillai’s trace = 0.63, $F_{[1,16]} = 27.1$, $P < 0.01$; Table 3; Fig. 2). In enclosures with Ishmael Lake water, zooplankton from Carlyle Lake had higher total abundance than zooplankton from Ishmael Lake at acidic pH (comparison of linear trends, Pillai’s trace = 0.64, $F_{[1,16]} = 28.7$, $P < 0.01$; Table 3; Fig. 2).

Community ordination

PCA indicated that ordination axes 1 and 2 explained 52.4% and 26.3% of variance in species data, respectively. A factorial ANOVA conducted on sample scores from the first PCA axis detected an overall influence of pH ($F = 24.29$, df = 1, $P < 0.01$) and a zooplankton source × pH interaction ($F = 5.63$, df = 1, $P = 0.03$) on zooplankton community composition. Zooplankton from historically acidified Carlyle Lake and buffered Ishmael Lake had opposite responses to acid treatment (Fig. 3a). For sample scores from PCA axis 2, we detected significant main effects of incubation lake ($F = 13.54$, df = 1, $P < 0.01$), zooplankton source ($F = 5.34$, df = 1, $P = 0.03$), and pH ($F = 14.67$, df = 1, $P < 0.01$). The response of the zooplankton community from Ishmael Lake depended on incubation lake on PCA axis 2 (Fig. 3b).

Individual species historically present during acidification

“Acid-tolerant” species present in Carlyle and Ishmael lakes in 1972 were *Bosmina* spp., *Daphnia* spp., *Diacyclops bicuspidatus thomasi*, *Diaphanosoma birgei*, *Holopedium gibberum*, *Leptodiaptomus minutus*, and *Mesocyclops edax* (Fig. 4; Sprules 1975). An effect of zooplankton source that was detected for *Bosmina* spp. (Table 3) in experimental enclosures was supported by differences in linear trends (Pillai’s trace = 0.53, $F_{[1,16]} = 18.4$, $P < 0.01$) because more individuals came from Carlyle Lake than from Ishmael Lake across treatments. Zooplankton source was also significant for *L. minutus* (Table 3; Fig. 5), but we could not detect differences in linear patterns among treatments. The abundance of all species, except *D. bicuspidatus thomasi*, was significantly different in acid treatments compared with controls (Table 3; Fig. 5). Unlike *D. birgei* (comparison of linear trends, Pillai’s trace = 0.21, $F_{[1,16]} = 4.29$, $P = 0.05$) and *M. edax* (comparison of linear trends, Pillai’s trace = 0.85, $F_{[1,16]} = 87.5$, $P < 0.01$), the abundance of which decreased in acid treatments, *H. gibberum* flourished in acidic enclosures (comparison of quadratic trends, Pillai’s trace = 0.52, $F_{[1,16]} = 17.2$, $P < 0.01$). Although we detected a significant
pH × time effect for *Bosmina* spp. and *L. minutus*, we could not detect differences in linear trends among pH treatments.

The pH response depended on acidification history for *H. gibberum*, *L. minutus*, and *M. edax*, as indicated by significant zooplankton source × pH and incubation lake × zooplankton source × pH time interactions (Table 3). Acid tolerances of *H. gibberum* differed between lakes (Fig. 5) because more individuals from Carlyle Lake than Ishmael Lake were detected in acidic enclosures (comparison of quadratic trends, Pillai’s trace = 0.57, $F_{[1,16]} = 21.0$, $P < 0.01$).

The acid tolerance of *L. minutus* was dependant on zooplankton source (Table 3; Fig. 5). In this case, *L. minutus* from Carlyle Lake was more abundant than from Ishmael Lake when incubated in acidic enclosures with Carlyle Lake water (comparison of linear trends, Pillai’s trace = 0.32, $F_{[1,16]} = 8.0$, $P = 0.01$). *Leptodiaptomus minutus* individuals from Carlyle Lake also occurred in higher concentrations than individuals from Ishmael Lake when incubated in acidic enclosures with Ishmael Lake water (comparison of linear trends, Pillai’s trace = 0.41, $F_{[1,16]} = 10.9$, $P < 0.01$). We detected a marginally significant time × zooplankton source × pH interaction for *M. edax* (Table 3) and could not detect significant differences in linear trends. *Mesocyclops edax* from both Carlyle (comparison of linear trends, Pillai’s trace = 0.59, $F_{[1,16]} = 23.4$, $P < 0.01$) and Ishmael (comparison of linear trends, Pillai’s trace = 0.81, $F_{[1,16]} = 70.4$, $P < 0.01$) lakes became more abundant in circumneutral enclosures than in acidic treatments (Fig. 5).

With the exception of *D. birgei* and *M. edax*, species abundance was dependant on the lake in which a species was incubated (Table 3; Fig. 5). A greater concentration of *H. gibberum* was detected in enclosures in Ishmael Lake than in Carlyle Lake (comparison of quadratic trends, Pillai’s trace = 0.21, $F_{[1,16]} = 4.29$, $P = 0.05$). Conversely, *D. bicuspidatus thomasi* (comparison of linear trends, Pillai’s trace = 0.34, $F_{[1,16]} = 8.10$, $P = 0.01$) and *Bosmina* spp. (comparison of linear trends, Pillai’s trace = 0.46, $F_{[1,16]} = 13.8$, $P < 0.01$) became more abundant in Carlyle Lake enclosures. For *L. minutus*, individuals from Carlyle Lake had greater abundance in Carlyle Lake than in Ishmael Lake (comparison of linear trends: Pillai’s Trace = 0.26, $F_{[1,16]} = 5.72$, $P < 0.02$), and individuals from Ishmael Lake had greater abundance in Ishmael Lake than in Carlyle Lake (comparison of linear trends, Pillai’s trace = 0.23, $F_{[1,16]} = 4.78$, $P < 0.03$; zooplankton source × incubation lake; Table 3; Fig. 5).

**Individual species not historically present during acidification**

Acid-sensitive species not historically present in Carlyle Lake or Ishmael Lake in 1972 were *Acanthocyclops vernalis*, *Leptodiaptomus sicilis*, and *Tropocyclops extensus*. *Epischura lacustris* was detected in Ishmael Lake (Sprules 1975). Ishmael Lake was a source of more *L. sicilis* individuals than Carlyle Lake (comparison of quadratic trends, Pillai’s trace = 0.63, $F_{[1,16]} = 27.4$, $P < 0.01$; Table 3). *Epischura lacustris* (comparison of linear trends, Pillai’s trace = 0.40, $F_{[1,16]} = 10.8$, $P < 0.01$) and *T. extensus* (com-
parison of linear trends, Pillai’s trace = 0.48, $F_{[1,16]} = 14.8$, $P < 0.01$) were more abundant in circumneutral enclosures than in acidic enclosures (Table 3; Fig. 6). Not surprisingly, these responses were not influenced by lake history as these taxa were historically absent in Carlyle during acidification and recolonized following lake-water recovery to pH ≥ 6.0. 

*Tropocyclops extensus* from Carlyle Lake incubated in acidic enclosures with “home” Carlyle Lake water increased until week 2 and then decreased. This treatment followed a similar time trend in circumneutral enclosures but increased again after week 3. Other treatment × time responses for this species were linear (Fig. 6).

Local environmental conditions influenced abundances of *L. sicilis* and *T. extensus* (Table 3). Although more *L. sicilis* individuals were detected in enclosures with Ishmael Lake water than Carlyle Lake water (comparison of quadratic trends, Pillai’s trace = 0.67, $F_{[1,16]} = 32.1$, $P < 0.01$), there were more *T. extensus* individuals in enclosures with Carlyle Lake water than Ishmael Lake water (comparison of linear trends, Pillai’s trace = 0.89, $F_{[1,16]} = 125$, $P < 0.01$; Fig. 6).

**Discussion**

We found differences in acid tolerances at the community level between lakes with different acidification histories. Al-
though there was a reduction in species richness in acidified treatments in historically acidified Carlyle Lake, the reduction was not as great as in buffered Ishmael Lake. As a result, the zooplankton community from acid-recovering Carlyle Lake was more speciose than the community from buffered Ishmael Lake at acidic pH, and the converse pattern held at circumneutral pH. This response was primarily driven by acid-sensitive species in Ishmael Lake that were not detected in Carlyle Lake 

(S. oregonensis and Sida crystallina). Total abundance was greatest in acidic enclosures in Ishmael Lake containing the zooplankton community with a history of acidification (Carlyle Lake), and this was likely driven by *H. gibberum*, which had peak abundances in acidified enclosures with Carlyle Lake as the zooplankton source. Therefore, local adaptation of key zooplankton species to past lake-water acidity may have driven community-level responses in Carlyle Lake for species richness and total abundance.

In our experiment, two key species, *H. gibberum* and *L. minutus*, contributed to community-level differences in acid tolerance of zooplankton with different lake acidification histories. Both species from Carlyle Lake appeared to be locally adapted to historical acidity, even though Carlyle Lake had a pH > 6 for 6 years. Fischer et al. (2001b) found a similar result for these taxa in Little Rock Lake, Wisconsin, after 2 years of recovery to pH 5.9. Acidic lakes with pH < 5.0 in the Killarney, Ontario, area frequently contain high abundances of *H. gibberum*, *L. minutus*, and *Bosmina* spp. (Sprules 1975; Holt and Yan 2003). Our data did not indicate any evidence for differences in acid tolerances among *Bosmina* spp. populations, which grew in abundance at both acidic and circumneutral pH regardless of population source. Other researchers have suggested that increases in *Bosmina* spp. following acidification occur because of indirect community effects caused by competitive release from larger cladoceran species that do not survive at acidic pH (Locke and Sprules 1993; Fischer et al. 2001b). It may be that *Bosmina* spp. can survive across a broad spectrum of pH but have an indirect response to acid. Acidification has been found to favor dominance by small zooplankton taxa, such as *Bosmina* spp. (Havens and Hanazato 1993).

We expected, but did not find, evidence of locally adapted acid tolerances in *D. bicuspidatus thomasi*, *D. birgei*, and *M. edax*. Acidic lakes with pH 5.0 to 5.2 often have zooplankton communities comprised of some combination of *D. bicuspidatus thomasi*, *D. birgei*, and *M. edax* in addition to other acid-tolerant species found in lakes with pH < 5.0 (Sprules 1975; Locke and Sprules 1993; Holt and Yan 2003). Past studies found evidence of increased acid tolerance in populations of *D. bicuspidatus thomasi* (Fischer et al. 2001b) and *M. edax* (Price and Swift 1985) from lakes with historic or current acidity. We found weak but statistically insignificant evidence for increased acid tolerance in *D. bicuspidatus thomasi* and no clear trends for *D. birgei* originating from Carlyle Lake. There are several hypotheses to explain why population-specific tolerance to acidity detected by Fischer et al. (2001b) in *D. bicuspidatus thomasi* and *M. edax* by Price and Swift (1985) was absent in our study: (i) geographical variation in population response; (ii) dispersal of acid-sensitive genotypes from other circumneutral lakes following pH recovery of Carlyle Lake; (iii) concentrations of individuals too low to detect differences; and (iv) loss of acid tolerance following lake recovery. Comparisons of the effects of experimental and atmospheric lake acidification in different regions have indicated similar declines in *D. bicuspidatus thomasi* and *M. edax* from lakes without a previous history of acidification (Schindler et al. 1991), and there is no evidence to suggest regional variation in population responses to pH for these species. Dispersal to Carlyle Lake from other circumneutral lakes is unlikely because, although the dispersal abilities of these cyclopoid copepods are unknown, many other copepod species are spatially dispersal-limited (Boilieu and Hebert 1991; Zeller et al. 2006). *Diacyclops bicuspidatus thomasi* concentrations were low and variable in our enclosures, and it is possible we did not have the statistical power to detect differences in acid tolerance among zooplankton sources. Our power to detect statistically significant differences at α = 0.05 was low (critical *F*14,64 = 2.52, power = 0.592), even after assuming a relatively high effect size (*f*2 = 0.35; Cohen 1988), reflecting trade-offs of logistics with conducting an experimental design with relatively low power. We suspect that differences in acid tolerance among *M. edax* populations from an acid pond and a circumneutral pond detected by Price and Swift (1985) could have been plastic responses adaptive in the maternal environment and lost within several generations under similar pH conditions. This is supported by results of Fischer et al. (2001b), who also failed to detect an adaptive response in *M. edax*. Loss of acid tolerance in years following recovery from acidification could be an explanation for the absence of adaptation to historical acidification in some species.

We did not measure the genetic component of acid tolerances in zooplankton but have circumstantial evidence to support genetically based local adaptation of acid tolerance in *H. gibberum* and *L. minutus*. Although both *H. gibberum* and *L. minutus* sexually produce resting eggs that accumulate in lake sediments with time, *Holopedium* spp. are cyclic parthenogens that alternate asexual reproduction with sex (Dodson and Frey 2001), and *L. minutus* is an obligately sexual species (Williamson and Reid 2001). Because most egg bank emergence occurs from the top 2 cm of lake sediment (Brendonck and De Meester 2003) and given that this depth interval involves the last 10 years of sedimentation in Killarney area lakes (Dixit et al. 1992), emergence from the egg bank into the water column would involve resting eggs produced when Carlyle Lake had recovered to around pH 6.0. Because at least several sexual generations of *H. gibberum* (one sexual generation in late summer per year) (Ward and Whipple 1959) and at least 50 to 75 generations of *L. minutus* likely occurred during that time, maternal effects from parents that directly experienced acidic conditions were likely minimized. Therefore, interpopulation differences in acid tolerance in *H. gibberum* and *L. minutus* were likely a result of local adaptation to historical lake-water pH.

Although there is evidence to suggest a genetic basis for adaptation to acidity in *H. gibberum* and *L. minutus*, this adaptation may be lost if it is associated with a fitness trade-off following lake-water recovery to pH 6.0. Our results showed that *L. minutus* was less strongly associated with acidic pH than *H. gibberum*. Other work found that *L. minutus* became locally adapted to historical acidification in many Killarney...
lakes but had a reduced acid tolerance after 6 to 8 years of recovered, circumneutral lake-water pH (Derry and Arnott 2007). This loss in acid tolerance with chemical lake recovery in *L. minutus* was associated with a physiological fitness trade-off (Derry and Arnott 2007). Adaptive reversals have also been found in other aquatic organisms, such as oligochaetes following the restoration of a metal-contaminated coastal marsh (Levinton et al. 2003). It is possible that *H. gibberum* maintained adaptation to historical pH in Carlyle Lake because this cladoceran was not subject to the same physiological trade-off as *L. minutus* or because this species has undergone fewer generations following lake recovery. A similar experiment done in an experimentally acidified lake basin that recently recovered to pH 5.9 (<2 years; Fischer et al. 2001b) found four species with responses dependant on acidification history of zooplankton source (H. gibberum, L. minutus, D. bicuspidatus thomasi, and *T. extensus*). Only two of these species appeared to be locally adapted to historical acidification in Carlyle Lake, which had been circumneutral for a longer period of time (6 years). Because *T. extensus* was not detected during the period of acidification in Carlyle Lake, we did not expect to detect increased acid tolerance in this species. However, it is possible that we did not detect increased acid tolerance in some species because of adaptive reversals in acid tolerance that may have occurred in years following lake recovery from acidification.

Recovery of historically acidified Carlyle Lake resulted in colonization of acid-sensitive zooplankton that were not present when the lake was acidic (Sprules 1975) — the copepods *A. vernalis*, *E. lacustris*, *L. sicilis*, and *T. extensus*. Other research indicated that the abundance of these species is lower in acidic lake water (Keller and Pitblado 1984; Marmorlok and Korman 1993). Although Fischer et al. (2001b) found evidence for increased acid tolerance in *T. extensus* populations following experimental acidification in Little Rock Lake, Wisconsin, this species appeared to be acid-sensitive in other regions (Schindler et al. 1991). The acid-sensitive copepods that we detected were either able to disperse into Carlyle Lake or were recruited from historic resting egg banks present in lake sediments (Williamson and Reid 2001) following lake-water recovery to pH 6.0. Because these copepods recently colonized the Carlyle Lake water column, it is not surprising that we did not detect local adaptation to low pH in these taxa.

Our experiment also tested if local environmental conditions affected zooplankton response when transplanted among lakes with similar physicochemical properties but different acidification histories. We examined this in two ways. (i) Did zooplankton, regardless of incubation lake, have higher abundances in either lake? (ii) Did zooplankton have higher growth and survival (and therefore higher abundance) in their “home” water compared with “transplant” water, particularly at circumneutral pH? We observed higher zooplankton abundance in Carlyle Lake likely because of higher algal concentrations. The establishment success of *D. bicuspidatus thomasi*, *Bosmina* spp., and *T. extensus* in Carlyle Lake enclosures compared with Ishmael Lake, despite the fact that more individuals were detected in Ishmael Lake than in Carlyle Lake enclosures during the initial week of the experiment, was probably related to the greater availability of food resources, as indicated by higher levels of chl a in Carlyle Lake. *Leptodiaptomus sicilis* was comparatively rare in the Carlyle Lake zooplankton community compared with the community from Ishmael Lake. This cyclopoid copepod established poorly in Carlyle Lake water compared with Ishmael Lake water and may be adapted to local water chemistry and (or) food resources in this lake. However, all other species, including those found only in the zooplankton community of Ishmael Lake (*S. oregonensis* and *S. crystallina*), were able to establish in enclosures with both home and transplant source water at circumneutral pH, suggesting that they were not specifically adapted to their similar home water conditions. In contrast, Forrest and Arnott (2006) found poor colonist establishment in an experiment in which zooplankton from nearby lakes were transplanted into enclosures in another Killarney lake. However, our enclosures did not contain the resident zooplankton community, whereas those of Forrest and Arnott (2006) did, suggesting that biotic interactions may be the primary determinant of colonist establishment in these circumneutral lakes. Further, high establishment of transplanted zooplankton in our experiment has important implications for the recovery of zooplankton communities because it suggests that local conditions in lakes of these types are suitable for most species and that other factors such as dispersal limitation and biotic interactions may be impeding recovery.

In conclusion, we found evidence for lasting effects of historical atmospheric acidification on the acid tolerances of some zooplankton taxa in a boreal shield lake after the passage of 6 years of recovered, circumneutral pH. Our findings support and extend the findings of Fischer et al. (2001b), who found similar responses for a zooplankton community after more recent recovery (<2 years) of a lake basin from experimental acidification. Adaptable acid tolerances in zooplankton communities may be important for maintaining certain ecosystem services, such as the maintenance of zooplankton biomass (Fischer et al. 2001a; Vinebrooke et al. 2003), in many lakes since the latest National Acid Rain Assessment (Meteorological Service of Canada 2004) reported that hundreds of thousands of lakes in eastern Canada will remain acidified at current levels of acid deposition. Of acidified lakes that do undergo chemical recovery, adaptation to past lake-water acidity may be advantageous when re-acidification events occur from drought and climate change (Arnott et al. 2001). On the other hand, zooplankton with adaptable acid tolerances may have monopolized resources in acidified and acid-recovering lakes and may be contributing to the delayed recolonization of other acid-sensitive taxa as lakes recover from acidification (Yan et al. 2003; Binks et al. 2005; Frost et al. 2006).

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