

## Distribution and Abundance of Benthic Macroinvertebrates and Zooplankton in Lakes in Kejimikujik National Park and National Historic Site of Canada, Nova Scotia

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As part of the Acid Rain Biomonitoring Program at Environment Canada, we sampled aquatic biodiversity in 20 acidic lakes in 2009 and 2010 in Kejimikujik National Park and National Historic Site of Canada and vicinity in Nova Scotia. We established an inventory of current aquatic macroinvertebrate and zooplankton species composition and abundance in each of the 20 study lakes. A total of 197 macroinvertebrate taxa were identified; the number of taxa observed was positively correlated with pH across the 20 lakes. Acid-tolerant taxa, such as isopods, amphipods, trichoptera, and oligochaetes, were common and abundant, while bivalves, gastropods, and leeches were lower in abundance. The number of isopods and amphipods collected was correlated with calcium concentration; a greater proportion of isopods than amphipods were collected from lakes with low calcium and low pH. Taxa with hard, calcareous shells, such as bivalves and gastropods, were not present in lakes with low calcium and low pH, with bivalves occurring only in lakes above pH 4.9. Odonates and ephemeropterans, which were low in abundance, were associated with a wide range of acidity. Coleopteran abundance was positively correlated with concentrations of dissolved organic carbon. A total of 26 zooplankton taxa were collected, but only cyclopoid abundance was correlated with lake pH. Results presented here provide a summary of aquatic biodiversity in lakes in Kejimikujik National Park and National Historic Site and vicinity and provide a baseline for future monitoring as acid deposition continues to affect this acid-sensitive region in Atlantic Canada.

Key Words: macroinvertebrates; Kejimikujik National Park and National Historic Site of Canada; water chemistry; acidic lakes; zooplankton; Nova Scotia

### Introduction

Acid deposition remains a widespread stressor of freshwater ecosystems across southeastern Canada despite legislated reductions in emissions of acidifying pollutants over recent decades in both Canada and the United States (Jeffries *et al.* 2004; Ginn *et al.* 2007). Analyses of critical loads of acid deposition in eastern Canada have suggested regions with carbonate-poor geology continue to be influenced by acid inputs into the environment (Doka *et al.* 2003; Jeffries *et al.* 2003; Dupont *et al.* 2005; Clair *et al.* 2007, 2011). The effects of acidification on the diversity of aquatic macroinvertebrate species have been well studied (e.g., Dermott 1985; Peterson 1987; Schell and Kerekes 1989; Lento *et al.* 2008), and changes in the composition of the aquatic food web can have an impact on higher trophic levels that rely on these groups for food (Weeber *et al.* 2004).

In the 1980s, Environment Canada implemented the Acid Rain Biomonitoring Program to study aquatic invertebrate species assemblages in acid-sensitive Boreal Shield lakes in Ontario (McNicol *et al.* 1995b; Jeffries *et al.* 2004). In 2009 and 2010, this biomonitoring program was expanded to include Kejimikujik National Park and National Historic Site of Canada, which has a long history of environmental and ecological monitoring (Kerekes 1975; Kerekes *et al.* 1994; Burgess and Hobson 2006; Wyn *et al.* 2010; Clair *et al.* 2011).

In the period from 2000 to 2007, the Kejimikujik region in southwestern Nova Scotia received an average of  $8 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$  to  $12 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$  of  $\text{SO}_4^{2-}$  deposition (wet and dry) (Clair *et al.* 2011). This level is relatively low compared to the rest of North America. However, the geology of Kejimikujik National Park and National Historic Site consists mainly of poorly weath-

erable bedrock that offers little buffering capacity, and this makes this ecosystem extremely sensitive to additional inputs of acid from the atmosphere (Clair *et al.* 2007). In addition, the landscape in Kejimikujik National Park and National Historic Site and the surrounding area is composed of naturally acidic habitats due to the prevalence of bog and fen wetlands. Therefore, even with further reductions in atmospheric acid deposition, recovery in these aquatic ecosystems is expected to be extremely slow (Whitfield *et al.* 2006; Clair *et al.* 2011).

Although information on the status of and trends in lake chemistry in Kejimikujik National Park and National Historic Site is well developed (Clair *et al.* 2011), only limited research has been completed on the aquatic biodiversity in these acid-sensitive lakes (Kerekes and Freedman 1989; Schell and Kerekes 1989). The purpose of this study was: (i) to determine the current composition and abundance of aquatic

invertebrate and zooplankton in 20 acid-sensitive lakes in Kejimikujik National Park and National Historic Site and vicinity and (ii) to identify potential indicator taxa with respect to biological responses to lake acidity.

### Study Area

Kejimikujik National Park and Historic Site is a protected area of 404 km<sup>2</sup> located in southwestern Nova Scotia (Figure 1). Twenty study lakes (17 within the Park and 3 in the vicinity) were selected to cover a range of water chemistry parameters. Lakes were chosen to cover the largest possible gradients of acidity/alkalinity, calcium, colour, and concentration of dissolved organic carbon in the study area. All of the 20 lakes were accessible by road or canoe (some back-country lakes in Kejimikujik National Park and Historic Site are not accessible by road, so accessibility was also a factor). Eight of the lakes were sampled in

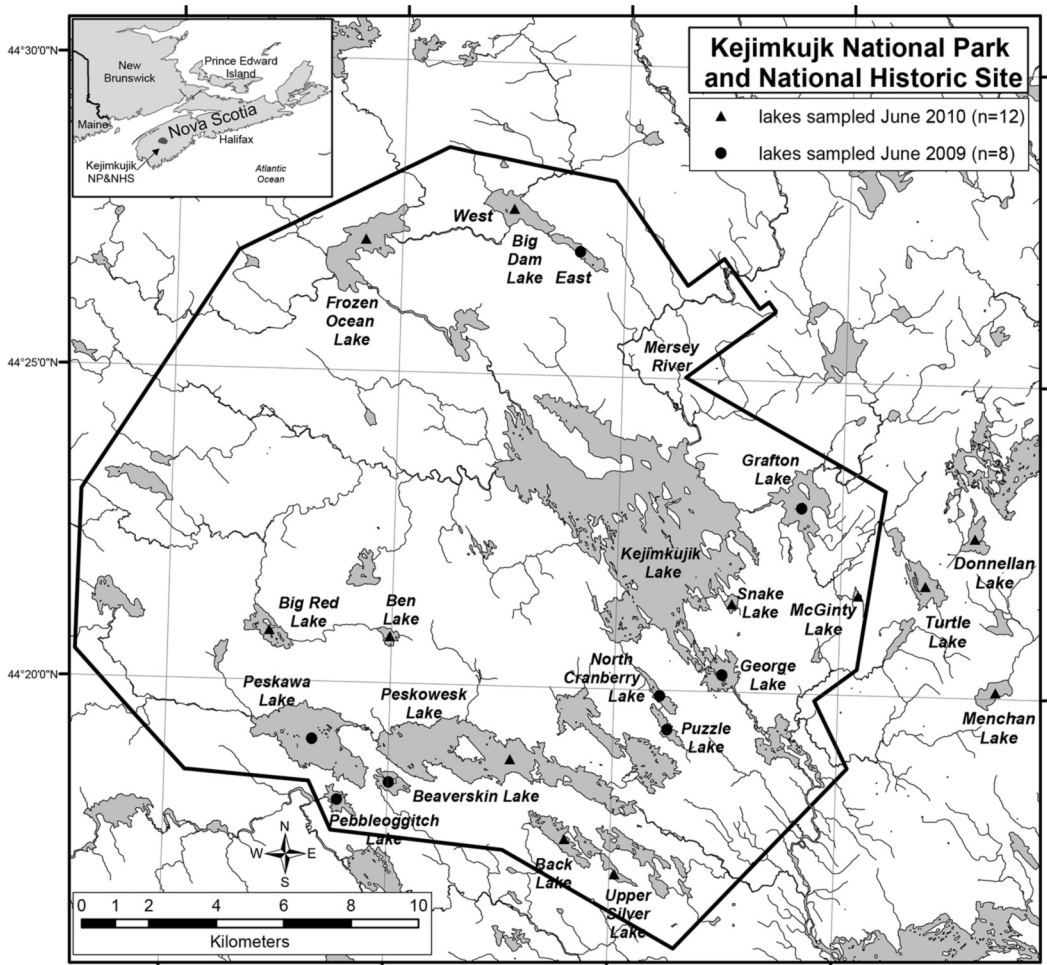


FIGURE 1. Location of 20 acid rain biomonitoring study lakes sampled during 2009 and 2010 in Kejimikujik National Park and National Historic Site of Canada and surrounding area, Nova Scotia.

June 2009 (Beaverskin, Big Dam East, George, Grafton, North Cranberry, Pebbleloggitch, Peskawa, and Puzzle) and the remaining 12 lakes were sampled in June 2010 (Back, Ben, Big Dam West, Big Red, Donnellan, Frozen Ocean, Menchan, McGinty, Peskowsk, Snake, Turtle, and Upper Silver).

## Methods

### *Sampling methods*

As part of an Environment Canada lake monitoring network, surface water samples were collected by helicopter from the centre of each lake during the spring and fall turnover periods each year (usually May and October) (Clair *et al.* 2011). Samples were collected at a depth of 0.5 m, kept cool, and shipped overnight to the Environment Canada Atlantic Laboratory for Environmental Testing (ALET) in Moncton, New Brunswick. At every 10th lake, triplicate samples were collected and compared to each other for quality control. All water samples were analyzed in the laboratory for various water chemistry parameters using unfiltered water following ALET protocols (Clair *et al.* 2011; Eaton *et al.* 2012).

For the collection of aquatic macroinvertebrates and zooplankton, we followed the sampling protocols of the Environment Canada Acid Rain Biomonitoring Program in Ontario and Quebec (see McNicol *et al.* 1995b). Sampling was completed in mid-June, as this is a time of high invertebrate biomass and richness and it is also when local waterbirds that depend on aquatic prey to raise their young are breeding (McNicol *et al.* 1996).

At each study lake we conducted 10 benthic drag samples, 10 water column sweeps, and 10 hoop samples, and we set 6 minnow traps (McNicol *et al.* 1996). All samples were taken at randomly selected sites. Benthic drag samples, which targeted odonates, ephemeropterans, bivalves, and gastropods, were conducted in water less than 1 m in depth. A D-frame dip net (0.85 mm mesh) was dragged over the substrate for a distance of 0.5 m to collect the top 1–2 cm of substrate (total sample area of 0.14 m<sup>2</sup>) (McNicol *et al.* 1996). If boulders or rocky substrates made benthic drag sampling impractical, a traveling kick and sweep sample was completed instead. For these samples, the sampler walked backwards for a distance of 1 m along the shoreline (maximum 1 m depth), kicking the bottom substrate and sweeping the dislodged detritus and invertebrates into the D-frame net (Rosenberg *et al.* 2000).

Both the benthic drag and the travelling kick and sweep samples were processed in the same way: detritus in the net was thoroughly rinsed to remove fine sediments and was transferred to a sample container, where it was first preserved with 10% buffered formalin for 48 hours and then transferred into 70% ethanol. Entire benthic samples were later sorted under a dissecting microscope. All observed macroinvertebrates were removed and preserved in 70% ethanol.

Sweep sampling targeted nektonic invertebrates active in the water column. Sweep sampling was conducted in open water less than 5 m from the shore. Sampling was completed by sweeping through the water column in 10 consecutive arcs using a D-frame dip net (0.85 mm mesh, 625 cm<sup>2</sup> capture area) over the bow of a forward-moving canoe traveling parallel to the shoreline. Each sweep described an arc from the water surface down to a maximum depth of 1 m and back to the surface, and a new section of the water column was sampled with each arc. Captured invertebrates were picked from the net using forceps and transferred to a sample container containing 70% ethanol.

Hoop sampling targeted trichopterans and gastropods. A circular hoop of coated wire (diameter of 0.64 m, area of 0.32 m<sup>2</sup>) was placed on the substrate in water <0.5 m deep. The hoop was visually searched for a total of 5 minutes, and all invertebrates observed on the surface of the substrate and vegetation were removed and preserved in 70% ethanol.

All benthic macroinvertebrates from hoops, sweeps, kick and sweep samples, and benthic drags were later identified to species (or lowest taxonomic level possible).

Minnow traps targeted large nektonic invertebrates. Six standard Gee's minnow traps were baited with dry dog food (Purina Puppy Chow®) and set for a total of 24 hours in near-shore sites where water depth was approximately 1 m. Specimens were preserved in 70% ethanol.

Zooplankton sampling was conducted at 15 of the 20 study lakes (5 of the study lakes were ≤2 m deep and were therefore too shallow for vertical zooplankton sampling to be carried out). A single vertical haul was completed at the deepest part of each lake, starting from 1 m above the sediment to the water's surface. Samples were collected using a non-metered zooplankton net (80 µm mesh, 26 cm in diameter). The contents of the net were rinsed into the bottom of the collection jar and then poured into a sample jar containing 33% sugared, buffered formalin. All zooplankton samples were identified to species (or lowest possible taxonomic level).

### *Data analysis*

Counts from all benthic invertebrate sampling procedures were pooled within each lake for the statistical analyses. The resulting data from the 20 study lakes were summarized with respect to mean, minimum, and maximum counts for each species, as well as the percentage of lakes where a given species was observed. Rare species ( $n = 72$  taxa) were defined as occurring in ≤ 10% of the study lakes, while common species ( $n = 125$  taxa) occurred in > 10% of the study lakes. The abundance and percentage composition of the most abundant taxonomic groups were determined for each lake, and boxplots were generated to show trends for individual taxonomic groups of interest. Taxonomic richness was calculated as the total number of unique taxa in each lake.

Associations between water chemistry parameters, as well as between the total number of macroinvertebrate taxonomic groups and lake acidity, were evaluated using Spearman rank correlations. This non-parametric method of statistical analysis was employed as some of the data did not meet assumptions of normality required for Pearson's correlations. All statistical analyses were completed using SYSTAT 13 (SYSTAT Software Inc., Chicago, Illinois).

Zooplankton data were summarized by mean density (number of individuals/m<sup>3</sup>) for each of the 15 lakes, and the percentage of lakes a given species was observed in was also calculated.

## Results

Fish were present in all 20 of the study lakes (Kerekes 1975; Drysdale *et al.* 2005). Mean water chemistry values for each lake are presented in Table 1. Many of the study lakes were oligotrophic and darkly coloured (99–202 Hazen units) due to dissolved organic compounds leached from nearby bogs. Mean lake pH varied from 4.3 (Big Red Lake) to 6.6 (McGinty Lake) (Table 1). pH and calcium concentrations were positively correlated in the study lakes ( $r_s = 0.747$ ,  $P < 0.001$ ); pH and dissolved organic carbon were negatively correlated ( $r_s = -0.715$ ,  $P < 0.001$ ).

A total of 26 zooplankton species were observed in the study lakes, with many of the common taxa observed across a wide gradient of acidity (Supplementary Table 1). Only the abundance of Cyclopoida was

significantly correlated with lake pH ( $r_s = 0.536$ ,  $P = 0.040$ ).

A total of 197 taxa of aquatic macroinvertebrates were observed (149 were identified to species, 38 to genus, and 10 to family) (Supplementary Table 2). The total number of taxa in each lake was positively correlated with both lake pH (Figure 2) ( $r_s = 0.554$ ,  $P = 0.011$ ) and calcium concentrations ( $r_s = 0.463$ ,  $P = 0.040$ ). Taxon richness was not significantly associated with dissolved organic carbon ( $r_s = -0.390$ ,  $P = 0.090$ ). Total abundance (number of individuals of all macroinvertebrates captured in each lake) was not correlated with any water chemistry parameter.

The most abundant benthic invertebrate groups in the 20 study lakes were Isopoda, Amphipoda, Oligochaeta, and Trichoptera (Figures 3A and 3B). Only one species of isopod was observed (*Caecidotea communis*), but it constituted up to 60% of the macroinvertebrates collected in some lakes (e.g., Peskowesk Lake) (Figure 3A). The abundance of isopods (*Caecidotea communis*) was lower in lakes with high pH and calcium levels and higher in lakes with low calcium levels (Figure 4A) ( $r_s = -0.614$ ,  $P = 0.004$ ). Amphipods were also abundant, with *Hyalella azteca* collected in 19 of the 20 lakes. There was a significant positive relationship between amphipod abundance and calcium levels (Figure 4B) ( $r_s = 0.776$ ,  $P < 0.001$ ). The proportion of isopods relative to amphipods decreased with increasing lake pH and calcium, with two exceptions (Big Dam East Lake and Turtle Lake) (Figure 4C).

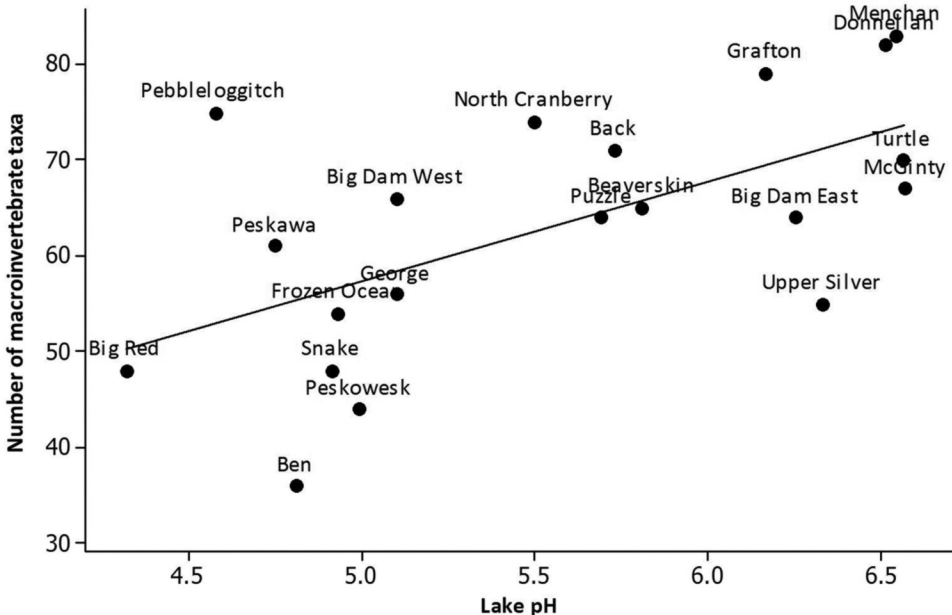


FIGURE 2. Total number of aquatic invertebrate taxa observed in relation to pH of 20 lakes sampled in June 2009 and 2010 in Kejimikujik National Park and National Historic Site of Canada and vicinity in Nova Scotia. Note the significant positive trend between lake pH and the number of invertebrate taxa ( $P = 0.005$ ,  $r_s = 0.36$ ).

TABLE 1. Mean water chemistry parameters for 20 lakes sampled in 2009 and 2010 in Kejimikujik National Park and National Historic Site of Canada, Nova Scotia, arranged from lowest to highest pH. Water samples were collected during spring and fall turnover in 2009 and 2010 as part of Environment Canada's acid rain monitoring program in Atlantic Canada (Clair *et al.*, 2011).

Lake	pH	Dissolved										Aluminum (Al) (mg/L)	Iron (Fe) (mg/L)	Potassium (K) (mg/L)	Chlorine (Cl) (mg/L)	Sodium (Na) (mg/L)	Calcium (Ca) (mg/L)	Magnesium (Mg) (mg/L)	Nitrogen (N) (mg/L)	Nitrogen organic carbon (mg/L)	Alkalinity (mg/L CaCO <sub>3</sub> )	SO <sub>4</sub> (mg/L)	Colour (Hazen) (units)	Area (ha)	Mean depth (m)	Maximum depth (m)	Elevation (m)
		Alkalinity (mg/L CaCO <sub>3</sub> )	SO <sub>4</sub> (mg/L)	Carbon (mg/L)	Nitrogen (mg/L)	Calcium (mg/L)	Magnesium (mg/L)	Sodium (mg/L)	Chlorine (mg/L)	Potassium (mg/L)	Iron (mg/L)																
Big Red	4.3	<0.01	1.07	19.53	0.38	0.28	0.33	2.80	3.58	0.26	0.20	0.30	202	70.5	1.0	2.2	160										
Pebbleloggitch	4.6	<0.01	1.04	13.70	0.31	0.28	0.28	2.66	3.38	0.22	0.22	0.30	150	33.4	1.4	2.5	120										
Peskawa	4.8	<0.01	1.30	9.83	0.28	0.29	0.26	2.63	3.69	0.27	0.18	0.30	99	388.5	3.2	9.0	120										
Ben	4.8	0.05	1.29	6.23	0.21	0.18	0.23	2.43	3.52	0.34	0.10	0.15	56	20.4	0.5	0.7	170										
Snake	4.9	0.41	0.53	14.73	0.40	0.66	0.41	2.74	3.86	0.16	0.43	0.13	159	12.7	1.4	2.5	90										
Frozen Ocean	4.9	0.34	1.06	13.28	0.32	0.62	0.38	3.30	4.36	0.32	0.27	0.29	128	228.0	1.9	7.6	105										
Peskowesk	5.0	0.45	1.38	6.90	0.22	0.30	0.28	2.71	3.88	0.25	0.17	0.25	69	685.0	3.9	13.0	105										
Big Dam West	5.1	0.58	1.08	12.63	0.31	0.73	0.39	3.72	5.09	0.30	0.26	0.27	114	105.0	2.5	9.5	120										
George	5.1	0.67	1.37	9.63	0.26	0.58	0.36	3.20	4.47	0.29	0.27	0.21	100	108.0	2.4	8.5	87										
North Cranberry	5.5	0.54	1.29	4.33	0.21	0.38	0.29	2.53	3.68	0.23	0.07	0.08	29	38.0	1.5	5.0	105										
Puzzle	5.7	0.79	1.10	3.60	0.16	0.38	0.28	2.42	3.63	0.28	0.07	0.05	13	36.0	2.7	6.1	120										
Back	5.7	0.66	1.49	4.13	0.20	0.46	0.32	2.62	3.89	0.22	0.06	0.08	28	64.9	2.2	5.8	100										
Beaverskin	5.8	0.56	1.41	2.83	0.18	0.29	0.30	2.61	3.86	0.24	0.02	0.04	11	39.5	2.2	6.3	120										
Grafton	6.2	1.55	1.59	6.43	0.25	0.94	0.49	4.36	6.34	0.23	0.28	0.09	52	270.4	2.8	10.0	100										
Big Dam East	6.2	1.34	1.38	4.45	0.21	0.61	0.38	3.09	4.25	0.27	0.05	0.09	24	45.5	2.3	4.2	120										
Upper Silver	6.3	1.38	1.49	3.60	0.17	0.64	0.33	2.77	3.86	0.25	0.03	0.07	18	24.3	2.3	5.8	90										
Donnellan	6.5	2.19	1.61	4.00	0.19	0.86	0.47	4.44	6.48	0.39	0.08	0.06	25	47.6	-	-	110										
Menchlan	6.5	2.32	1.82	2.90	0.16	0.74	0.46	2.65	3.47	0.38	0.04	0.03	13	51.1	6.5	12.0	126										
Turtle	6.6	2.49	1.57	3.90	0.17	0.98	0.48	2.78	3.93	0.41	0.08	0.06	24	85.2	4.5	9.0	103										
McGinty	6.6	3.11	0.97	7.70	0.32	1.12	0.51	2.70	3.69	0.27	0.37	0.08	61	4.4	1.4	4.0	105										

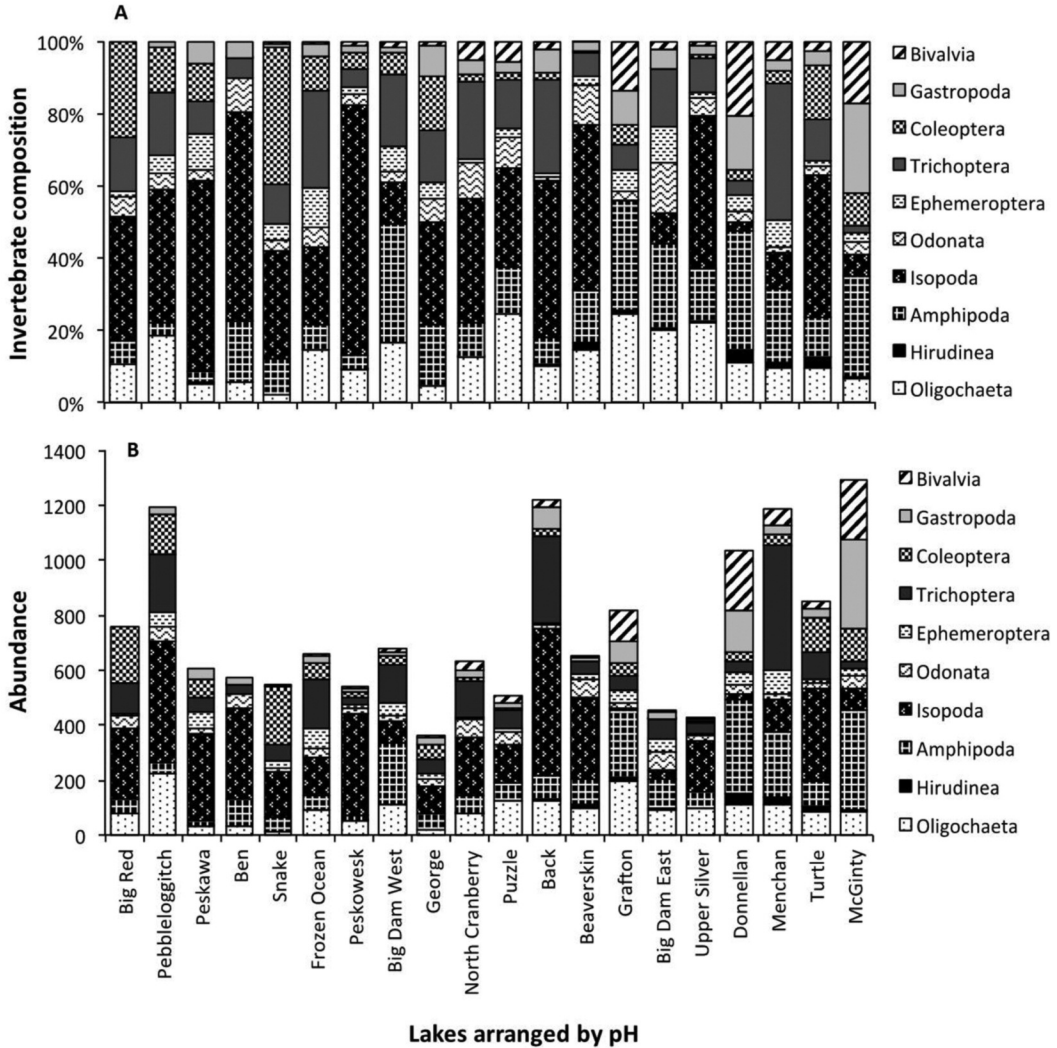


FIGURE 3. Percentage composition (panel A) and total abundance (panel B) of various taxonomic groups sampled in June 2009 and 2010 in 20 lakes in Kejimikujik National Park and National Historic Site of Canada and vicinity in Nova Scotia. Total abundance is the total number of individuals collected per lake. Lakes are arranged from the most acidic (Big Red Lake) (pH 4.3) to the least acidic (McGinty Lake) (pH 6.6).

Lakes with high pH and calcium concentrations had a larger number of bivalves, gastropods, and leeches (Hirudinea) (Figure 5). Bivalves were observed only in lakes with pH greater than approximately 4.9, and abundance was significantly correlated with lake acidity (Figure 5A) ( $r_s = 0.775, P < 0.001$ ). Gastropod abundance was also significantly correlated with pH (Figure 5B) ( $r_s = 0.539, P = 0.014$ ). Similarly, Hirudinea abundance was significantly correlated with lake pH

(Figure 5C) ( $r_s = 0.789, P < 0.001$ ). No leeches were collected from lakes with pH  $< 5.5$ , with the exception of a few individuals from the Erpobdellidae family captured in Peskawa Lake (pH 4.8) and Peskowesk Lake (pH 5.0). In contrast, abundance of coleopterans was significantly correlated with dissolved organic carbon (Figure 6) ( $r_s = 0.650; P = 0.002$ ), but not with pH or calcium ( $P > 0.05$ ).

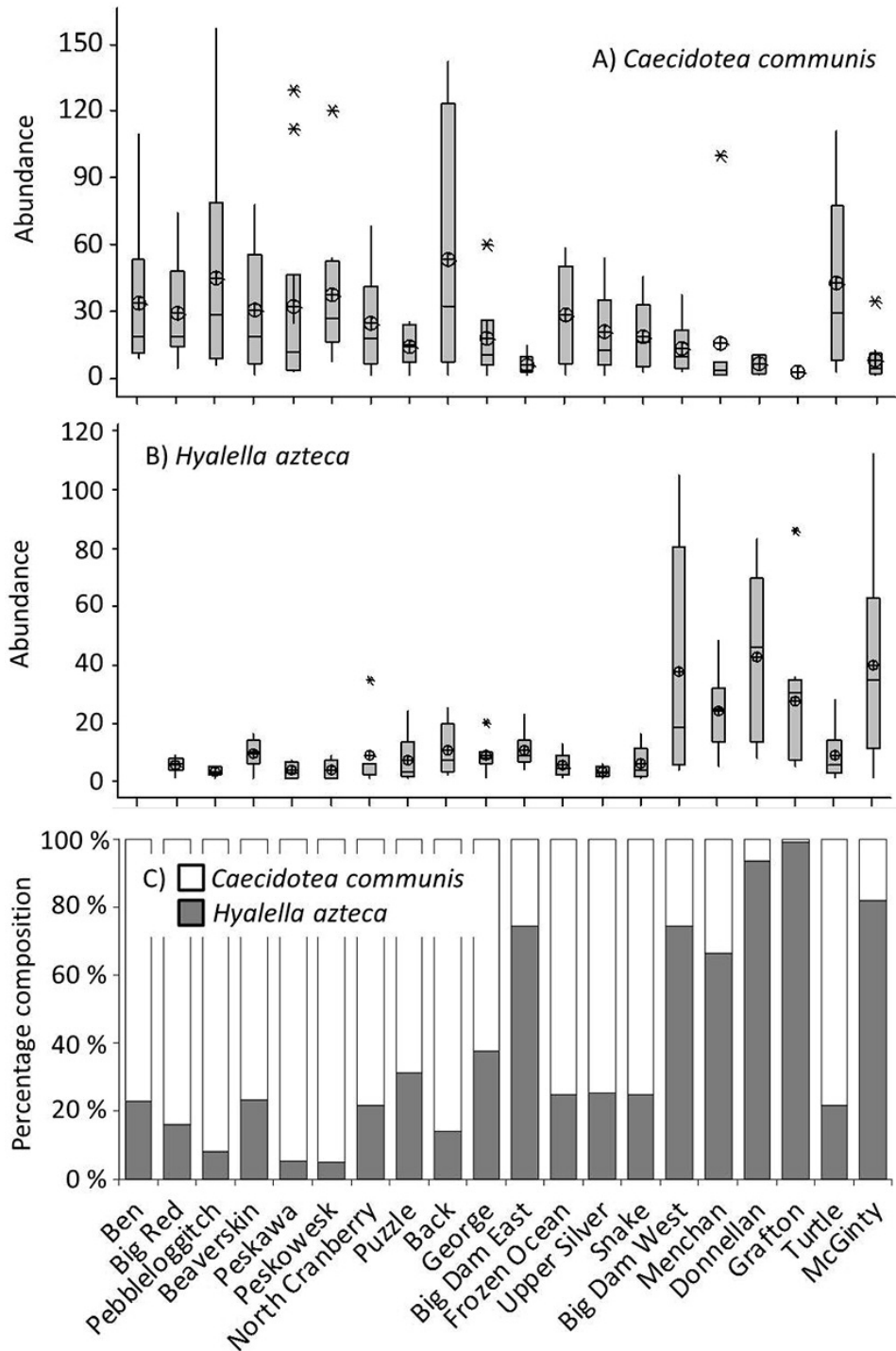


FIGURE 4. Abundance of the isopod *Caecidotea communis* (panel A), the amphipod *Hyalella azteca* (panel B), and the corresponding proportions of these two species (panel C) observed in 20 lakes sampled in June 2009 and 2010 in Kejimikujik National Park and National Historic Site of Canada and vicinity in Nova Scotia. Lakes are arranged by level of calcium from the lowest (Ben Lake) (0.18 mg/L) to the highest (McGinty Lake) (1.12 mg/L). For panels A and B, the horizontal line indicates the median, ⊕ indicates mean, box indicates 25th and 75th percentiles, whiskers indicate minimum and maximum data points within 1.5 × the box height from the bottom or top (respectively), and asterisks mark outliers.

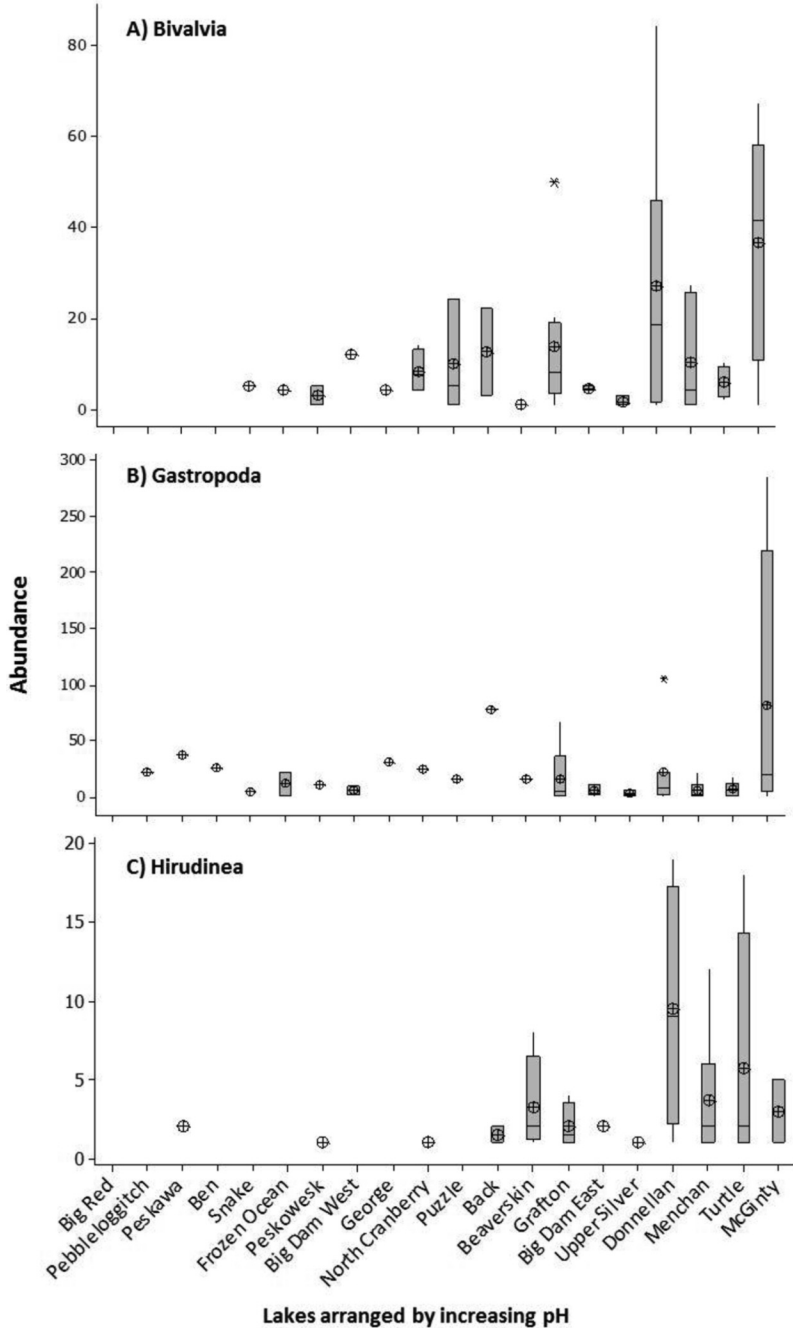


FIGURE 5. Abundance of Bivalvia (panel A), Gastropoda (panel B) and Hirudinea (leeches) (panel C) in 20 lakes sampled in June 2009 and 2010 in Kejimikujik National Park and National Historic Site of Canada and vicinity in Nova Scotia. Lakes are arranged from the most acidic (Big Red Lake) (pH 4.3) to the least acidic (McGinty Lake) (pH 6.6). Horizontal line indicates the median,  $\oplus$  indicates mean, box indicates 25th and 75th percentiles, whiskers indicate minimum and maximum data points within  $1.5 \times$  the box height from the bottom or top (respectively), and asterisks mark outliers.



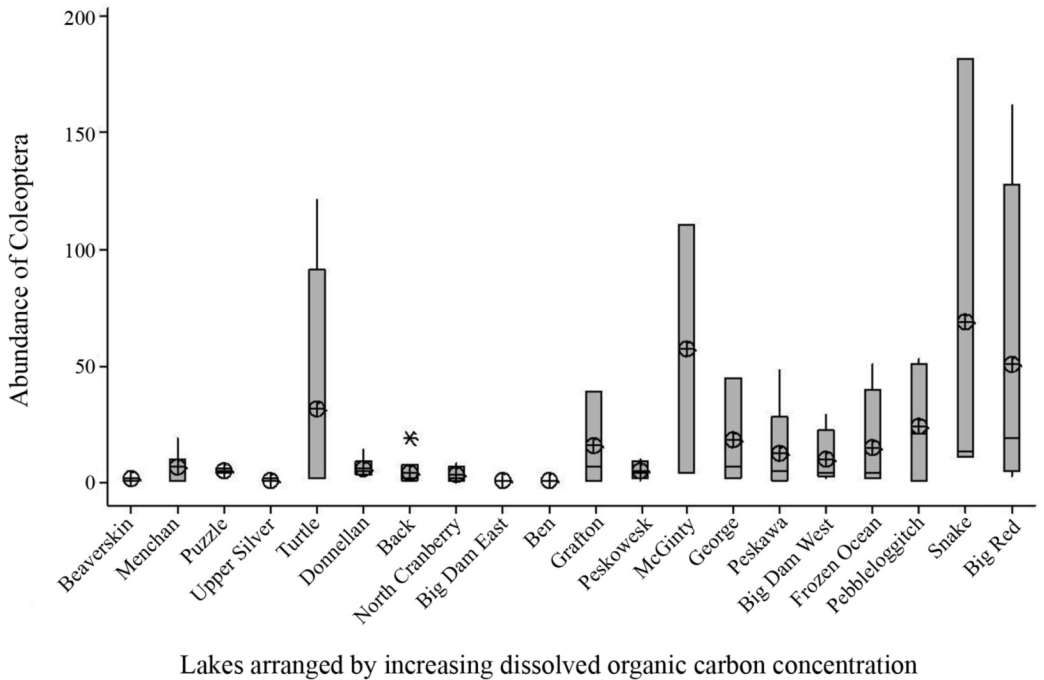


FIGURE 6. Abundance of Coleoptera in 20 lakes sampled in June 2009 and 2010 in Kejimikujik National Park and National Historic Site of Canada and vicinity in Nova Scotia. Lakes are arranged by concentration of dissolved organic carbon from the lowest (Beaverskin Lake) (2.8mg/L) to the highest (Big Red Lake) (19.5mg/L). Horizontal line indicates the median, ⊕ indicates mean, box indicates 25th and 75th percentiles, whiskers indicate minimum and maximum data points within  $1.5 \times$  the box height from the bottom or top (respectively), and asterisks mark outliers.

## Discussion

We found that both pH and calcium were significantly correlated with the number of aquatic macroinvertebrate taxa observed in the study lakes. Lakes that were less acidic and lakes with higher calcium concentrations tended to have greater species richness. These findings are consistent with other studies, which also reported fewer aquatic invertebrate taxa in more acidic lakes (McNicol *et al.* 1995a; Doka *et al.* 1997). However, the relationship between chemical conditions and the abundance of macroinvertebrates was less clear.

Fish were present in all of the study lakes (Kerekes 1975; Drysdale *et al.* 2005), and the presence of fish likely influenced the macroinvertebrate and zooplankton species richness. The most frequently collected taxa were isopods, amphipods, and trichopterans. Gastropods, bivalves, and ephemeropterans, commonly considered to be more sensitive to acidity, were collected less frequently during the study. Lakes with lower pH had fewer taxa (consisting mostly of isopods, coleopterans, and oligochaetes), while lakes with higher pH had greater taxa richness.

### *Isopoda and amphipoda*

Only one species of isopod was collected (*Caecidotea communis*), but this species was present in all 20

study lakes. *Caecidotea communis* was also the most abundant taxon in many of the study lakes, comprising  $\geq 30\%$  of the macroinvertebrates collected in 11 of the lakes. The abundance of this species was negatively correlated with calcium concentrations, and the highest numbers were found in the most acidic lakes (e.g., Peskawa Lake, Ben Lake, Peskowesk Lake). Schell and Kerekes (1989) also reported Isopoda in Nova Scotia lakes with pH as low as 4.4.

Isopods are known to be acid tolerant (Merritt *et al.* 2008), but their high frequency of occurrence in lakes in this study contrasts with their relative rarity in lakes monitored in Ontario (RCW *et al.*, unpublished data). Potential explanations include differences in the species found in the two datasets (Ontario isopods were identified only to order), regional differences in species habitat affinities, or a relative dominance of substrate type or other habitat conditions that encourage isopod abundance in lakes in this study area. Because sampling methods were similar in the two regions, we do not believe sampling variation is likely to be responsible for these differences.

Amphipods were also common, and their abundance was greater in lakes with high pH and high calcium concentrations. Two species of amphipods were col-

lected: *Crangonyx richmondensis* (collected in 55% of study lakes) and *Hyaletta azteca* (collected in 95% of study lakes). In this study, *H. azteca* was present across a broad pH range (i.e., 4.3 to 6.6). Studies in Ontario have identified this species as acid sensitive (McNicol *et al.* 1995a), with a minimum pH threshold of 5.6 or higher (Stephenson *et al.* 1986; Rosenberg *et al.* 1997; Snucins 2003). In this study, however, *H. azteca* appeared to be very acid tolerant and was observed in lakes with pH as low as 4.3.

Peterson (1987) also observed *Hyaletta* in lakes with low pH (4.5–5.5) in Nova Scotia and New Brunswick, and reported that *Hyaletta* species in lakes in the Maritimes appear to be more tolerant of acidic conditions than other Amphipoda. However, the lakes in that particular study had higher concentrations of calcium than the study lakes with low pH in southwestern Nova Scotia or in acidified lakes in Ontario (Peterson 1987). The lakes in this study with low pH also had low calcium concentrations.

It may be possible that a localized population of *H. azteca* has adapted to the acidic environment in the lakes in Kejimikujik National Park and National Historic Site. A genetic study by Witt and Hebert (2000) examined populations of *H. azteca* from various locations across North America and found a complex of at least seven species rather than a single species as previously believed. Grapentine and Rosenberg (1992) also suggested that populations of *H. azteca* may have adapted to acidic conditions in some regions of Canada.

Interpretation of regional variation in *H. azteca* habitat associations and identification of their potential role in biological monitoring of lakes in this study area would benefit from an improved understanding of the geographic variation in their genetic profile and the consequences for their tolerance of acidic conditions.

When we compared the relative proportions of isopods and amphipods across the 20 study lakes, we found that isopods were dominant in lakes with low pH and low calcium concentrations while amphipods were dominant in lakes with high pH and high calcium concentrations. Both amphipods and isopods are photosensitive and avoid bright light by moving into crevices or under rocks, leaves, and roots (Covich and Thorp 2001, page 791), where they are less exposed (complex substrates provide protection from predation by fish and crayfish) (Covich and Thorp 2001, page 791). The substrate in many of the study lakes consists of cobbles and boulders, which may partially explain the high abundance of these two taxa.

#### *Bivalvia and gastropoda*

Invertebrate taxa with hard, calcareous shells such as bivalves and gastropods were generally collected only from less acidic lakes. A total of 10 species of bivalves and 12 species of gastropods were collected. Bivalve abundance was correlated with lake pH: bivalves were observed only in lakes where pH was greater than 4.9.

Because many lakes in the study area are acidic and have low calcium concentrations, low abundance of calcium-dependent macroinvertebrate taxa was expected. Our results are consistent with a previous study of 8 acid-sensitive Nova Scotia lakes by Schell and Kerekes (1989), which found that bivalves did not occur below a pH of 5.0.

This exclusion of calcareous species in acidic lakes has also been noted for other acid-sensitive regions of eastern Canada (Weeber *et al.* 2004; Jeziorski *et al.* 2008). As calcium concentrations in many acidified lakes continue to decline (Jeziorski *et al.* 2008), this may further reduce the abundance and distribution of calcium-rich taxa such as bivalves and gastropods in lakes in the study area.

Gastropods were also generally more abundant in lakes with high pH and high calcium concentrations; this finding is consistent with results from Ontario (Bendell and McNicol 1993). One exception to this is *Ferrissia fragilis*, which was the only species collected in lakes in the study area with pH lower than 6. Bendell and McNicol (1993) also reported *Ferrissia* as an acid-tolerant gastropod in study lakes in Ontario, where it was the only gastropod taxon observed in lakes with pH below 6. That study also suggested that, above the minimal pH thresholds, gastropod abundance in small oligotrophic lakes was not limited by acidity or calcium concentrations but rather by food resources. Predation, substrate type, and macrophyte biomass can also play a large role in gastropod distributions (Brown 2001, page 310). In our study lakes, the abundance of *Ferrissia fragilis* also did not appear to be associated with pH, calcium, or dissolved organic carbon and thus is likely limited by some other constraint such as predation or availability of food resources.

#### *Hirudinea*

A total of 12 species of leeches were collected from the study lakes, with only 4 of those species being common (i.e., occurring in >10% of the lakes). Counts were generally low, and abundance was correlated with lake pH. Hirudinea were not observed in lakes with pH < 5.5, with the exception of two *Mooreobdella fervida* collected in Peskawa Lake (pH 4.8) and one *Erpobdella punctata* collected in Peskowsk Lake (pH 5.0).

Bendell and McNicol (1991) observed similar reductions in the diversity and abundance of Hirudinea in acidic conditions below pH 5.5. However, they suggested that acidity alone does not predict the distribution of leech species and that predation and availability of suitable prey also influenced their distribution (Bendell and McNicol 1991). In addition, other studies have shown that, although leeches are sensitive to low pH, their occurrence and abundance are also influenced by other factors, such as lake productivity (Schalk *et al.* 2001). Lakes in Kejimikujik National Park and National Historic Site are oligotrophic and generally have low

productivity (especially at the lower pH range), and lower abundance of preferred prey may therefore play an important role in the distribution of leeches there.

#### *Coleoptera*

Although lower in abundance than other groups, coleopterans appeared to be tolerant of acidity and were collected in all 20 study lakes. A total of 14 taxa were observed (8 were common and 6 were uncommon). The abundance of this taxonomic group was correlated with dissolved organic carbon. A study of Ontario lakes by Lento *et al.* (2008) also suggested a strong correlation between macroinvertebrate abundances and dissolved organic carbon, especially in acidic lakes. Wood *et al.* (2011) reported that dissolved organic carbon can protect against the deleterious effects of low pH on organismal function via physiological mechanisms. Dissolved organic carbon can alter the permeability of cell membranes in acidic conditions and also influence transport physiology (Wood *et al.* 2011).

Other studies have suggested that water chemistry is not as important a stressor on coleopterans as predation by fish (Bendell and McNicol 1987; Arnott *et al.* 2006). The darkly coloured water of some lakes in the study area (due to high concentrations of dissolved organic carbon) may provide coleopteran taxa with some protection from predation by fish and other visual predators.

#### *Trichoptera*

Trichopterans were common and taxonomically diverse in the study lakes, with 23 of the 30 taxa occurring in >10% of the lakes. Trichopteran species collected included taxa from 10 families, with the most common and abundant families being Hydroptilidae, Leptoceridae, and Limnephilidae. The trichopterans collected in the study lakes generally had a high apparent tolerance to acidity, with many of the observed species occurring across a wide gradient in lake pH.

Trichoptera abundance can be strongly influenced by fish predation, and trichopterans generally associated with fishless conditions, such as the leptocerid *Triaenodes* and phryganeid *Banksiola* (Bendell and McNicol 1995), were rare in the study lakes. Both of these organisms are quite large and thus are likely to be attractive prey for insectivorous fish. In contrast, the leptocerid *Nectopsyche* was quite abundant. They are smaller in size and construct cases with bristling twigs or elongate sticks attached that may make them more difficult for fish to consume as prey (Wiggins 2004).

#### *Ephemeroptera and odonata*

Ephemeroptera generally had low abundance in the 20 study lakes, with a total of 10 taxa collected. This is likely due to the acidity of the lakes, as ephemeropterans are recognized as being sensitive to acidity (Carbone *et al.* 1998). Seven of the ephemeropteran taxa were common, and 3 were uncommon. The most frequently collected species were *Caenis diminuta* and the genus *Eurylophella*, which have been reported to have

at least some tolerance to acidity (Carbone *et al.* 1998). No ephemeropterans were collected from Ben Lake, which is low in pH (4.8) and had the lowest calcium levels of the 20 study lakes (0.18mg/L).

Odonates were taxonomically diverse in the study lakes, with a total of 30 species observed (22 species were common and 8 were uncommon). However, counts were generally low, and odonates did not make up a large proportion of macroinvertebrates in terms of abundance. The most abundant family of damselflies (suborder Zygoptera) was Coenagrionidae, which was observed across a wide gradient of acidity. Larvae in this family are relatively small (Hilsenhoff 2001, page 671) and thus may be less visible to predators such as fish or larger predatory odonates.

Within the suborder Anisoptera (dragonflies), the most common families observed in the study lakes were Corduliidae, Gomphidae, and Libellulidae, while Aeshnidae were rare. Anisoptera taxa also occurred across a wide gradient of acidity; for example, *Cordulia shurtleffi* was observed in 65% of the study lakes (pH 4.3–6.6). Bendell and McNicol (1995) also found that abundance of this particular taxon was not related to lake acidity in Ontario lakes.

#### *Diptera*

With the exception of chironomids, Diptera were not abundant in the study lakes. Ceratopogonidae were present in all 20 study lakes, and no correlation with acidity was detected. Chironomidae were frequently collected in all of the study lakes, but were not targeted in our sampling and sorting, so specimens were not identified to species level.

#### *Hemiptera*

Very few water striders were captured in the study lakes. The only species with high abundance was *Rheumatobates rileyi*, in particular in Upper Silver Lake. Although the abundance of this particular species has been shown to have a strong correlation with pH (Bendell 1988), acidity did not appear to be the main driver in the presence of this particular species in the study lakes.

#### *Zooplankton*

Of the 26 zooplankton species observed in the 15 study lakes, many were common and occurred across a wide gradient of acidity. Daphniids were the only taxonomic group that showed a clear correlation with acidity in the study lakes: they were not observed below a pH of 5.5. This finding is consistent with previous studies, which have shown daphniids to be acid sensitive (Yan *et al.* 2008; Korosi and Smol 2012). In addition, daphniids are sensitive to calcium levels (Jeziorski *et al.* 2008), and this may also explain their absence in the lakes that had low pH and low calcium concentrations.

With the exception of daphniids, zooplankton abundance in the study lakes did not appear to be correlated with acidity alone. Dissolved organic carbon has been

shown to affect zooplankton populations, and the high concentrations of dissolved organic carbon in some of the study lakes may provide some protection from visual predators (Yan *et al.* 2008). Using paleolimnological methods in 3 lakes in Kejimikujik National Park and National Historic Site, Korosi and Smol (2012) found that there was a more pronounced change induced by acidification in the assemblage of cladocerans in clearwater lakes with lower concentrations of dissolved organic carbon over time than in assemblages in dark water lakes with more dissolved organic carbon. Zooplankton can also be influenced by a large variety of natural factors, such as the availability of food, competition with other zooplankton species, the presence of parasites, and the presence of both vertebrate and invertebrate predators (Yan *et al.* 2008).

#### *Future directions and conclusions*

These results provide a summary of the aquatic macroinvertebrate and zooplankton assemblages in acid-sensitive lakes in Kejimikujik National Park and National Historic Site and surrounding area in southwestern Nova Scotia. Although some of the overall trends of macroinvertebrate species richness with respect to varying pH were similar to results reported in other regions of eastern Canada, several differences were noted.

Some of the lakes in the study area had physical characteristics that differed from acid-sensitive lakes in other regions of eastern Canada, and these physical characteristics influenced the type and abundance of benthic macroinvertebrates that were collected. pH can vary spatially within each lake as well as seasonally due to runoff, with pulses of acidity in the spring and fall (Clair *et al.* 2007). These pulses also coincide with lower temperatures, and at these times of the year organisms may be less active and therefore more tolerant of their acidic environment (Stephenson and Mackie 1994). Although benthic microhabitats near the lake bed can have lower acidity than the upper water column (Grapentine and Rosenberg 1992), lakes in the study area are shallow with a large surface area which often allows for mixing throughout the open-water period. Therefore, benthic organisms would likely be exposed to high acidity throughout the active growth period in the summer.

All aquatic sampling methods have inherent biases in their sampling efficiencies for different invertebrate taxa. We employed multiple sampling methods in order to collect a wide range of taxa, but there was likely to have been variation in efficiency among the sampling methods with respect to particular taxa. Because the same suite of methods was used in all lakes, we assume the effects of this variation were consistent across the 20 study lakes, and we emphasize comparisons of invertebrate taxa patterns between lakes, rather than within lakes.

Our sampling methods, which were initially developed to collect benthic invertebrates from thick organic sediments in small Boreal Shield lakes in Ontario

(McNicol *et al.* 1995b), may not have been as suitable for lakes with rocky substrates. Although regional variation in species' habitat affinities may have contributed to particular differences between the findings from this study and reports from other regions (e.g., isopods, *H. azteca* distributions), substrate or other differences in the habitat also may have been a factor. Hoop sampling (visual searches in a confined area along the shoreline) worked particularly well in our lakes for sampling species of Trichoptera. Future studies should incorporate traditional benthic drag sampling with other methods such as kick and sweep, rock picking, or artificial substrates.

Carbone *et al.* (1998) successfully sampled macroinvertebrates in shallow, rocky littoral habitats using substrate cages filled with native rocks to match the rocky littoral substrate of sample lakes. This method might work well in Kejimikujik National Park and National Historic Site, where the littoral zone of many lakes is extremely shallow and consists of cobble and boulders. Many species collected in the study were rare (occurring in only one or two of the lakes) and had low counts. Increasing sampling effort, especially in the large lakes with varying substrate types, would reduce the likelihood of missed taxa.

Another interesting difference between the lakes in Kejimikujik National Park and National Historic Site and the lakes in the Boreal Shield in Ontario is the high concentration of dissolved organic carbon due to naturally occurring bogs and wetlands in the watersheds. The extremely dark waters of some lakes in the study area may benefit particular invertebrate species through physiology, protection from visual predators, or other reasons.

The data presented here establish a baseline for future monitoring in Kejimikujik National Park and National Historic Site as acid deposition continues to affect this region. Because the lakes are naturally acidic and are extremely vulnerable to additional acid inputs, recovery is slower than in other regions in eastern Canada affected by acid deposition (Clair *et al.* 2011). Additional effort may be required to reduce the impacts of acidification on the aquatic organisms that live in these ecosystems.

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SUPPLEMENTARY TABLE 1. Abundance of zooplankton species observed in 15 lakes sampled in June 2009 and 2010 in Kejimikujik National Park and National Historic Site of Canada and vicinity in Nova Scotia. Abundance is presented as density (number of individuals/m<sup>3</sup>). Lakes are arranged by pH from the lowest (Peskawa Lake) (pH = 4.8) to the highest (McGinty Lake) (pH = 6.6).

Classification and Family	LAKE NAME															Number of lakes present	% of lakes present	mean density	min density	max density		
	Peskawa	Frozen Ocean	Peskowsk	Big Dam West	George	North Cranberry	Back	Beaverskin	Grafton	Big Dam East	Upper Silver	Donnellan	Menchan	Turtle	McGinty							
Taxa Name	4.8	4.9	5.0	5.1	5.1	5.1	5.5	5.7	5.8	6.2	6.2	6.3	6.5	6.5	6.6	6.6	6.6	6.6	6.6			
<b>C. Branchiopoda,</b> <b>O. Diplostraca</b> Bosminidae	4198	12055	10157	3128	1973	5206	1378	155	3622	8873	2755	66	45	2678	7232	6830	15	100	4235	45	12055	
		4956		741	78	103			132	724		19					7	47	1922	19	6830	
					129												2	13	131	129	132	6830
					26												1	7	26	26	26	26
							35										1	7	35	35	35	26
																	8	53	2290	595	6062	6062
																	1	7	1185	1185	1185	1185
																	3	20	513	310	827	827
							310										1	7	402	402	402	402
																	15	100	6121	66	24909	24909
																	2	13	15	5	26	26
																	1	7	134	134	134	134
																	4	27	128	26	268	268
																	4	27	128	26	268	268
																	15	100	3064	38	14865	14865
<b>C. Maxillopoda,</b> <b>O. Calanoida</b>	412	1205	2153	659	362	755	310	4742	1712	3803	5511	38	194	9242	14865		15	100	3064	38	14865	14865
	23744	30144	12387	3949	5172	119708	7230	31093	27701	21710	6197	1507	2411	61173	60212		15	100	27622	1507	119708	119708
	7904	36127	14451	25063	4138	17209	15415	49748	26382	40525	66231	4820	38635	938	22910		15	100	24700	938	66231	66231
	4116	13019	4736	247	621	13808	10607	13965	9051	8330	58685	603	5089	8519	1205		15	100	10173	247	58685	58685
				3539					329	1087	1378				8035		5	33	2874	329	8035	8035
								466							1205		1	7	466	466	466	466
																	1	7	1205	1205	1205	1205
																	2	13	677	86	1268	1268
																	8	53	294	26	905	905
																	12	80	650	9	3582	3582
<b>C. Maxillopoda,</b> <b>O. Cyclopoida</b> Cyclopidae	27	1741		1646	1940	1811	620	414	527	3079	4133	61	15	6829	9038		14	93	2277	15	9038	9038
	82	670		412	52	453	138	26	395	362	551		74	134	1607		13	87	381	26	1607	1607
	55	1473	258	1646	2199	2603	138	517	2633	3863	3031	1130	2545	10845	57803		15	100	6049	55	57803	57803
							69				551				268		4	27	423	69	804	804
															804		2	13	69	52	86	86

SUPPLEMENTARY TABLE 2. List of aquatic macroinvertebrate taxa observed in 20 lakes sampled in June 2009 and 2010 in Kejimikujik National Park and National Historic Site of Canada and vicinity in Nova Scotia. Lakes are arranged by pH from the most acidic (Big Red Lake) (pH 4.3) to the least acidic (McGinty Lake) (pH 6.6). Invertebrate taxa are organized by order, family, genus, and species (where possible) and include the total number of individuals captured in each lake as well as mean, minimum, and maximum counts and the percentage of lakes in which each taxon was observed.

Table with columns: Classification and Family, Taxa Name, pH, and Lake Name (listing 20 lakes: Big Red, Pebbletoegitch, Ben, Peskawa, Snake, Frozen Ocean, Peskowesk, Big Dam West, George, North Cranberry, Puzzle, Back, Beaverskin, Grafton, Big Dam East, Upper Silver, Donnellan, Menchan, Turtle, McGinty), and summary columns: Number of lakes present, % of lakes present, mean count, min count, max count.













SUPPLEMENTARY TABLE 2. (continued)

Classification and Family	Taxa Name	LAKE NAME														% of lakes present	Number of lakes present	mean count	min count	max count														
		Big Red	Pebble	Ben	Peskawa	Snake	Frozen Ocean	Peskowsk	Big Dam West	George	North Cranberry	Puzzle	Back	Beaverskin	Grafton						Big Dam East	Upper Silver	Donnellan	Menchan	Turtle	McClinty								
Limnephilidae	<i>Triaenodes nox</i>	4.3	4.6	4.8	4.8	4.9	4.9	5.0	5.1	5.1	5.1	5.7	5.7	5.8	6.2	6.2	6.3	6.5	6.5	6.6	6.6	6.9	6.9	3	15	1.0	1	1						
	<i>Triaenodes</i> species <i>indet.</i>	1								1	1																							
	<i>Leptoceridae</i>																																	
	<i>Glyptopsyche irrorata</i>	21	2	3	3	6	6	4	4	4	11	10	72	3			8									13	65	14.1	2	72				
	<i>Grammatotulus</i>															4																		
	<i>Hydatophylax</i>																																	
	<i>Limnephilus</i>	22	44	5	7	18	7	2	2	47	10	14	5	2				5	11							16	80	12.5	1	47				
	<i>Phanocelia canadensis</i>	1					3																				2	10	2.0	1	3			
	<i>Platycentropus amicus</i>	2	6	3	1	8	3	6	2	2	4	9	3	1				1	5							16	80	3.9	1	9				
	<i>Molanna ulmerina-unicapilla</i>	1	16	4	2			4	2	4	2	1		2												9	45	3.6	1	16				
Phryganeidae	<i>Agrypnia straminea</i>	6	5		1					1	6	3	1	2												6	45	2.9	1	6				
	<i>Banksiola crotchii</i>																									6	30	1.5	1	3				
	<i>Banksiola smithi</i>	2	3	1	1	1	2					1	4	1												8	40	1.9	1	4				
	<i>Nyctophylax</i>																										2	10	2.0	1	3			
	<i>Polycentropus</i>	7	8	1	6	3	1			3	4	1	2													15	75	3.3	1	8				
Sericostomatidae	<i>Agarodes distinctus</i>	10	3					2	3	12	15							5	4							13	65	4.6	1	15				
<b>O. Coleoptera</b>																																		
	<i>Chrysomeiidae</i>																																	
Curculionidae	<i>Donacia</i>	1																																
	<i>Pyrrhalta</i>																																	
	<i>Phyllotribus oblongus</i>												1																					
Dytiscidae	<i>Coptotomus</i>																																	
	<i>Manus ovatus</i>	1																																
Elmidae	<i>Neoporus undulatus</i>	3	11	9		2	4	2				1							3							9	45	4.0	1	11				
	<i>Ancyronyx variegata</i>			1			2				6	4														5	25	3.4	1	6				
	<i>Dubiraphia vitata</i>																									11	55	57.9	1	182				
	<i>Macronychus glabratus</i>																									3	15	3.0	1	6				
	<i>Stenelmis - larvae</i>	162	53	1	48	11	2	10	29		2	8	4	2	1	1										11	55	21.1	1	162				
Gyrinidae	<i>Stenelmis crenata - adult</i>	27																								10	50	14.7	1	53				
	<i>Stenelmis musgravei - adult</i>																									1	5	1.0	1	1				
	<i>Gyrinus</i>																									1	5	1.0	1	1				

SUPPLEMENTARY TABLE 2. (continued)

Classification and Family	Taxa Name	LAKE NAME																Number of lakes present	% of lakes present	mean count	min count	max count								
		Big Red	Pebllelogitch	Ben	Peskawa	Snake	Frozen Ocean	Peskowsk	Big Dam West	George	North Cranberry	Puzzle	Back	Beaverskin	Grafton	Big Dam East	Upper Silver						Donnellan	Menchan	Turtle	McCinty				
Hydrophiliidae Psephenidae <b>O. Diptera</b> Ceratopogonidae	<i>Berosus</i>	11	30	5	4.8	4.8	4.9	4.9	5.0	5.1	5.1	5.1	5.5	5.7	5.7	5.8	6.2	6.2	6.3	6.5	6.5	6.6	6.6	10	50	9.5	3	30		
	<i>Ectopria nervosa</i>												1			2					5	7			5	25	3.4	1	7	
	<i>Bezzia</i>	1	20	5	3	3	4	7	4	7	4	1	2	7	2	5	11	10	2	3	1	3	1	5	20	100	5.3	1	20	
	<i>Dasyhelea</i>																1				1	2			4	20	1.3	1	2	
	<i>Monohelea</i>	1	2																						2	10	1.5	1	2	
	<i>Polpomyia</i>										5							2							3	15	2.7	1	5	
	<i>Probezzia</i>	5	5	2	3	1					3	1					3								9	45	2.8	1	5	
	<i>Sphaeromyia</i>															4										1	5	4.0	4	4
	<i>Chaoborus punctipennis</i>										2															1	5	2.0	2	2
	Chironomidae		16	26	13	10	11	5	5	5	19	2	12	6	19	9	8	15	14	13	7	13			20	100	12.8	2	33	
	<i>Rhaphium</i>															2										1	5	2.0	2	2
Dolichopodidae																									1	5	2.0	2	2	
Emphididae													3	1			1								4	20	2.3	1	4	
Tabanidae		3	1	2									6	1	1		1								9	45	1.9	1	6	
<b>C. Gastropoda</b>																														
Ancyliidae		22	26	37	4	22	10	9	31	25	15	78	16	1	9	7								17	85	18.8	1	78		
<i>Ferrissia fragilis</i>																									3	15	5.3	1	8	
<i>Gyraulus deflectus</i>																									6	30	83.0	3	286	
<i>Annicola limosa</i>																66	3	105	21	17					1	5	2.0	2	2	
<i>Cincinnatia integra</i>																									2	10	1.0	1	1	
<i>Helisoma anceps</i>																									2	10	1.0	1	1	
<i>Menetus dilatatus</i>																									5	25	10.4	1	22	
<i>Physa</i>																									2	10	6.0	1	11	
<i>Planorbella trivolvis</i>																	11								1	5	11.0	11	11	
<i>Planorbella campanulata</i>																									1	5	2.0	2	2	
<i>Probythinella emarginata</i>																	6								1	5	6.0	6	6	
<i>Valvata perdepressa</i>																	1								1	5	1.0	1	1	
<i>Valvata sincera</i>																									1	5	1.0	1	1	
<i>Campeloma decaisum</i>																	4	2	2	6	2	1	16	7	35	4.7	1	16		

