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Abiotic and biotic processes in lakes recovering from acidification: the relative roles of metal toxicity and fish predation as barriers to zooplankton re-establishment

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SUMMARY

1. Recovery of acidified aquatic systems may be affected by both abiotic and biotic processes. However, the relative roles of these factors in regulating recovery may be difficult to determine. Lakes around the smelting complexes near Sudbury, Ontario, Canada, formerly affected by acidification and metal exploration, provide an excellent opportunity to examine the factors regulating the recovery of aquatic communities.
2. Substantial recovery of zooplankton communities has occurred in these lakes following declines in acidity and metal concentrations, although toxicity by residual metals still appears to limit survival for many species. Metal bioavailability, not simply total metal concentrations, was very important in determining effects on zooplankton and was associated with a decrease in the relative abundance of cyclopoids and *Daphnia* spp., resulting in communities dominated by *Holopedium gibberum.*
3. As chemical habitat quality has improved and fish, initially yellow perch and later piscivores (e.g. smallmouth bass, walleye), have invaded, biotic effects on the zooplankton are also becoming apparent. Simple fish assemblages dominated by perch appear to limit the survival of some zooplankton species, particularly *Daphnia mendotae*.
4. Both abiotic (residual metal contamination) and biotic (predation from planktivorous fish) processes have very important effects on zooplankton recovery. The re-establishment of the zooplankton in lakes recovering from stress will require both improvements in habitat quality and the restoration of aquatic food webs.

Keywords: metal toxicity, planktivory, recovery, Sudbury, zooplankton

Introduction

The area surrounding Sudbury, Ontario was one of the most severely industrially damaged regions in North America. Since the implementation of emission controls on mining and smelting operations in the early 1970s, lake water quality in this large zone has improved, and there is evidence of recovery at all levels of the food web (Gunn *et al*., 1995). Although this recovery is encouraging, the rate and extent of recovery of the zooplankton differ among regions and species (McNicol, Bendell & Mallory, 1995; Schartau, Halvorsen & Walseng, 2007). In general, recovery in the most severely damaged Sudbury lakes (those closest to the smelters) remains limited, despite their having reached near-neutral pH (see Keller & Yan, 1998; Keller *et al*., 2007 for a review). Across a gradient of severity of historical damage, there is still high variation in recovery.

Studies on individual lakes and also in laboratory and mesocosm experiments have suggested that recovery from acidification is affected by many factors. Metal toxicity can be ameliorated by dissolved salts and organic carbon (Brezonik, King & Mach,

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1991). The exigencies of species reintroduction can play an important and stochastic role (Binks, Arnott & Sprules, 2005; Forrest & Arnott, 2006). Food web effects such as intense predation by macroinvertebrates and planktivorous fish predators can also greatly affect zooplankton community composition in formerly acidified lakes (Keller *et al.*, 2007; Linley, 2008).

While the effects of all of these factors have been explored in small-scale experiments and a few well-studied lakes, there has not yet been a broad assessment of their relative importance across the recovering landscape for zooplankton. We use data from an extensive survey of lakes across the gradient of historical industrial damage and apply a multivariate approach to test hypotheses about recovery and its variation.

Abiotic factors potentially limiting zooplankton recovery include increased UV penetration (Williamson *et al.*, 1999), declines in calcium (Keller, Dixit & Heneberry, 2001) and metal toxicity (Yan *et al.*, 1996). Metal toxicity is of particular concern in lakes closest to the smelters, where copper and nickel concentration often exceed Ontario's Provincial Water Quality Objective (PWQO) of 5 $\mu g\ L^{-1}$ for copper and 25 $\mu g\ L^{-1}$ for nickel (MOEE, 1994). Zooplankton, especially cladocerans, are among the most sensitive aquatic organisms to metal contamination (Brix, DeForest & Adams, 2001). In laboratory experiments, copper increases cladoceran mortality, decreases growth rate, delays maturity and decreases brood size in many species (Winner & Farrell, 1976; Koivisto, Ketola & Walls, 1992; Koivisto & Ketola, 1995). However, community-level studies of zooplankton responses to metal contamination in natural systems are few.

Current water quality standards are based on total metal concentration. However, there is increasing evidence that total metal concentration is not a good predictor of either metal bioavailability or metal toxicity (Janssen *et al.*, 2000). Water chemistry [pH, dissolved organic carbon (DOC), calcium] can substantially affect the toxicity of copper to aquatic biota (De Schamphelaere, Heijerick & Janssen, 2002; Borgmann, Nowierski & Dixon, 2005; Boeckman & Bidwell, 2006). The same is true for several other metals. The Biotic Ligand Model is an index that accounts for water chemistry and provides a better estimate of metal toxicity to aquatic organisms (Paquin *et al.*, 2002). As yet, studies of Biotic Ligand Model-predicted copper toxicity in lakes appear to be lacking, and a model for nickel, another important Sudbury metal is still being developed.

Predation is well known to affect the zooplankton strongly and might be particularly important during species recolonisation (Nilssen & Wærvågen, 2002). Recovering lakes often have impoverished fish communities, and such food web alterations have been hypothesised to influence zooplankton recovery in the Sudbury area (Keller *et al.*, 2002; Holt & Yan, 2003; Yan *et al.*, 2004). Acidification has profound effects on fish communities, including reductions in species richness and failures in recruitment (Beamish, 1976; Somers & Harvey, 1984). The elimination of piscivores can have strong, cascading effects on lower trophic levels through alterations in predator–prey interactions (e.g. Carpenter, Kitchell & Hodgson, 1985; Tonn & Paszkowski, 1986; Hall & Ehlinger, 1989). Piscivorous fish can indirectly increase the abundance of some zooplankton species and change the size structure of the community through reducing the density of planktivores (Hambright, 1994) or changing their foraging behaviour (Turner & Mittelbach, 1990). The potential effect on zooplankton communities of yellow perch (*Perca flavescens* Mitchill), dominant in Sudbury area lakes, has been suggested but has not been studied across a range of lakes.

We tested the above hypotheses about the effects on zooplankton of residual acidification and metals, and the effects of altered predation regimes. We also tested whether the Biotic Ligand Model provides a better index of copper toxicity than total metal concentration. Uniquely, for research in the Sudbury area, our approach was not based on either mesocosm experiments or a small set of lakes, but featured a broad survey of the zooplankton in lakes across a gradient of past industrial damage. In the first part of our study, we used multivariate analyses to test for evidence of the effects of metals (copper and nickel) in determining the recovery of zooplankton. We also compared the effects of measured copper concentrations with available copper predicted from the Biotic Ligand Model. We also tested the hypothesis that fish assemblages in chemically recovering lakes play a role in controlling reassembly of the zooplankton. The data set was restricted to lakes for which we have quantitative data on fish abundance.

Methods

Study area

The 46 lakes studied are located in the City of Greater Sudbury and the surrounding District of Sudbury, ranging from latitude N46°–47° and longitude W80°–81°. This is a region of historically high acid and metal deposition from a variety of local and regional sources. The largest smelters were located right in the city and thus the gradient of greater industrial pollution near the city and less farther away was overlapped by a gradient of urban influence. A few lakes were never acidified but most were and are now in various stages of recovery (pH 5.22–7.58). In four lakes, experimental manipulation (i.e. liming) was undertaken to hasten chemical recovery. The lakes lie 3–100 km from the Sudbury smelters, and metal concentrations covered a wide range (Cu 0.3–31.0 μg L^{-1} Ni 1.5–224.0 μg L^{-1}). Most lakes are oligotrophic to mesotrophic [total phosphorus (TP) < 24 μg L^{-1}], with two lakes with TP < 30 μg L^{-1}. TP as well as ionic strength was higher in lakes closest to the smelters because of the increased urban influence. The lakes covered a wide range of surface area (mean = 279.6 ha, 11.9–1315.4 ha) and maximum depth (mean = 19.3 m, 5.7–39 m) (Table 1).

Table 1 Summary statistics of the physical and chemical characteristics of the study lakes collected in 2005 (n = 41) and 2006 (n = 5)

Variable	Abbreviation	Mean	Range
Aluminium (μg L^{-1})	Al	29.9	5.2–105.3
Calcium (mg L^{-1})	Ca	4.7	1.2–17.4
Dissolved organic carbon (mg L^{-1})	DOC	3.2	1.4–7.1
Conductivity (uS cm)	Cond	102.0	20–575.2
Copper (μg L^{-1})	Cu	7.6	0.36–31.7
Accumulation Potential	AP	3.3	0.09–52.9
Iron (μg L^{-1})	Fe	69.3	6.9–822.0
Manganese (μg L^{-1})	Mn	29.5	2.3–89.6
Nickel (μg L^{-1})	Ni	44.0	0.75–224.0
pH	pH	6.5	5.22–7.6
Total phosphorus (μg L^{-1})	TP	6.9	2.0–29.4
Sulphate (mg L^{-1})	SO_4	10.5	5.75–48.6
Zinc (μg L^{-1})	Zn	6.0	0.7–14.1
Secchi depth (m)	Secchi	5.4	1.25–11.2
Surface area (ha)	Area	280.0	11.9–1315.4
Maximum depth (m)	Depth	19.3	4.5–36.0
Distance from smelter (km)	Smelter	27.4	5–100.1

Collection of zooplankton and analysis of water chemistry

Sampling of zooplankton and water chemistry was carried out in July of 2005 (41 lakes) and 2006 (5 lakes). Zooplankton samples were collected at the deepest site on each lake using either a 12.5 cm diameter metered tow net with an 80-μm mesh size or a 30 cm diameter conical tow net with an 80-μm mesh. Each haul was taken from 1 m above the bottom to the surface. Samples were preserved in a 14% buffered formalin solution. The potential for selective sampling resulting from nets of different sizes was evaluated by Holt & Yan (2003); they found no differences between the sampling methodologies for either species richness or abundance of most zooplankton species.

Zooplankton were identified to species and measured using the software ZEBRA2, a MSDOS-based measuring program (Garry Allen Computing, Kingston, ON, Canada) that takes input from electronic calipers. Images of zooplankton were displayed using a video camera and a Leica MZ16 dissecting microscope.

Water for chemical analyses was collected by immersion of a plastic bottle below the surface or as a non-volume weighted composite sample collected from epi- and meta-limnion. The two different methods produce comparable results in these lakes (Keller & Pitblado, 1986; Keller *et al.*, 2006). All chemical analyses were performed at the Ontario Ministry of the Environment's Dorset Environmental Science Center in Dorset, Ontario (2005 samples) and Testmark Laboratories, Sudbury, Ontario (2006 samples). Both laboratories followed procedures outlined by the Ontario Ministry of the Environment and Energy (1995). Physical lake parameters include distance to smelter, surface area, maximum depth and Secchi depth. Distance to smelter (km) was measured between the lake centre and the Copper Cliff smelter (Vale Inco, Ltd.). Concentrations of metals and SO_4 in these lakes are strongly related to distance from the smelters (Keller & Pitblado, 1986), with lakes <30 km the most severely affected (Keller *et al.*, 1999), thus distance was used as a proxy for the severity of pollution. The rate and extent of recovery in these lakes have been shown to be related to the initial severity of pollution (Yan *et al.*, 1996).

Metal toxicity depends on metal availability, not simply total metal concentration, as metals can be complexed by a variety of natural chemical processes. The Biotic Ligand Model was used to quantify toxicity based on total dissolved concentration of copper in the water column and a range of chemical parameters including pH, DOC and ions (http://www.hydroqual.com/wr_blm.html). This index was developed to incorporate copper speciation as well as the protective effects of cations into predictions of metal toxicity (Di Toro *et al.*, 2001).

The model was used to derive a critical threshold, called the Final Acute Value (FAV), where accumulation of copper leads to acute toxicity in a hypothetical genus more sensitive than 95% of the tested genera (US EPA, 2007). The FAV represents the site (lake)-specific water quality criterion for copper. Accumulation Potential (AP) was calculated as the ratio of total dissolved copper in the water to the site-specific FAV, with AP values >1 indicating that copper concentrations exceeded this threshold. AP values were used as an additional chemical parameter in statistical analyses. In addition to copper, nickel is known to have serious detrimental effects on aquatic biota (Wong, 1992; Munzinger, 1994; Pyle, Swanson & Lehmkuhl, 2000), and nickel concentrations in these lakes were quite high. Unfortunately, no Biotic Ligand Model was available for nickel. Pyle *et al.* (2000) found that, like copper, nickel toxicity decreased in response to increasing pH, calcium and suspended solids, which may indicate that nickel responds similarly to copper in terms of accumulation and toxicity.

Fish sampling

Data on fish assemblages were available for 23 lakes from 2001 to 2004. Of the remaining lakes, nine were sampled for fish in 2005 and 14 in 2006. The fish in each lake were assessed using the Nordic multimesh gillnetting method, a standardised sampling method developed in Scandinavia and modified for use with North American species (Morgan & Snucins, 2005). The method uses a stratified, randomised, sampling design in which the sampling effort (number of nets) is adjusted to lake surface area and maximum depth.

The relative abundance of each fish species was calculated as mean catch-per-net and expressed as catch-per-unit-effort (CPUE). The relative abundance of lake trout and burbot was combined into a single metric (trout/burbot). Four fish indices (fish species richness, piscivore species richness, total CPUE, and planktivore CPUE) were used in addition to species data. Classification of piscivores and planktivores followed Masson, Pinel-Alloul & Dutilleul (2004). Fish species classified as piscivores included walleye (*Sander vitreus* Mitchill), smallmouth bass (*Micropterus dolomieui* Lacépède), largemouth bass (*Micropterus salmoides* Lacépède), northern pike (*Esox lucius* Linnaeus), lake trout (*Salvelinus namaycush* Walbaum) and burbot (*Lota lota* Linnaeus).

Statistical analyses

A variety of statistical approaches were used to determine the effects of physical and chemical lake characteristics, as well as fish community composition on zooplankton community structure. To satisfy the assumption of normality and homoscedasticity of the residuals, transformations were applied where appropriate. Zooplankton and fish abundance data were $\log_{10}$-transformed, except for fish species richness and piscivore richness, which were normally distributed. Physical and chemical lake parameters, except for pH and Secchi depth, were also log-transformed. Pearson correlation was used to examine the relationships among the zooplankton variables and the physical, chemical and biological lake characteristics.

Redundancy analysis (RDA) and partial redundancy analysis (pRDA) were used to test whether the zooplankton community was significantly related to the physicochemical variables and fish community composition, and how much variation was shared between the two data sets (pRDA). Species occurring in <5% of the lakes were removed from the analyses. To avoid multicollinearity, variables with a variance inflation factor >20 were removed from the analysis. Only 14 of the 17 potential physicochemical variables were included in the RDA after accounting for correlation among variables (Al, DOC, conductivity, AP, Cu, Fe, Mn, Ni, pH, TP, Secchi, area, depth, distance to smelter). Similarly, seven fish community variables were included in the analyses, including the CPUE of northern pike, walleye, smallmouth bass, yellow perch, cyprinids and lake trout/burbot, fish species richness and piscivore richness.

The variance partitioning method of Borcard, Legendre & Drapeau (1992) was followed. When two variable groups (in this case abiotic and biotic variables) are analysed using this method, one is treated as a set of covariates, while the other is treated as explanatory variables. This allows estimation of the percent variance explained by each of the variable groups. To reduce the effects of the difference in the number of variables in each of the two sets, we only used those variables which were significant ($P < 0.05$) based on a forward selection (Økland & Eilertsen, 1994). RDA was conducted with standardisation by sample (lake) norm which takes into account species proportions and not total abundance. Significance of the RDAs and pRDAs was assessed using Monte Carlo permutation tests based on 499 unrestricted permutations. For ordination analyses, we used CANOCO version 4.02 (ter Braak & Smilauer, 2002).

Results

Abiotic correlates of zooplankton composition

Thirty-two zooplankton species were identified from the 46 lakes with a maximum of 14 occurring in any one lake (mean species richness = 8.6). Changes in species composition were projected onto an ordination graph with the first axis constrained by lake distance from the Sudbury smelters (Fig. 1). Species composition was significantly related to distance from the nearest smelter ($P < 0.003$), which explained 10% of the variance. Cladoceran richness and the relative abundance of some *Daphnia* species, such as *Daphnia catawba* (Coker) and *Daphnia ambigua* (Scourfield), increased with distance from the smelting complexes. while the relative abundance of *Tropocyclops extensus* (Kiefer), *Bosmina freyi* (De Melo & Hebert) and *Diaphanosoma birgei* (Korinek) was greater near the smelters.

Total copper and nickel concentrations were highly correlated with distance from the smelters and decreased substantially in lakes farther than 25 km away (Fig. 2). Copper and nickel concentrations were often quite high, reaching 32 and 224 μg L^{-1}, respectively, with a total of 24 of the 46 lakes containing copper and/or nickel concentrations above current PWQO. The site-specific water quality criterion (FAV) was on average higher than the PWQO for copper

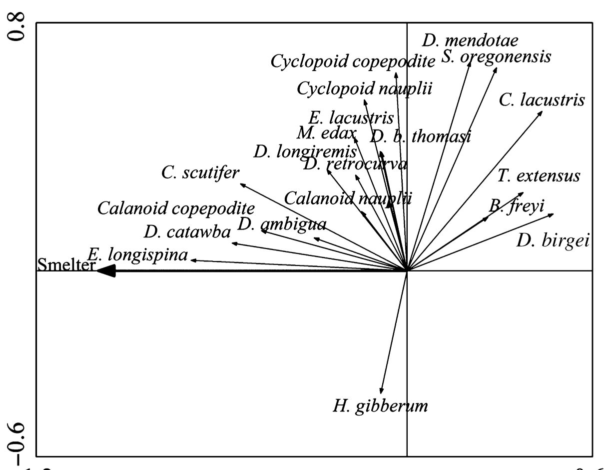

Fig. 1 Redundancy analysis biplot of the single explanatory variable, distance from smelter (Smelter), explaining 10% of the variance in zooplankton community composition.

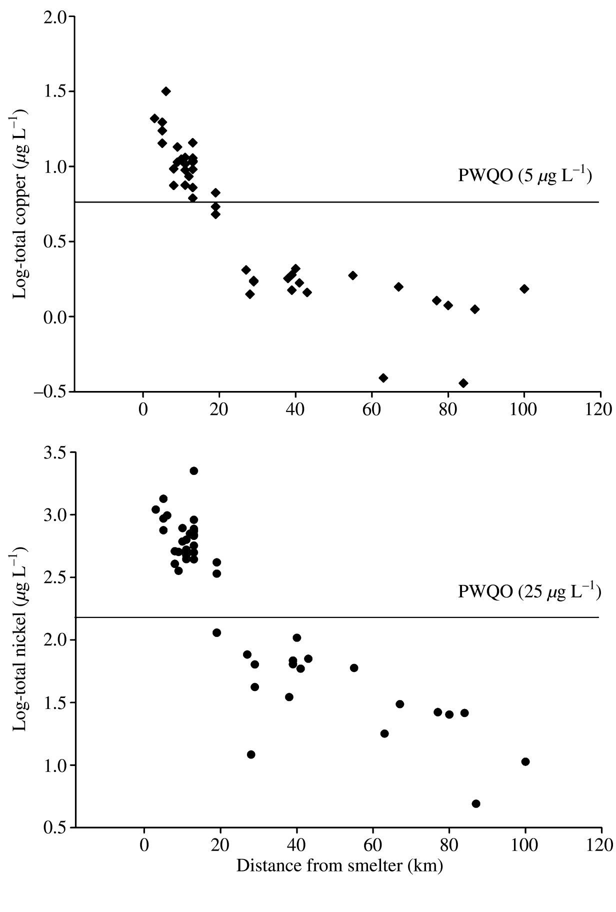

Fig. 2 Concentrations of total copper and total nickel (log10-transformed) in the study lakes as a function of distance from nearest smelter (km). Provincial Water Quality Objectives (PWQOs) for the protection of aquatic life for copper (5 μg L^{-1}) and nickel (25 μg L^{-1}) are indicated.

(mean = 10.42 μg L^{-1}, SD = 12.22), although the values varied considerably in response to the wide range of lake water chemistry. Final Acute Values ranged from 0.24 to 49.28 μg L^{-1}. The Biotic Ligand Model indicated that 20 lakes contained copper concentrations which reached or exceeded the FAV (lake-specific water quality criterion), indicating a potential for accumulation on the biotic ligand (AP >1, Fig. 3). There was no correlation between AP and either total copper or distance from smelter ($P > 0.05$).

An RDA was applied to examine the relationship between the physicochemical (abiotic) variables and zooplankton species composition (Fig. 4). The variables most strongly related to relative zooplankton abundance were pH, AP, conductivity, depth and aluminium concentrations. These five variables explained 36% of a possible 56.5% of the variance among lakes (Table 2). Higher pH lakes were characterised by a high relative abundance of *Skistodiaptomus oregonensis* (Lilljeborg) and *Daphnia mendotae* and a high abundance of cyclopoids (Fig. 4). Because of the confounding effect of urbanisation, lakes high in metal concentrations (Cu, Ni, Zn) were also often higher in conductivity and TP and were characterised by an increased abundance of *Diaphanosoma birgei*, *T. extensus* and *B. freyi*.

Accumulation Potential, which indicated the potential for copper bioaccumulation on the biotic ligand, displayed relationships with zooplankton species not seen with total copper concentrations. Whereas copper and nickel concentrations were not related to any measures of relative abundance ($P > 0.05$), high AP values were associated with a decrease in the relative abundance of cyclopoids and *Daphnia* spp. The relative abundance of *Holopedium gibberum*

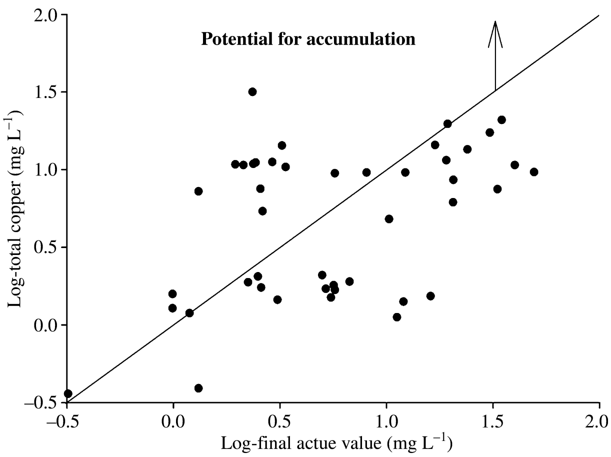

Fig. 3 Relationship between log10-total copper concentrations and the final acute value (FAV) in the 46 study lakes with a 1 : 1 line indicated. Lakes which fall below the 1 : 1 line (FAV < total copper concentrations) exceed the critical threshold of available copper.

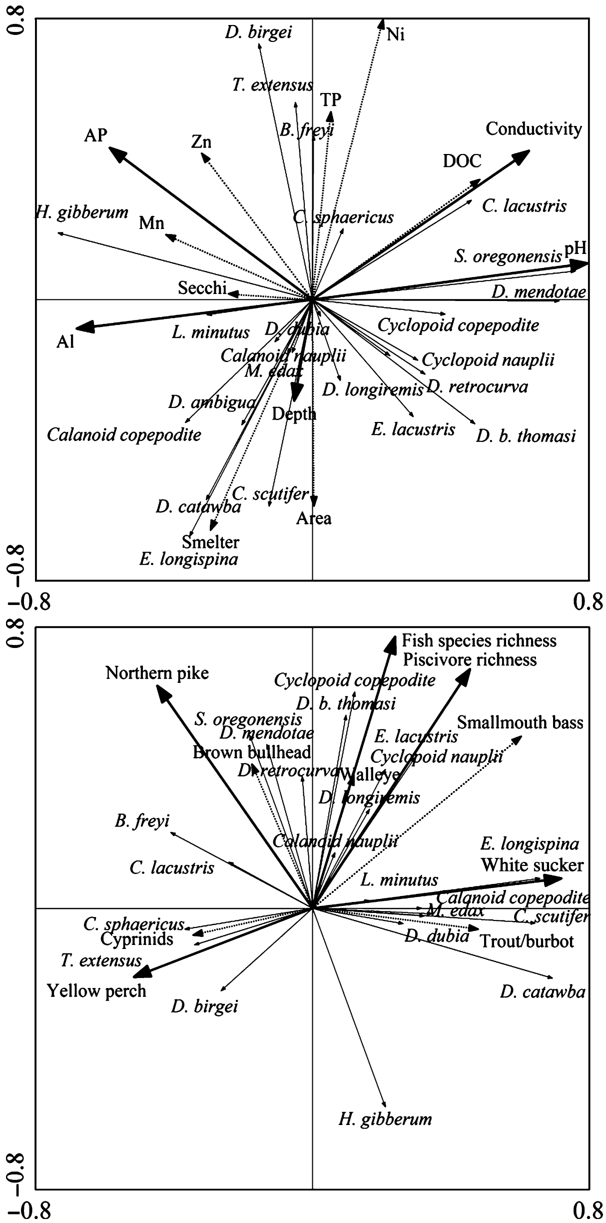

Fig. 4 Redundancy analysis biplot of fish community composition (upper panel) and physical and chemical variables (lower panel) in relation to zooplankton species relative abundance in the 46 study lakes. Variables found to be significant by forward selection are shown with bold arrows and are explained in Table 2.

Table 2 Summary of the redundancy analyses (RDA) and partial redundancy analyses (pRDA) on zooplankton community composition and size structure using both abiotic and biotic explanatory variables. See Table 1 for an explanation of the variable names

			% Variance explained	
Variables	Covariables	Significant variables	Significant variables	All variables
RDA				
Abiotic and biotic		pH, AC, DOC, fish richness, sucker CPUE, pike CPUE, perch CPUE, Al	50	65.8
Biotic		Fish richness, pike CPUE, sucker CPUE, perch CPUE, piscivore richness	26	38.5
Abiotic		pH, AP, Conductivity, DOC, Depth, Al	35	56.5
pRDA				
Biotic	Abiotic	Perch CPUE	5	15.8
Abiotic	Biotic	pH, AP, DOC, Fe	19	27.6

CPUE, catch-per-unit-effort.

increased with increasing AP (Fig. 4). *Holopedium gibberum* appeared to be the more metal tolerant than *Daphnia* spp. Of the 20 lakes with AP values >1, *Daphnia* was absent from 12, whereas *H. gibberum* was absent from only four. When present in these lakes, the *Daphnia* assemblage was primarily composed of *D. mendotae*.

Fish community composition and its potential impact on zooplankton recovery

A total of 35 fish species were caught in the 46 study lakes, with a maximum of 16 species coexisting in any one lake (mean fish species richness 6.6). Changes in fish community composition were related to the gradient of distance from the smelters. The abundance of yellow perch decreased ($r = -0.52$, $P < 0.003$) while that of piscivorous fish increased ($r = 0.37$, $P < 0.03$) with increasing distance. Yellow perch was the most common species, occurring in 80% of the lakes ($n = 37$), and in four lakes was the sole species collected. Most lakes ($n = 33$) had both piscivorous and planktivorous fish; however, 13 lakes had no piscivores, containing one or more of yellow perch, Iowa darter (*Etheostoma exile* Girard) and various cyprinids. The abundance of yellow perch was inversely correlated with the abundance of piscivores ($r = -0.45$, $P = 0.002$).

An RDA using biotic (fish species composition) variables explained a significant proportion (38.5%) of the variance in zooplankton species composition, albeit much lower than the variation explained by abiotic factors (Table 2, Fig. 4). Piscivorous fish species richness, the CPUE of pike, white sucker (*Catostomus commersoni* Lacépède) and yellow perch, and fish species richness were the most significant fish variables. The number of piscivorous fish, as well as overall fish species richness, displayed a positive relationship with many of the zooplankton species, including the relative abundance of most cyclopoid copepod and *Daphnia* species. In contrast, the relative abundance of *H. gibberum* showed a significant decrease with increasing piscivore and fish species richness.

Variance partitioning and the relative roles of abiotic and biotic processes

Although fish community composition and physicochemical lake variables were used as explanatory variables, these two data sets were not independent. Lake area, manganese, conductivity, total copper and AP explained 65% of the variance in fish community composition (Fig. 5). Lake area explained the largest amount of variance (23%) and was positively related to the abundance and richness of piscivores. The lakes closest to the smelting complexes (those high in Cu and Ni) were characterised by a high CUE of yellow perch and cyprinids. Of those high metal lakes, those with a high AP lacked in piscivores but had abundant yellow perch.

Variance partitioning analysis revealed that much of the variation explained by the abiotic and biotic

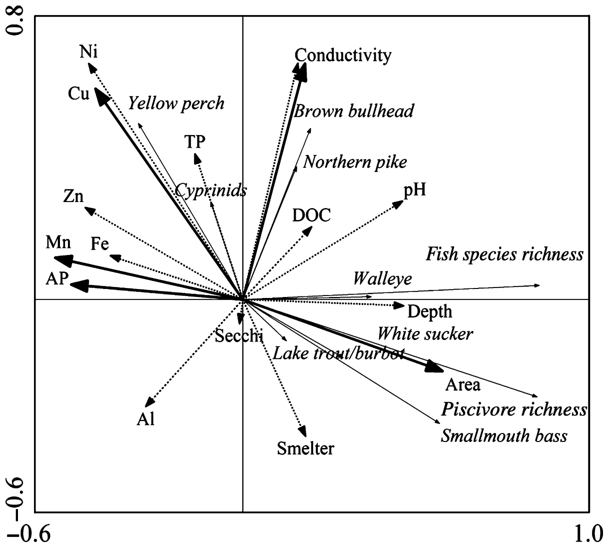

Fig. 5 Redundancy analysis biplot of 14 physical and chemical variables in relation to fish community composition in the 46 study lakes. Variables found to be significant by forward selection are shown with bold arrows.

variables was shared (22.4%), reflecting the strong role abiotic factors have in shaping the fish assemblage. Despite the interaction between these two groups of variables, fish species composition continued to explain a significant proportion of the variance in zooplankton species composition, after the variance attributed to the abiotic variables was removed (Table 2). Only one fish variable, the CPUE of yellow perch, was statistically significant and it alone explained 16.6% of the variance in zooplankton species abundance among lakes. Lakes with a high abundance of perch contained relatively more immature calanoids, *Leptodiatomus minutus* (Lilljeborg) and *H. gibberum* than lakes with few or no yellow perch, even after accounting for the higher metal concentrations often seen in these perch-dominated lakes.

Discussion

Distance from smelters is perhaps a simplistic measure of industrial damage, and the fact that it explained only 10% of the variation in zooplankton species composition indicates the patchiness in lake recovery caused by the exigencies of recolonization and other anthropogenic effects. Nevertheless, a strong spatial gradient was evident in many of the characteristics of the study lakes that was directly related to proximity to the Sudbury smelting complexes. Metal concentrations, both in the soil and water, were high near the smelters (Hutchinson & Whitby, 1977; Nriagu, Wong & Coker, 1982). Lakes located close to the smelters (<25 km) had higher concentrations of SO_4, copper and nickel and low abundance of piscivorous fish compared to lakes further away (>25 km). Many of the study lakes had been acidified and are currently recovering, with most of the lakes now above pH 6, an accepted threshold for zooplankton recovery (Holt, Yan & Somers, 2003).

The Biotic Ligand Model was used as a way to estimate the level of free cupric ion, which is considered the most toxic form of copper to aquatic life (Borgmann & Ralph, 1984). The FAV is the maximum allowable copper concentration for the protection of sensitive aquatic biota and was estimated from the Biotic Ligand Model. In laboratory tests, the FAV calculated under reference chemistry was 4.2 μg L^{-1} of copper (US EPA, 2007) (pH = 7.5, DOC = 0.5 mg L^{-1}, Ca = 14.0 mg L^{-1}, Mg = 12.1 mg L^{-1}, Na = 26.3 mg L^{-1}, K = 2.1 mg L^{-1}, SO_4 = 81.4 mg L^{-1}, Cl = 1.90 mg L^{-1}, and alkalinity = 65.0 mg L^{-1}). This is similar to the current PWQO of 5 μg L^{-1}. In this study, many of the lakes had FAVs that exceeded the PWQO, ranging from <1 μg L^{-1} in acidic lakes to over 20 μg L^{-1} in some of the more productive lakes. This indicates that PWQOs may overestimate toxicity in hardwater lakes with higher DOC while underestimating it in acidic, clear, softwater lakes.

AP values, estimated from the Biotic Ligand Model, displayed significant relationships with many aspects of zooplankton community composition that were not seen using total copper. This perhaps is not surprising as AP values incorporate total copper, pH, DOC, major anions and cations and alkalinity into a single metric. The relative abundance of cyclopoid copepods decreased significantly with increasing available copper, with *T. extensus* often the only cyclopoid found in lakes with high AP values. Among the cladocerans, the relative abundance of *Daphnia* decreased, while the relative abundance of *H. gibberum* was positively related to increasing AP values. Although copepods are generally considered less sensitive to metals than cladocerans (Kerrison *et al.*, 1988; Boeckman & Bidwell, 2006), this may not be consistent across all species, and the differential response of cyclopoid copepods and cladocerans warrants further examination.

A comparison of the relative abundance of *H. gibberum* and *Daphnia* to the physicochemical lake characteristics revealed that these two taxa responded very differently to the underlying environmental gradients. The abundance of *H. gibberum* increased with increasing concentrations of aluminium and decreased with increasing pH and conductivity, while *Daphnia* decreased significantly with increasing metal concentrations. The exception to the overall trend for *Daphnia* was the species *D. mendotae*. Both *D. mendotae* and *H. gibberum* tolerated high metal concentrations in lakes close to the smelters and were found in lakes with copper concentrations as high as 20 and 31 μg L^{-1}, respectively. *Holopedium gibberum* was found in the lakes with the highest AP.

Yan *et al.* (2004) attributed the success of *D. mendotae* over *H. gibberum* in Middle Lake to a greater susceptibility of the latter to either metals or fish predation. However, metal toxicity, at least for copper, appeared to be relatively low in Middle Lake because of its high pH and calcium levels. Moreover, *H. gibberum* was found in lakes with the highest available copper values compared to *D. mendotae*; this species occurred primarily in lakes with little or no available copper. The absence of *H. gibberum* from lakes with low available copper may reflect its inability to compete with *Daphnia* at the high pH and calcium concentrations that characterise lakes with low available copper (Hessen, Faafeng & Andersen, 1995).

The importance of biotic factors in influencing rates of biological recovery has received substantial attention in recent years (Keller *et al.*, 2002; Binks *et al.*, 2005; Monteith *et al.*, 2005). Although it is well known that community composition is determined by both the physical and chemical properties of the water body and the ecological interaction among the different organisms, most earlier work seeking to explain patterns in zooplankton distribution has focused primarily on abiotic factors (Keller & Pitblado, 1984; Shaw & Kelso, 1992; Siegfried & Sutherland, 1992). Studies of recovery have largely continued this bias. Despite this emphasis on physical and chemical properties, Menge & Sutherland (1987) predicted that biotic processes should dominate the regulation of recovering communities. Some studies have failed to find evidence of a strong influence of biotic interactions on recovery rates for stream fish communities (Detenbeck *et al.*, 1992), periphyton (Vinebrooke, 1996) and zooplankton (Lukaszewski, Arnott & Frost, 1999). Work in both Swan and Sans Chambre Lake (Sudbury, Ontario) has, however, provided evidence for the primacy of ecological limitations to recovery of some zooplankton species. Recovery in these lakes appears to be limited by food web alterations caused by an absence of fish and an abundance of macroinvertebrate predators (Yan *et al.*, 1991; Keller & Yan, 1998; Linley, 2008).

In Sudbury's lakes, acidification eliminated the large piscivores, smallmouth bass, walleye and lake trout (Beamish, 1976). In contrast to the piscivores, yellow perch is quite tolerant to both acidification (Rahel, 1984) and metal contamination (Taylor, Wood & McDonald, 2003). In highly contaminated lakes where even perch disappeared, they returned as the first fish colonists during lake recovery. Yellow perch was the most common fish species in our study, occurring in 80% of the lakes and reaching very high densities in lakes lacking piscivores. Predation by planktivores such as yellow perch may play a key role in structuring the zooplankton community, especially with respect to size structure, since fish prey selection is typically size dependent. In lakes without piscivores, yellow perch biomass is higher and perch shift from zooplankton to benthic invertebrates at a larger size (Lippert, Gunn & Morgan, 2007). Both these factors may increase the predation potential by yellow perch on the zooplankton community.

Several authors have reported significant changes in the zooplankton community following a change in the abundance of planktivorous fish (Mazumder *et al.*, 1990; Vanni *et al.*, 1990; Sarvala *et al.*, 1998). In our study, the CPUE of piscivorous fish, which may be considered a proxy for predation pressure on zooplankton from planktivores, was positively related to the relative abundance of *Daphnia* spp. and cyclopoids. Similar patterns were seen when piscivore richness was used, indicating that the presence of piscivorous fish is enough to effect a change in the zooplankton community. Whether this shift in zooplankton composition truly reflects reduced foraging of planktivorous fish on zooplankton or is the result of improved water quality in these lakes is difficult to unravel.

Increased planktivory may explain the significant negative association between the abundance of *H. gibberum* and *Daphnia*. This observation has been noted previously in field studies by Allan (1973), Yan *et al.*

(1988) and Hessen *et al.* (1995). In this study, *H. gibberum* abundance usually surpassed *Daphnia* abundance only when piscivores were absent and presumably planktivory was high. Yan *et al.* (1988) hypothesised this effect – that *Daphnia* would outcompete *H. gibberum* except when under intensive fish planktivory. We found that *H. gibberum* was often the largest species in lakes without piscivores, which were primarily characterised by relatively small species (*T. extensus*, *Chydorus sphaericus* O.F. Müller and *Diaphanosoma birgei*).

Overall, fish species composition was able to explain a significant proportion of the variation in the zooplankton. Recent work has shown that the fish assemblage can explain as much or more of the variation in the zooplankton among lakes as can water chemistry (Amsinck, Jeppesen & Landkildehus, 2005; Finlay *et al*., 2007). Much of the explanatory power of the fish variables was a result of the relationship between fish species composition and water quality and lake depth. The relationship between lake area and fish species richness was a confounding factor in this study, as larger lakes generally contained a greater diversity of fish (e.g. Eadie *et al*., 1986), and these large lakes were also located further from the smelters. However, after accounting for the variation in the physical and chemical lake properties, the abundance of yellow perch explained the dominance of *H. gibberum* and immature calanoids seen in some lakes.

The Sudbury area has enjoyed a substantial degree of recovery in comparison with other acidified areas, thus an understanding of the recovery process in these lakes may serve as a model for other similarly affected regions. Because both abiotic and biotic factors affect abundance and species composition, it is very difficult to isolate the mechanisms that determine zooplankton community composition in recovering lakes. Continuing metal toxicity appears to be the primary cause of the absence of many *Daphnia* species, other than *D. mendotae*, and also many cyclopoid species from the heavily contaminated lakes close to the smelters. AP estimated by the Biotic Ligand Model was better able to explain the differences in zooplankton community composition in these lakes and may be more useful in estimating toxicity than total metal concentrations. We did not find evidence for a greater sensitivity to metals in the Cladoceran community in comparison with copepods as seen by Yan *et al.* (2004). Our study suggests that the introduction of piscivores into the recovering lakes may be necessary to facilitate the return of *D. mendotae*, although, reduction in metal levels and metal toxicity will still be important in allowing the complete return of all components of the zooplankton community.

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