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Recovery in a multiple stressor environment: using the reference condition approach to examine zooplankton community change along opposing gradients

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Lakes around the metal smelters of Sudbury, Ontario, Canada offer a unique opportunity to study recovery processes in stressed aquatic ecosystems. Following major reductions in the atmospheric deposition of sulphur and metal particulates, chemical and biological recovery have been observed in many lakes; however, the magnitudes and trajectories of biological recovery are variable. Crustacean zooplankton communities have proven to be particularly valuable in tracking the biological recovery patterns in Sudbury lakes. In a survey of 87 lakes, zooplankton community structure revealed strong gradients related to acidification, metal contamination, trophic status and depth. The study lakes could be divided into four groups related to these gradients: acidified, recovered, urban and reference lakes. Community composition in recovered (to pH $>$6.0) lakes did not differ from reference lakes for copepods and cladocerans. In contrast, urban lakes subjected to the effects of watershed development as well as atmospheric metal contamination had either copepod or cladoceran communities that differed from reference conditions. Among the acidified lakes, most lakes with pH $<$5.5 contained species poor copepod communities; however, substantial community recovery was evident in lakes with pH $>$5.5. Our study illustrates the complexity of biological recovery processes in lakes subjected to multiple environmental stressors. Although substantial zooplankton community recovery clearly occurs as lakes recover from acidification, other stressors including metal contamination and eutrophication in urban environments may greatly affect recovery processes and outcomes.

KEYWORDS: recovery; test site analysis; zooplankton; acidification

INTRODUCTION

The widespread acidification of lakes throughout acid sensitive regions in Europe, the northeastern USA and southeastern Canada has resulted in dramatic losses of aquatic biota, including fish, benthic invertebrates and zooplankton (see Havas and Rosseland, 1995 for a review). Acidification in the Sudbury, Ontario, region

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began in the 1920s and quickly accelerated throughout the 1960s when the Sudbury metal smelters were one of the world's largest point sources of SO_2. By the 1970s, many of the lakes were severely acidified. With the implementation of large reductions in SO_2 and metal emissions, water quality has improved (Keller, 1992). Numerous studies have documented both the effects of acidification (e.g. Roff and Kwiatkowski, 1977; Keller and Pitblado, 1984) and the subsequent chemical recovery on aquatic communities (e.g. Keller *et al.* 1992a). Zooplankton communities in particular have been shown to be excellent indicators of acidification, yielding predictable and consistent responses to changes in lake pH and other chemical parameters affected by acid deposition (e.g. Webster *et al.*, 1992; Keller *et al.*, 2002; Walseng *et al.*, 2003).

Evidence of zooplankton recovery in the Sudbury area following emission reductions has been mounting (Keller *et al.*, 1992b; Havas *et al.*, 1995; Keller *et al.*, 2002). In this region, a pH of 6 has been used as a threshold at which significant biological damage is known to occur (Neary *et al.*, 1990). In a synthesis of studies of zooplankton recovery from lakes across Eastern North America and Central, and Northern Europe, Gray and Arnott (Gray and Arnott, 2009) found that zooplankton communities in all regions appear to be at least partially recovering in lakes with pH values above 6.0. While increases in pH have led to an overall recovery of zooplankton across a broad geographic scale, there exists a patchwork of partially recovered zooplankton communities in lakes across the landscape (see Keller *et al.*, 2007 for a review). This variability may be due to physiological effects of residual metal toxicity, physical effects resulting from reductions in dissolved organic carbon (DOC) concentrations and resultant changes in UV penetration, and ecological effects of altered predator communities. All of these multiple stressors interact with the spatial heterogeneity of the landscape and with chance events to create uneven recovery (Diamond, 1975; Keller and Yan, 1998; Yan *et al.*, 2003; Young *et al.*, 2005). It is clear that habitat restoration through chemical recovery of lakes may not be enough to ensure rapid and full ecological recovery.

Yan *et al.* (Yan *et al.*, 2004) observed a surprising pattern in the recovery of the zooplankton community in Middle Lake, which was severely contaminated by acid and metal emissions from the Sudbury smelters. They found that the cladoceran community, unlike the copepod community, had yet to recover, despite 30 years of non-acidic conditions. This delayed recovery of cladocerans was unexpected due to the quick generation time and production of hardy, desiccation-resistant resting eggs of many species. Differences in recovery rates between taxonomic groups have been shown in manipulative experiments (Frost *et al.*, 2006), but this aspect of recovery has yet to be tested at a regional scale.

Our study contributes to the growing body of work examining zooplankton recovery in response to increases in pH (e.g. Locke *et al.*, 1994; Yan *et al.*, 1996, 2004; Holt *et al.*, 2003; Frost *et al.* 2006). In contrast to most past studies in the Sudbury area that focused on one or a few lakes, we used a broad, synoptic survey of 87 impacted and reference lakes to determine the relative importance of a suite of different environmental parameters in affecting the species composition of zooplankton. Our first objective was to determine whether zooplankton communities had recovered to a state typical of neutral lakes once pH rose above 6. We followed the recommendations of Yan *et al.* (Yan *et al.*, 1996) who found that multivariate measures of species composition provided the best indicator of recovery of zooplankton from acidification. Multivariate indicators of recovery were calculated separately for the cladoceran and copepod community.

Our second objective was, if delays in recovery could be identified, to determine whether the pattern of delayed cladoceran versus copepod recovery observed by Yan *et al.* (Yan *et al.*, 2004) was a widespread phenomenon in the Sudbury area. We hypothesized that the cladoceran community would recover once lake pH had risen above 6; however, recovery would be delayed despite reaching this threshold if metal levels were sufficiently high. To achieve these objectives, we used indirect and direct gradient analyses and test site analysis (TSA) to analyze survey data on zooplankton abundance and abiotic variables collected from 87 lakes. TSA is a bio-assessment approach commonly used to assess damage and recovery in benthic invertebrate communities (Bowman and Somers, 2006) and allows for a direct assessment of the biological condition of each lake in comparison to an established reference condition.

METHOD

Study lakes

The 87 lakes chosen for study spanned a gradient of acidification (pH 4.7−7.6) and metal contamination (Cu 0.4−31.7 $\mu g\ L^{-1}$ and Ni 0.75−134 $\mu g\ L^{-1}$). The lakes are located on the Canadian Shield; however, they varied considerably in chemistry, morphometry and basin geology (Table I). The lakes varied slightly in elevation (182−486 m ASL) but differed considerably in

Table I: Average values (and standard errors) for the 18 physicochemical variables in each of the four lake groups and their proximity to the smelting complexes (>15 km or <15 km)

Variable	Abbreviation (units)	>15 km			<15 km
		Acid	Recovered	Reference	Urban
Alkalinity	Alk (mg L^{-1})	**−0.1** (0.20)	**0.9** (0.1)	4.5 (0.5)	10.6 (2.3)
Aluminum	Al (μg L^{-1})	**122.1** (21.1)	**36.5** (4)	18.3 (2.3)	20.5 (3)
Dissolved inorganic carbon	DIC (mg L^{-1})	0.3 (0.1)	0.4 (0.03)	1.2 (0.1)	3.0 (0.6)
Dissolved organic carbon	DOC (mg L^{-1})	**1.7** (0.4)	3.3 (0.2)	3.7 (3.7)	3.6 (0.2)
Conductivity	Cond (uS cm)	**22.1** (0.8)	**23.3** (0.8)	30.1 (1.6)	**159.3** (29.7)
Copper	Cu (μg L^{-1})	1.7 (0.30)	1.8 (0.3)	1.6 (0.2)	**11.8** (1)
Iron	Fe (μg L^{-1})	42.6 (12.7)	37.2 (5.3)	36.7 (11.4)	**118.4** (38.7)
Manganese	Mn (μg L^{-1})	**74.4** (8.6)	**27.0** (4.4)	8.1 (0.7)	**36.3** (10.7)
Nickel	Ni (μg L^{-1})	**7.2** (1.7)	5.9 (1.8)	2.4 (0.6)	**62.8** (4.7)
Total nitrogen	TKN (μg L^{-1})	**147.1** (14.8)	221.4 (13.9)	230.9 (15.6)	277.7 (20.2)
pH	pH	**5.3** (0.1)	**6.3** (0.04)	6.9 (0.04)	6.8 (0.1)
Total phosphorus	TP (μg L^{-1})	2.9 (0.4)	4.5 (0.4)	5.1 (0.4)	**10.7** (1.3)
Sulfate	SO_4 (mg L^{-1})	7.1 (0.2)	6.6 (0.3)	6.4 (0.3)	**11.5** (0.9)
Zinc	Zinc (μg L^{-1})	**6.9** (1.0)	4.4 (0.9)	2.8 (0.8)	**6.9** (0.9)
Secchi depth	Secchi (m)	**10.9** (1.2)	5.3 (0.4)	5.2 (0.4)	4.2 (0.3)
Distance from smelter	Smelter (km)	55.4 (4.5)	48.2 (6.6)	55.9 (6.8)	**9.9** (0.6)
Area	Area (ha)	286.3 (58.8)	300.5 (85.3)	236.1 (34.3)	**123.9** (41.2)
Maximum depth	Depth (m)	24.1 (3.9)	21.3 (3.3)	18.2 (2.0)	13.3 (1.4)

Means significantly different from the reference lake group are indicated in bold (ANOVA, $P < 0.002$).

size (8.1−1315.4 ha) and maximum depth (8.0−90.3 m). They were located between 5 and 120 km from the Sudbury smelters.

The 87 lakes were divided into four non-overlapping groups with regard to stress from atmospheric contaminant deposition. Three of the groups were located outside the major metal deposition area. The reference group contained lakes which never acidified to a pH less than 6 ($n = 25$) and, therefore, represent normal conditions for this area (chemically and biologically) under minimal disturbance. The acid lake group included lakes that exhibited slow rates of chemical recovery and remained below pH 6 ($n = 23$). The recovered lake group included lakes which were once acidified but had returned to pH values of 6 or above ($n = 17$) by the time of our study. The last group, the urban lake group, contained lakes located inside the high metal deposition zone ($n = 22$). These lakes were above pH 6 but, due to their proximity to the smelters (<15 km), they have copper and nickel levels which exceeded Ontario's Provincial Water Quality Objectives (PWQOs) (5 μg L^{-1} for copper and 25 μg L^{-1} for nickel) (MOEE 1994). Additionally, due to their position within the heart of Sudbury, they were exposed to multiple urban stresses not found in the other lakes (e.g. nutrient inputs, road salt runoff, shoreline development).

These four lake groups were used to create two gradients, one characterizing acidification and the other metal contamination. The lakes located outside the metal deposition zone (acid lake and recovered lake groups) were compared with the reference lake group to determine patterns in recovery from acidification. The lakes located inside the metal deposition zone (urban lake group) were compared to the reference lake group to examine patterns in recovery from metal contamination. Characterization of past acidification was based on examination of long-term data on water chemistry (Keller *et al.*, 2006).

Data collection

Environmental variables

To reduce confounding effects of seasonal changes in composition, sampling took place in 2005 during the relatively stable midsummer period (28 June to 4 August), with the order of lakes sampled at random so as not to confound sampling time and lake group. Fourteen chemical parameters were measured in addition to four physical lake descriptors: Secchi depth, distance from nearest smelter, lake area and maximum depth (Table I). Morphometric variables were obtained from various published and unpublished sources (Keller *et al.*, 2006).

Lake water was sampled with two different methods because of different monitoring needs. Sixty-five lakes were sampled with "surface grabs" by immersing a 4 L polyethylene jug at the deep station. The other 22 lakes (urban lake group) were sampled with an integrated plastic tube sampler that was deployed through the epi- and metalimnion. A comparison of the two sampling methods for 43 lakes did not reveal any statistically significant differences in the levels of pH, SO_4, Cu and Ni (Keller and Pitblado, 1986). A more recent comparison of 15 lakes revealed that 8 out of the 22 chemistry variables showed significant differences between surface and tube composite samples (Keller *et al.*, 2006). In light of the Keller *et al.* (Keller *et al.*, 2006) study, the variables aluminum, iron, manganese and zinc were expected to be slightly higher in our tube composite samples (urban lake group) in comparison to surface grab samples. However, the absolute differences would be very small and were not likely to be biologically meaningful.

Fish community assessments had been done on a number of the lakes between 2000 and 2006 and this information was used to aid in the interpretation of the results. A detailed description of these methods can be found in Valois *et al.* (Valois *et al.*, 2010). These assessments were primarily done on the urban lakes ($n = 22$); however, some of the acid ($n = 4$), recovered ($n = 9$) and reference ($n = 3$) lakes had fish data as well.

Zooplankton composition

Zooplankton were sampled with a single vertical haul from 1 m above the bottom to the lake surface. Either a metered 12.5 cm diameter net with an 80 μm mesh (65 lakes) or a non-metered, 30 cm diameter tow net with an 80 μm mesh (22 lakes—urban lake group) was used. Holt and Yan (Holt and Yan, 2003) investigated the effect of net size on zooplankton community descriptions (12.5 versus 30 cm diameter), and found no significant differences in species richness or abundances between the samples from the two nets, except for the abundance of *Holopedium gibberum*, which was significantly higher in the larger net. The variance in abundances of *H. gibberum* between lakes was significantly lower than for the other species, suggesting that the variance associated with net size was not necessarily greater for this species. Nevertheless, we interpret any difference we find in this species in samples from the urban lake group cautiously.

There has been some debate surrounding the accuracy of using a single haul as representative of the zooplankton community of an entire lake. Single samples have been shown to detect 50% of the annual species pool, missing highly variable species that are present infrequently throughout the sampling period (Arnott *et al.*, 1998, 1999). Single samples are useful for determining relationships with important drivers of zooplankton composition, such as productivity, metal toxicity and predator density (Arnott *et al.*, 1998). However, our sampling lacked the sensitivity to detect early patterns in recovery including the immigration of new species (which initially remain at low abundances) as well as failed colonization attempts (Yan *et al.*, 2004).

Crustacean zooplankton were identified according to Smith and Fernando (Smith and Fernando, 1978) and Balcer *et al.* (Balcer *et al.*, 1984). Immature stages of Copepoda were identified to suborder. A Folsom plankton splitter was used to generate subsamples which were processed using ZEBRA2, an MSDOS-based measuring program developed by the MOE Dorset Environmental Science Center (DESC). The ZEBRA2 program was run on a computer linked to electronic calipers, a Leica MZ16 dissecting microscope and a video camera. Abundance estimates were corrected for net efficiency when possible; net efficiency averaged 83%.

Data analyses

Environmental variables were $\log_{10}$ transformed to satisfy the assumptions of normality and equal variance. Species data were $\log_{10}(x + 1)$ transformed. This reduces the influence of the most dominant species and takes into account the less abundant species as well. Very rare species, found in <5% of samples, were removed from the analyses. Statistical analyses were completed using CANOCO for Windows version 4.5 (ter Braak and Smilauer, 2002) with a significance level of $\alpha = 0.05$.

Zooplankton structure and lake groups

Before applying ordination methods, principal components analysis (PCA) and redundancy analysis (RDA), the zooplankton data were checked for suitability. A preliminary detrended correspondence analysis indicated that the zooplankton data set exhibited relatively short gradients, less than two standard deviations, implying that RDA, which assumes a linear response of species to underlying gradients, was suitable for analyzing the data alone (ter Braak, 1995). We used PCA to search for overall patterns in both the environmental data and the zooplankton assemblages. A PCA on a correlation matrix with centering and standardization of the data was performed to detect the major environmental and biological gradients in the 87 lake data set.

Identifying delays in recovery

A common definition of recovery is a change in species composition to one typical of non-acidified or reference lakes (Holt and Yan, 2003). As no pre-stress data exist for lakes in this area, we used a reference condition approach in which non-acidified lakes were used to determine what communities would be expected in this area in the absence of acidification. The reference lakes were selected to contrast with the impacted lakes in terms of pH and metal concentrations; however, they also differed in other variables indirectly related to acidification (e.g. Al, DOC, conductivity, TKN and water clarity). Urban lakes were more productive and smaller than the reference lakes and so differences in zooplankton communities between these lake groups may also reflect inherent differences in productivity and size. We examined whether any of the impacted lakes were significantly outside of the normal range (reference condition) for selected metrics. We used PCA to calculate our multivariate metrics of recovery.

To determine whether the lakes with pH above 6 (recovered and urban groups) had similar community composition to the reference lakes, each lake was compared to the reference group using TSA. The TSA method has been used previously to evaluate the recovery of stream and lake benthic invertebrate communities from acidification (Bowman *et al.*, 2006; Szkokan-Emilson *et al.*, 2009) as well as impacts of mining activity on sediment quality, benthic algae, benthic invertebrates and fish communities, and slimy sculpin (*Cottus cognatus*) condition (Bowman *et al.*, 2010). A TSA uses a generalized distance (D^2) to quantify the difference between a potentially impacted site (test site) and a reference site (Bowman and Somers, 2006). Kilgour *et al.* (Kilgour *et al.*, 1998) proposed that the difference between the mean of the test site and the reference sites should be significantly greater than the normal range of variation among the reference sites. This normal range is defined as the confidence region enclosing 95% of the reference sites. A non-central interval test is used to generate a probability that each metric, in this case the position defined by PCA1 and PCA2, is inside the normal range. The non-central P-value measures if the difference between the test and reference lakes is significant, categorizing each lake as significantly impaired ($P < 0.05$), potentially impaired ($0.05 < P < 0.95$) or in reference condition ($P > 0.95$). Using the first two axes of the ordination, two TSAs were run, one for cladoceran species and the other for copepods. TSAs were performed on cladocerans and copepods separately to determine if one group recovered before the other. TSA calculations were performed using Microsoft Excel 2003 (Microsoft Corp.). Four urban lakes were sampled twice; Clearwater, Crooked, Silver, and Tilton Lakes. Analyses were re-run with these samples to determine whether results could be attributed to sampling error. The results of the TSA did not change.

Physicochemical gradients

RDA was used to relate cladoceran and copepod community structure to the underlying environmental gradients in order to detect differences among these two groups in terms of their response to acidification and metal contamination. Statistical tests of significance were carried out by Monte Carlo permutation tests with 499 unrestricted permutations. In the Monte Carlo test, the distribution of the test statistics under the null hypothesis is generated by random permutations of cases in the environmental data (Gittens, 1985).

RESULTS

Among the 87 study lakes, a total of 36 crustacean zooplankton species were collected, comprised of 24 cladocerans and 12 copepods. Seven species were found in more than half of the lakes and included the cladocerans *Bosmina freyi*, *H. gibberum*, *Eubosmina longispina*, *Diaphanosoma birgei* and *Daphnia mendotae* as well as the copepods, *Leptodiaptomus minutus* and *Mesocyclops edax*. In all samples, immature copepods dominated by numbers forming up to 90% of the zooplankton community in some lakes. Total zooplankton abundance ranged from 4184 to 124 112 individuals m^3. The relative occurrence of each species in each of the lake groups is summarized in Table II.

Table II: Summary of the average relative occurrence (%) of common zooplankton (those found in >10% of samples) in the four lake groups (acid, recovered, reference, urban)

	>15 km			<15 km
Species	Acid	Recovered	Reference	Urban
Cladocerans				
Bosmina freyi	78	100	96	93
Ceriodaphnia lacustris	0	6	4	33
Chydorus sphaericus	17	6	16	9
Daphnia ambigua	13	35	12	0
Daphnia catawba	57	41	24	0
Daphnia dubia	0	0	36	4
Daphnia longiremis	0	12	40	3
Daphnia mendotae	13	35	84	57
Daphnia retrocurva	17	18	44	13
Daphnia birgei	35	47	80	83
Eubosmina longispina	83	82	68	3
Holopedium gibberum	83	100	84	53
Copepods				
Cyclops scutifer	35	53	44	13
Diacyclops biscuspidatus thomasi	17	59	84	40
Epichura lacustris	9	47	64	20
Leptodiaptomus minutus	100	100	92	93
Mesocyclops edax	65	76	92	60
Skistodiaptomus oregonensis	4	18	44	43
Tropocyclops extensus	13	53	44	67

In general, the relationships between zooplankton species and lake groups were well characterized by the first two axes of PCA plots for both cladocerans and copepods. The first two axes of a PCA of cladoceran species abundance explained 41.7% of the variation among lakes (Fig. 1) and there were substantial differences in the loadings of the various species on the first two axes. The first axis primarily separated many of the urban lakes, which contained high abundances of *D. mendotae*, *Diaphanosoma*, *B. freyi* and *Cerodaphnia lacustris* from all other lakes. In contrast to the recovered lake group, the acid lakes were characterized by higher abundances of *E. longispina*, *H. gibberum*, *D. ambigua* and *D. catawba*. Many of the reference lakes loaded positively on the second axis. These lakes were characterized by *D. longiremis*, *D. dubia* and *D. retrocurva*. Some of the most severely acidic (pH <5.3) lakes and the urban lakes loaded negatively on this axis with a noticeable absence of many *Daphnia* species.

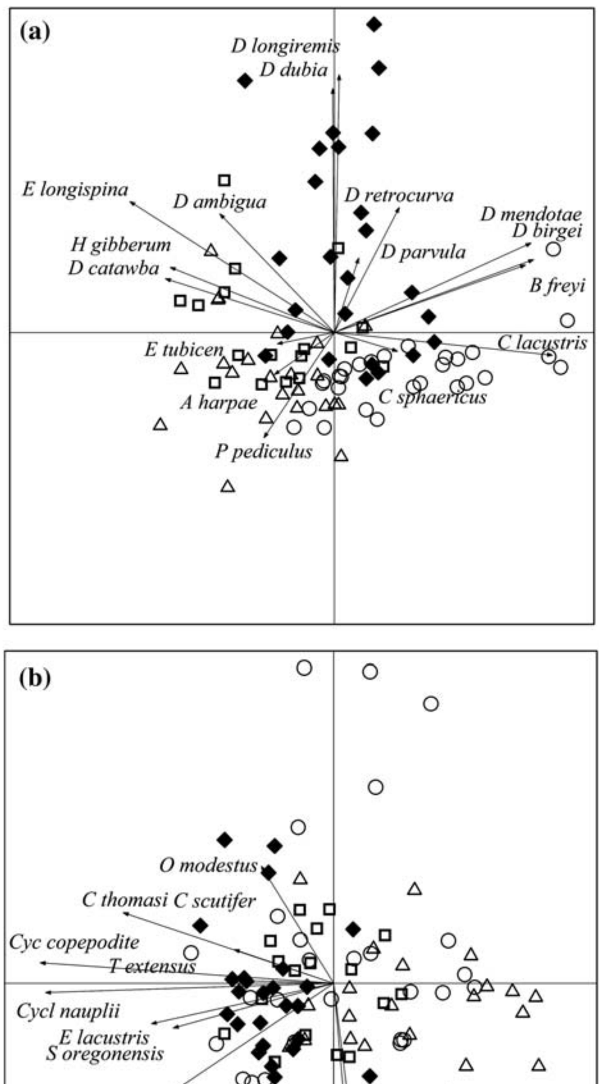

Fig. 1. Principal component analysis biplot on (**a**) cladoceran zooplankton and (**b**) copepod zooplankton in the 87 study lakes. Lake groups are indicated by the following symbols: acid lakes (triangle), recovered lakes (square), reference lakes (diamond) and urban lakes (circle).

The first two axes of a PCA of copepod species abundance explained 48.8% of the variation among lakes (Fig. 1). The first component, explaining 34.3% of the variance, was characterized by negative loadings of *Epischura lacustris*, *Skistodiaptomus oregonensis, Diacyclops bicuspidatus thomasi* and immature cyclopoids. Many of the acid lakes loaded positively on this axis and were characterized by low abundances or absences of most copepods except *L. minutus*. The second component, explaining 14.5% of the variance, was characterized by negative loadings of *L. minutus*, *M. edax* and immature calanoids. The most common copepods in the reference lake group included *M. edax* and *D. b. thomasi*. The recovering lake group, while similar to the reference group, had a decreased occurrence of *D. b. thomasi*. The urban lakes were characterized by higher abundances of *S. oregonensis* and a noticeable decrease in *Cyclops scutifer.*

Test site analysis

To illustrate the difference in PCA lake scores between each lake group and the reference lakes, the first two axes were plotted, and the 95% confidence ellipses were used to show the variability within each group (Fig. 2). The reference lakes were much more variable in their cladoceran communities in comparison to their copepod communities, primarily along axis two. The reference lakes varied in their daphniid communities, with most lakes containing a number of species especially *D. longiremis* and *D. dubia*, while some were primarily dominated by *D. mendotae*. Using the axis scores and the TSA method, we found that the majority of recovered lakes were not impaired. Two lakes, Annie and White Oak Lakes, were found to have significantly impaired copepod communities, lacking most cyclopoid copepod species.

Surprisingly, the cladoceran community of the acid lake group was not significantly impaired with more than half of the acid lakes in reference condition (some lakes were found to be potentially impaired). In 13 of

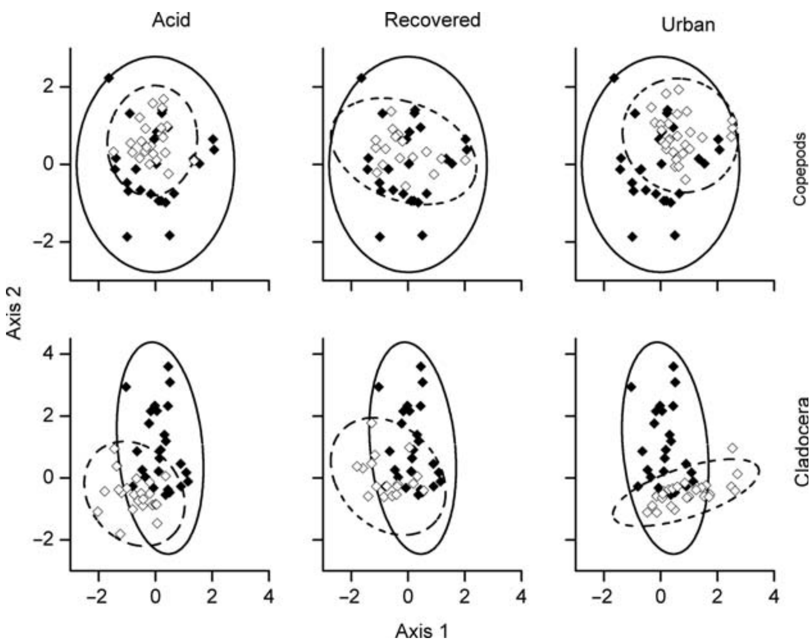

Fig. 2. Biplot of axis scores (PCA1 and PCA2) for the cladoceran and copepod communities in the reference lakes (closed symbols) relative to the test lakes (open symbols). Ellipses represent 95% confidence regions for the data values for each lake group (acid, recovered, urban—dotted lines) compared to the reference lake group (solid lines).

the acid lakes, copepod community composition was significantly impaired, with many lakes lacking *T. extensus*, *D. b. thomasi*, *S. oregonensis* and *Epischura lacustris* (Table II). The difference between the acid lakes and the reference lakes increased with decreasing pH (Fig. 3), with most lakes with a pH <5.5 containing impaired copepod communities. Unlike the recovered lakes, TSA analysis revealed that most urban lakes differed significantly from reference, and either cladoceran or copepod communities differed. Only one lake, Johnny Lake, a fishless lake, was found to be significantly impaired for both copepods and cladocerans. Copepod communities in these lakes were highly variable (Fig. 2). Urban lakes with impaired copepod communities were characterized by low pH and low DOC levels in addition to elevated metal concentrations. These lakes contained few *Daphnia* species other than *D. mendotae* and lacked (or contained low abundances) of *H. gibberum* and characterized by elevated pH and conductivity. There was no relationship between increasing metal concentrations (copper is shown) and distance from reference condition for the cladoceran community ($r = -0.07$, $P > 0.05$), although distance from reference condition appeared to increase for copepods ($r = 0.35$, $P = 0.07$) (Fig. 4). The four lakes with additional samples; Clearwater, Crooked, Silver, and Tilton Lakes had similar results when the TSA was re-run, with significant impairment found in the copepod but not the cladoceran community.

Zooplankton responses to environmental gradients

All 18 physical and chemical variables had significant marginal effects with the abundance of cladocerans. Forward selection indicated that five variables were significant; alkalinity, copper, manganese, conductivity and aluminum, explaining 29% of the variation (Table III). The most important variable was alkalinity, which alone explained 15% of the variation. An RDA of copepod species indicated that 16 variables exhibited significant marginal effects, explaining a total of 49.5% of the variation. Forward selection identified nine significant variables; pH, area, DOC, conductivity, depth, area, aluminum, iron and DIC which together explained 40.5% of the variation (Table III). Cyclopoid copepods such as *D. b. thomasi* and *M. edax* were strongly, negatively correlated with pH. The abundance of *D. b. thomasi* was negatively correlated with metals (copper and nickel). The first two axes were significant ($P = 0.002$) in the Monte Carlo permutation test with 499 permutations for both the cladoceran and copepod analyses.

The gradients identified in this analysis were consistent with the general environmental factors on which the lakes had been originally classified. The first axis was primarily an axis of acidification variables separating high pH, low aluminum reference lakes from the high aluminum, low DOC and acid lakes. The recovered lakes fell somewhere between these two extremes. These

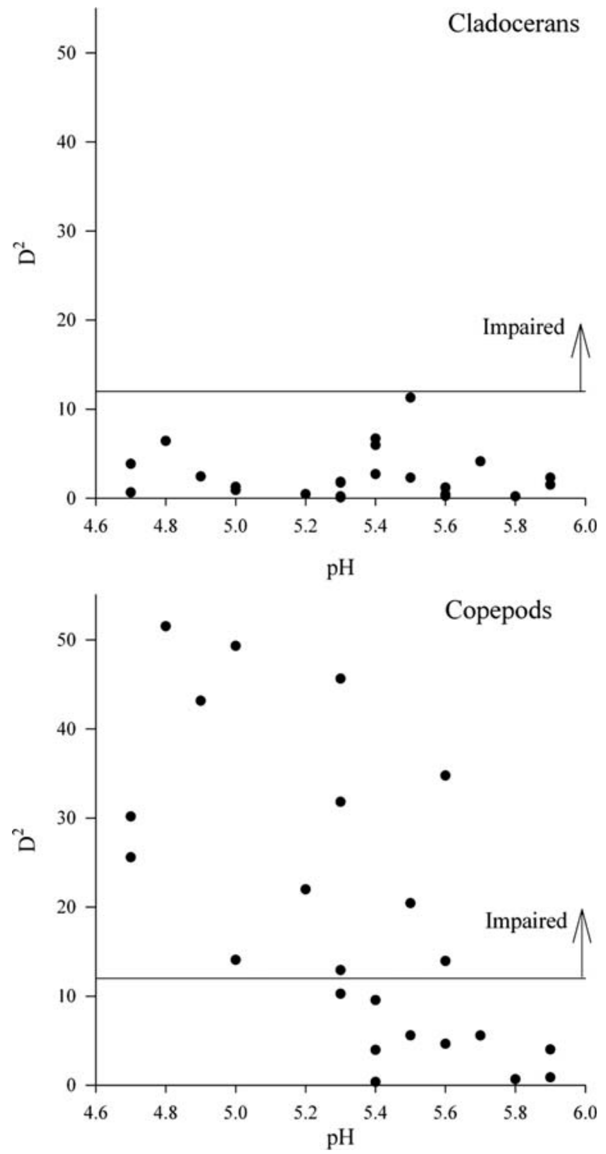

Fig. 3. Relationship between pH and each lake distance from reference condition in the acid lakes group. Distance (D^2) is a generalized Euclidean distance calculated using the first two axes of a PCA of cladoceran and copepod species abundance. Lakes falling above the horizontal line are significantly different from reference.

lakes contained low abundances of *Diaphanosoma birgei* and many *Daphnia* species, especially *D. mendotae*. On the other hand, the second RDA axis can be defined as a gradient of trophic status, metals and depth, separating the shallow, metal contaminated, high nutrient lakes within the city from the low nutrient, deeper lakes outside the urban zone. Many of the urban lakes also resembled the acid lakes. Although *D. mendotae* was quite common in many of the urban lakes, other *Daphnia* species were lacking.

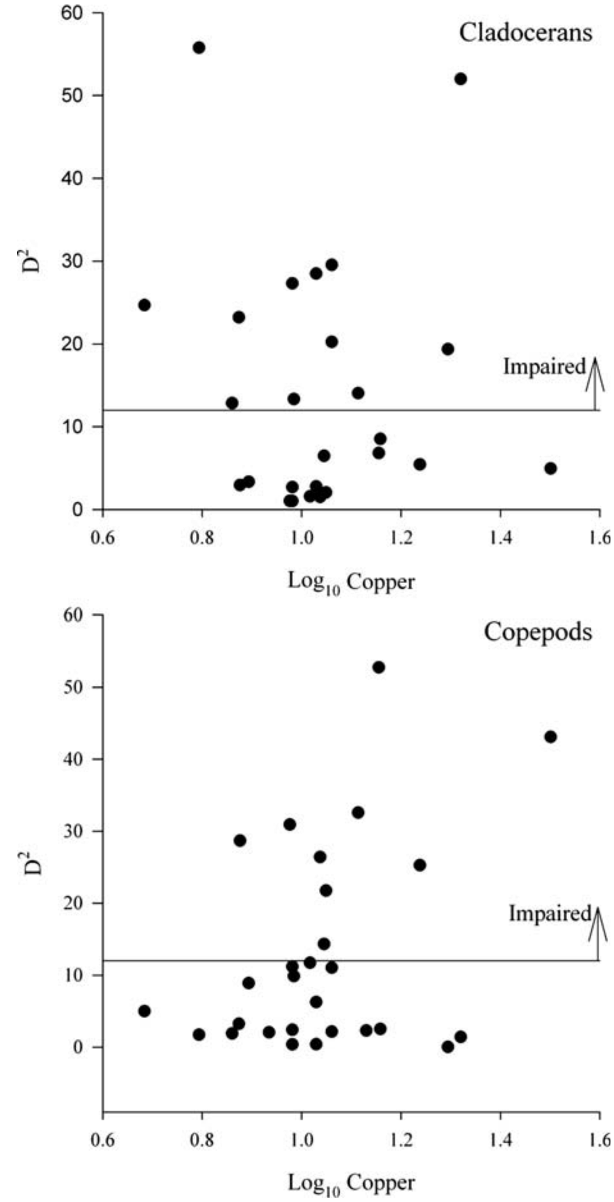

Fig. 4. Relationship between copper concentrations (log-transformed) and each lake distance from reference condition in the urban lakes group. Distance (D^2) is a generalized Euclidean distance calculated using the first two axes of a PCA of cladoceran and copepod species abundance. Lakes falling above the horizontal line are significantly different from reference.

DISCUSSION

Biological recovery from the acidification of lakes can follow unpredictable trajectories. Although zooplankton communities in many Sudbury area lakes are recovering as water chemistry improves, incomplete and divergent trajectories have been observed (e.g. Keller *et al.*, 2002; Yan *et al.*, 2004). In a review of studies from regions severely affected by acidification, Gray and Arnott

Table III: Marginal and conditional effects of forwardly selected environmental variables produced by RDA for the cladoceran and copepod community data

	Cladocerans			Copepods		
	Marginal	Conditional		Marginal	Conditional	
Variables	*P*-value	% explained	*P*-value	*P*-value	% explained	*P*-value
Alk	0.002	15	0.002	0.002	1	0.666
Al	0.002	1.5	0.18	0.002	2	0.016
DOC	0.002	1	0.38	0.002	3.5	0.002
DIC	0.002	0.5	0.84	0.002	2	0.010
Cond	0.002	3	0.002	0.002	2.5	0.014
Cu	0.002	3	0.002	0.002	6	0.002
Fe	0.008	1.5	0.120	0.008	2	0.036
Mn	0.002	4	0.002	0.002	0.5	0.856
Ni	0.002	1	0.360	0.002	0.5	0.930
TKN	0.002	1.5	0.100	0.002	1.5	0.224
pH	0.002	1	0.340	0.002	16	0.002
TP	0.002	0.5	0.850	0.002	1.5	0.118
SO_4	0.002	1	0.300	0.008	1.5	0.230
Zn	0.002	1	0.590	0.002	0.5	0.736
Secchi	0.002	4	0.002	0.002	1.5	0.084
Smelter	0.002	1	0.270	0.002	0.5	0.748
Area	0.002	1.5	0.070	0.004	2.5	0.016
Depth	0.002	1.5	0.070	0.002	4	0.002

(Gray and Arnott, 2009) found that the importance of water quality, dispersal and community-level impediments in influencing zooplankton recovery may differ among regions. By documenting recovery patterns in relation to water quality improvements at a regional level, we have the opportunity to increase our understanding of the processes regulating limnetic community structure and biodiversity.

Appropriate interpretation of community data relies on the assumption that sampling methods provided an adequate estimate of community composition and that any sampling biases were at least consistent across lakes (Magalhaes *et al.*, 2002). Previous zooplankton studies have shown that a potential drawback to synoptic surveys is the small per-lake sampling effort, which is suitable for common species but tends to miss rare species (Arnott *et al.*, 1999). Accounting for spatial heterogeneiety may add to the amount of explained variation but would also require a huge increase in sampling effort. On the other hand, in our study, the substantial number of lakes within each lake group did mitigate against missing important patterns. Moreover, some of the best-known long-term, comparative studies of zooplankton communities have used data collected with a variety of methods without any apparent biases caused by sampling gear (e.g. Shurin *et al.*, 2000).

Through the use of a reference condition approach, our findings support the hypothesis of Gray and Arnott (Gray and Arnott, 2009) that zooplankton communities do recover when lake pH rises above 6, at least in systems uncontaminated with metals. The TSA results revealed that the recovered lake group was not significantly different from reference condition for the cladoceran community, as well as for the copepod community in all but two lakes (Annie and White Oak Lakes). White Oak Lake only reached the recovery threshold within the last year, possibly indicating a delay between chemical and biological recovery. Time lags have been observed in many other studies (Havas *et al.*, 1995; Snucins *et al.*, 2001; Monteith *et al.*, 2005). Snucins (Snucins, 2003) found that sensitive benthic invertebrates took 4–8 years to re-colonize two recovering lakes after the estimated pH threshold for these species was reached. Moreover, the time before successful reestablishment throughout the lake was much longer, from 11 to 22 years. Annie Lake, on the other hand, has been at pH > 6 for 7 years, and the reasons for the absence of *C. scutifer* and low abundance of immature cyclopoids are unknown.

Surprisingly, many of the acid lakes appear to have recovered, particularly lakes above pH 5.5. Clearly, substantial improvements in zooplankton communities can occur before a level of pH 6.0 is reached. In the most severely acidified lakes (pH <5.5), it was the copepod and not the cladoceran community which deviated significantly from reference. In these lakes, cyclopoid copepods were absent or quite reduced in numbers. Different recovery rates for different taxonomic groups have also been found in other studies. In Swan Lake (Sudbury, Ontario), recovery was most rapid in the algae and rotifer communities, with slow and incomplete recovery in crustacean zooplankton (Arnott *et al.*, 2001). In a comparison of three taxonomic groups (cladocerans, copepods and rotifers), copepod recovery was slowest in Little Rock Lake (Wisconsin, USA) (Frost *et al.*, 2006). Metal toxicity is of particular concern in lakes closest to the smelters where copper and nickel concentration are often very high. Zooplankton communities in many of the urban lakes were found to be impaired despite years of non-acidic conditions, which may be attributable to elevated metal concentrations. Impairments were seen in both the cladoceran and copepod communities. Lakes with impaired cladoceran communities were lacking or contained very small populations of *H. gibberum*, a common species for Canadian Shield lakes. The affected lakes were often shallow, with elevated pH and conductivity, suggesting that the delays in cladoceran recovery we found were caused by increased urban stresses. The absence of *H. gibberum* from Middle Lake observed by Yan *et al.* (Yan *et al.*, 2004) was most likely the result of urban stresses and not metal levels as this species was often quite abundant in many of the metal-contaminated lakes nearby. To better assess recovery in these lakes would require the use of reference lakes subjected to similar stressors (e.g. shoreline modifications, with inputs of fertilizer and road salts).

Urban lakes with impaired copepod communities had elevated metal levels as well as low concentrations of DOC and ions, which can increase metal toxicity through increased bioavailability (Borgmann *et al.*, 2005). Copepod recovery may have been limited by residual metal toxicity. These lakes lacked many large calanoids (e.g. *Epischura lacustris*, *S. oregonensis*) which agrees with the results of Roch *et al.* (Roch *et al.*, 1985) who found that some calanoid copepods, especially the juvenile stages, were particularly sensitive to metal contamination. A striking similarity in the copepod communities of both acidic and highly metal contaminated lakes was found, with both lake types dominated by *L. minutus*. This small calanoid appears to be well-adapted to both metal and acid stress (Derry *et al.*, 2010), although some studies have found *L. minutus* to decrease under severe acid stress (e.g. Fischer *et al.*, 2001).

In addition to the chemical factors considered here, biological factors may also be important in determining zooplankton community recovery (e.g. Valois *et al.*, 2010). It appears that the recovery of zooplankton communities cannot necessarily be expected until the return of the pre-acidification predator community has occurred (Yan *et al.*, 1991; Marmorek and Korman, 1993; Keller *et al.*, 2002). Binks *et al.* (Binks *et al.*, 2005) suggested that high abundances of macro-invertebrate predators may be contributing to the failed recolonization of copepod communities in lakes lacking planktivorous fish, and that the recovery of the fish community may be necessary to facilitate the reestablishment of zooplankton in these systems. Of the non-acidic lakes, Johnny Lake was the only lake known to be fishless and thus should have supported a high abundance of invertebrate predators, e.g. *Chaoborus* larvae. This lake was found to be significantly impaired for both the cladoceran and copepod community, lacking large cladocerans (*Daphnia*, *H. gibberum*) and with low abundances of calanoid copepods. The absence of large cladocerans is very unusual and not likely the result of high predation pressure by *Chaoborus*, which generally exclude or reduce small zooplankton (Dodson, 1974; Vanni, 1988; Arnott and Vanni, 1993). Calanoid copepods have been shown to be vulnerable to invertebrate predators (Neill, 1981; Nyberg, 1984) and the conspicuous reduction in *L. minutus* may be a result of predation effects. Some of the severely acidified lakes were expected to be fishless and we were unable to determine what role predation by invertebrate predators may have played in zooplankton recovery in these lakes.

Fish are also known to have strong direct effects on zooplankton communities. Although the majority of the lakes in this study contained fish, many of the urban lakes had unusual fish communities dominated by yellow perch (*Perca flavescens*) (Gunn and Keller, 1990). In these lakes, perch may have relied heavily on zooplankton as a result of a benthic community that was impaired by sediment toxicity (Luek *et al.*, 2010). Valois *et al.* (Valois *et al.*, 2010) attributed the dominance of *H. gibberum* in some of these lakes to the ability of this species to out-compete *D. mendotae* at high levels of planktivory. We had information on fish communities for 9 of the 12 lakes with significantly impaired cladoceran communities (Valois *et al.*, 2010). Seven of these lakes contained relatively diverse fish communities, with well-established piscivore populations (smallmouth bass, brook trout, lake trout or northern pike) and low abundances of yellow perch. Little Raft Lake contained a high abundance of yellow perch; however, the lack of *H. gibberum* in this lake was more likely a result of its small size (19 ha) and higher productivity (TP = 13 μg/L). It does not appear that fish planktivory played a dominant role in delaying recovery in these lakes. In comparison, most of the lakes with significantly impaired copepod communities were also dominated by yellow perch. This was most likely a result of elevated metal toxicity, which appears to exclude both piscivorous fish and cyclopoid copepods from establishing in these lakes (Valois *et al.*, 2010).

Multiple stressors can result in complex, interactive effects on lake communities (Folt *et al.*, 1999; Christensen *et al.*, 2006). Stressors, such as shoreline development (Keller *et al.*, 1999), and changes in UV penetration (Christensen *et al.*, 2006) and calcium concentrations (Smol, 2010) have been shown to act antagonistically (shoreline development) or synergistically (increased UV and decreased calcium concentrations) on plankton communities recovering from acid stress. Although it appears that changes in nutrient concentrations resulting from urbanization and alterations in fish community structure are contributing to the lack of recovery in some of the urban lakes, the magnitude and consequences of these interactions are unknown. Multifactorial experiments (e.g. Strecker and Arnott, 2005; Christensen *et al.*, 2006) are needed to identify the nature (synergy versus antagonism) of these potentially interactive effects.

Summary

The fundamental objective of emission reductions and protective legislation is the restoration of ecosystem structure and function, often measured as a return in the community to either its pre-disturbance state or to one similar to un-impacted communities in the area. However, assessing community recovery is difficult. Barriers which may stall or prevent the return of the pre-disturbance communities once the stress is removed may be unevenly distributed across damaged sites. Moreover, confounding effects of multiple stressors may obscure recovery patterns. In this study, we found that the zooplankton communities often recovered once lake pH reached 5.5; however, delays were evident in lakes with elevated metal concentrations. The delayed recovery of cladocerans, observed by Yan *et al*. (2004), was not localized to Middle Lake, or even solely to the cladoceran community, but was common to both cladocerans and copepods in many of the urban lakes.

Although clear relationships could be established between alterations in the copepod community and effects of acidity and elevated metal levels, these effects were not as clear for the cladoceran community. Given their larger size, cladocerans are often much more susceptible to fish predation (e.g. Vinebrooke *et al*., 2001; Nilssen and Waevergan, 2002) which was not accounted in this study. The results of this study add to the growing body of evidence that recovery of the zooplankton community occurs fairly well given sufficient water quality improvements, with evidence of recovery in lakes with pH $>$5.5. However, untangling the complex effect of urbanization, including its effect on metal toxicity, and the effect of altered predator communities will require the use of more appropriate reference lakes, toxicity testing and *in situ* experiments (i.e. mesocosms). Clearly, further studies are needed to increase our understanding of the roles and interactions of multiple stressors (metal toxicity, shoreline development, fish community composition) in affecting zooplankton recovery from acidification.

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