Demographic and genetic evidence of the long-term recovery of *Daphnia galeata mendotae* (Crustacea: Daphniidae) in Sudbury lakes following additions of base: the role of metal toxicity[1]

N.D. Yan, P.G. Welsh, H. Lin, D.J. Taylor, and J.-M. Filion

Abstract: Twenty-year records of changes in abundance of *Daphnia galeata mendotae* are presented for Middle and Hannah lakes, two metal-contaminated lakes near Sudbury, Ontario, whose acidity was neutralized by additions of base in the mid–1970s. A comparison of allozyme frequencies and abundances of *D. g. mendotae* in Middle, Hannah, and numerous reference populations indicates that the taxon has fully recovered at both the population and genetic levels of analysis. However, the timing and pace of recovery of *D. g. mendotae* differed between the two Sudbury lakes. Two 21-day bioassays were conducted to examine the survival and brood production of *D. g. mendotae* in five treatments simulating 18 years of changes in Cu, Ni, and Cd concentrations in the lakes. The bioassays indicated that metal concentrations, i.e., habitat quality, regulated the pace of recovery of this important zooplankton taxon in the study lakes.

Résumé : Nous présentons les données recueillies en 20 ans sur les changements d'abondance de *Daphnia galeata mendotae* dans les lacs Middle et Hannah, deux nappes d'eau polluées par des métaux faisant partie du groupe de lacs de Sudbury soumis à un traitement de neutralisation par l'addition de d'une base vers le milieu des années 1970. L'étude comparative de la fréquence des alloenzymes et de l'abondance de *D. g. mendotae* des lacs Middle et Hannah et dans de nombreuses populations de référence a révélé que cette espèce s'est complètement rétablie et ce, tant au point de vue de l'analyse des populations que de l'analyse génétique. Cependant, l'époque où le rétablissement s'est effectué et la vitesse du processus de rétablissement ne sont pas les mêmes dans les deux lacs. Nous avons réalisé deux épreuves biologiques de 21 jours pour étudier la survie et le rendement de reproduction de *D. g. mendotae* dans des groupes expérimentaux soumis à cinq traitements reproduisant 18 années de changement des concentrations de Cu, Ni et Cd dans les lacs : ces épreuves ont révélé que les concentrations de métaux ou, en d'autres mots, la qualité de l'habitat, ont déterminé la vitesse du rétablissement de cette importante espèce zooplanctonique dans les lacs étudiés.
[Traduit par la Rédaction]

Introduction

Daphnia galeata Sars, 1864 *mendotae* Birge, 1918 is commonly abundant in temperate lakes in central and eastern North America (Brooks 1957; Carter et al. 1980; Keller and Conlon 1995). Because it is also relatively large, the species contributes more than any of its congeners to the average biomass of crustacean zooplankton on the southern Canadian Shield (Yan et al. 1988). *Daphnia galeata mendotae* is also sensitive to acidity, suffering ionoregulatory failure at pH levels below 6 (Havens 1992). In consequence, many of its populations have been damaged by the acidification of lakes in eastern North America. Evidence of this damage is provided by the universal (Marmorek and Korman 1993) rarity of *D. g. mendotae* in acidic lakes (Sprules 1975; Carter et al. 1986; Pinel-Alloul et al. 1989; Keller et al. 1990; Siegfried and Sutherland 1992), by the correspondence of field and laboratory assessments of its extreme acid sensitivity (Keller et al. 1990; Havens et al. 1993), and by its decimation in lake acidification experiments (Schindler et al. 1985; Locke and Sprules 1993).

Because of the severity of the acid deposition problem, the governments of Canada and the United States have legislated

Received August 23, 1995. Accepted January 17, 1996.
J13051

N.D. Yan.[2] Ontario Ministry of Environment and Energy, Science and Technology Branch, Dorset Research Centre, P.O. Box 39, Dorset, ON P0A 1E0, Canada.
P.G. Welsh. Department of Biology, University of Waterloo, Waterloo, ON N2L 3G1, Canada.
H. Lin. Shanghai Research Institute of Environmental Protection Science, Shanghai, 200233, People's Republic of China.
D.J. Taylor.[3] Department of Zoology, University of Guelph, Guelph, ON N1G 2W1, Canada.
J.-M. Filion. École sécondaire Algonquin, 555 avenue Algonquin, North Bay, ON P1B 4W8, Canada.

[1] This paper is dedicated to the late Dr. Bill Geiling.
[2] Author to whom all correspondence should be addressed. e-mail: yanno@epo.gov.on.ca
[3] Present address: Department of Biology, University of Michigan, Ann Arbor, MI 48109–1048, U.S.A.

substantial reductions in atmospheric emissions of SO_2, the major acid source. There is a widespread expectation (Jeffries et al. 1992) that water quality will improve in many acidified lakes in response. Unfortunately, ecologists do not know how rapidly aquatic biota will follow suit. Many factors may regulate the recovery of biota in damaged ecosystems, including the severity and duration of stress, the presence of refuges, the availability of colonists, their productivity and dispersal ability, and barriers to their dispersal (Cairns 1990; Detenbeck et al. 1992). Few of these factors have been investigated for the stress of acidification (Schindler et al. 1991; Yan et al. 1995).

The best evidence for the recovery of aquatic biota from acidification comes from the region of Sudbury, Ontario. Historical SO_2 and metal emissions from Sudbury's Cu and Ni smelters damaged thousands of local lakes. Reductions in emissions during the 1970s and early 1980s were followed by substantial improvements in water quality (Keller and Pitblado 1986; Keller et al. 1992*a*). Aquatic biota have begun to recover in response (reviewed by Keller et al. 1992*b*).

Yan et al. (1996) compared recovery of the zooplankton communities between the acidic (pH < 4.5) and heavily metal-contaminated Middle and Hannah lakes, near Sudbury, that followed whole-lake neutralization (liming). At the community level of analysis, Hannah Lake was recovering more rapidly than Middle Lake. The principal difference between the lakes was the timing and success of colonization by *D. g. mendotae*. The first objective of this paper is to determine if the *D. g. mendotae* populations of Middle and Hannah lakes have, in fact, recovered.

The recovery of a species should be assessed at both the population and genetic levels (Noss 1990). We have no preacidification data for *D. g. mendotae* from Middle and Hannah lakes to set recovery targets. Hence, to assess recovery at the population level, we compare the standing stocks of their *D. g. mendotae* populations with both temporal and spatial reference lakes. The temporal reference lakes provide the normal range of interannual variability, a yardstick we use to assess the significance of the changes in *D. g. mendotae* abundance in Middle and Hannah lakes. The spatial reference lakes provide our recovery targets, i.e., the range in population sizes of *D. galeata mendotae* typically observed in nonacidic lakes in Ontario.

Genetic analyses can strengthen our observations in several ways. First, they provide unequivocal species identifiers. Diagnostic allozyme markers can eliminate confusion among morphologically cryptic hybrids, species, and invading taxa (Wolf and Carvalho 1989; Taylor and Hebert 1993). Second, they may provide sensitive indicators of damage and recovery. Genotypic frequencies of *D. galeata* populations rarely deviate from Hardy–Weinberg (H–W) expectations (Mort and Wolf 1986; Hebert et al. 1989; Taylor and Hebert 1993); hence, an observation of H–W deviations or reduced clonal diversity indicates genetic disturbance that may not be evident in the demographic or morphological data. To assess recovery at the genetic level we compare the genotypic frequencies of five variable allozyme loci of the *D. g. mendotae* in Middle and Hannah lakes with the published genetics literature on *D. g. mendotae*, related taxa, and their hybrids in eastern temperate North America.

Levels of Cu, Ni, Al, and other metals were reduced dramatically by the additions of base to Middle and Hannah lakes (Yan and Dillon 1984). Concentrations declined further during the 1980s in response to reductions in metal emissions from the smelters and catchment neutralization, but the patterns of decline were lake specific. Yan et al. (1996) hypothesized that differences in habitat quality between Middle and Hannah lakes, manifested as differences in metal concentrations, regulated the pace and extent of recovery by *D. g. mendotae*. Our second objective is to test this hypothesis. Using laboratory bioassays, we quantify the survival and brood production of *D. g. mendotae* in Hannah and Middle lakes across a range of Cu, Ni, and Cd concentrations that mimic the two decades of changes in metal concentrations that followed additions of base.

Study lakes

Middle and Hannah lakes

Yan and Miller (1984) described the geography, morphometry, and premanipulation chemistry and biology of Middle and Hannah lakes. Middle Lake has an area of 28.2 ha and mean and maximum depths of 6.2 and 15 m, respectively. Hannah Lake has an area of 27.3 ha and mean and maximum depths of 4 and 8.5 m, respectively. The outflow of Hannah Lake is an unobstructed, 0.8-km channel that drains into Middle Lake.

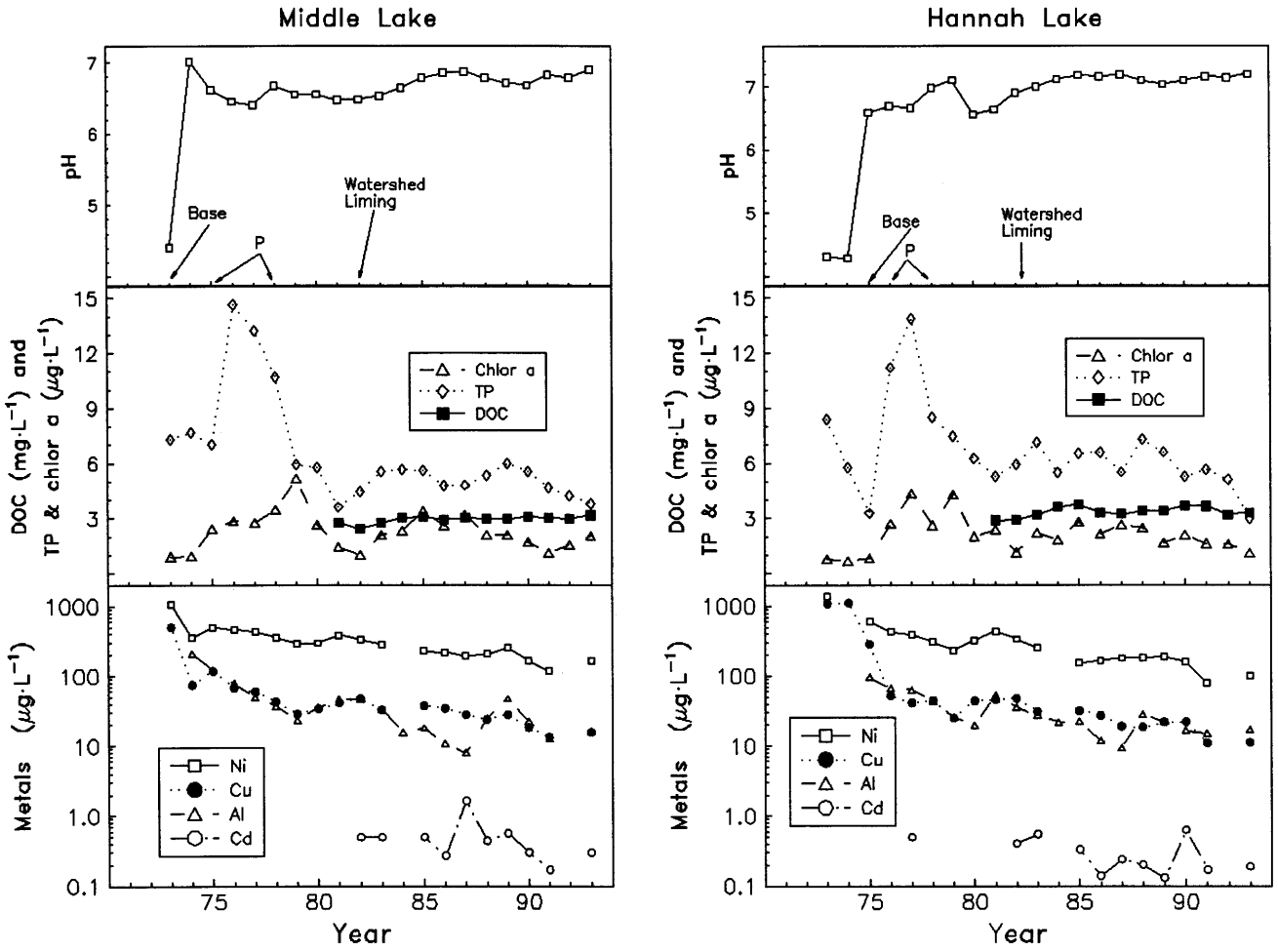
Middle and Hannah lakes acidified (Dixit et al. 1987, 1990) and were contaminated with metals (Dillon and Smith 1984) 60–80 years ago after the first tall stacks were erected by local industries. The resulting acid, Cu, and Ni contamination was severe (Table 1), dramatically reducing the diversity of all trophic levels and eliminating all fish (Yan and Welbourn 1990).

Middle and Hannah lakes have been experimentally manipulated in four ways. $CaCO_3$ and $Ca(OH)_2$ were added to Middle Lake in the fall of 1973 and to Hannah Lake in the spring of 1975 (Dillon et al. 1979). In an attempt to accelerate biological recovery (Blomqist et al. 1993), small amounts of P were added to the lakes in the mid–1970s (Yan and Lafrance 1984). Concentrations of P and chlorophyll rose in response but only during the treatment years (Fig. 1). In 1976, fisheries managers stocked Middle Lake with smallmouth bass (*Micropterus dolomieu*), Iowa darter (*Etheostoma exile*), and brook stickleback (*Culaea inconstans*). Metal toxicity apparently prevented the survival of all introduced fish (Yan et al. 1979). Finally, the catchments of the lakes were limed, fertilized, and planted in the early 1980s as part of regional land restoration efforts (Lautenbach et al. 1995). Because of the short-lived effects of nutrient additions and the rapid mortality of the stocked fish, we consider the additions of base to the lakes and their catchments to be the only manipulations with long-term effects.

The pH levels (average of at least monthly collections) of Middle and Hannah lakes have remained well above 6 since the mid–1970s (Fig. 1). *Daphnia galeata mendotae* prospers under such conditions (Keller et al. 1990; Havens et al. 1993); hence, the stress of acidity has been eliminated.

Total Cu and Ni levels dropped dramatically after the additions of base (Fig. 1) but were still very high in comparison with lakes remote from Sudbury. Concentrations of Cu, Ni, Cd, and other metals declined further during the 1980s and

Fig. 1. Long-term changes in selected chemical variables in Middle and Hannah lakes. Values are ice-free season averages of pH (the negative log of mean [H^+]), total chlorophyll *a*, total phosphorus (TP), dissolved organic C (DOC), and total Ni, Cu, Al, and Cd. The pre–1980 data were taken from Yan and Miller (1984) and Yan and Lafrance (1984). Approximate dates of manipulations are indicated in the pH panels.



early 1990s (Fig. 1), most likely because of the catchment liming and the reductions in acid and metal emissions from the smelters (Dillon et al. 1986).

Reference lakes

The 47 spatial reference lakes are located in south-central Ontario, 200 km southeast of Sudbury. The lakes are in the same biogeographic region as Sudbury for zooplankton (Sprules 1977) but beyond the influence of its smelters (Zeng and Hopke 1994). The pH levels of the lakes range from 5.3 to 6.7 with a median of 5.9. Levels of Cu and Ni in precipitation and surface waters are one or two orders of magnitude lower than in Sudbury (Scheider et al. 1981; LaZerte et al. 1989).

Harp, Blue Chalk, and Red Chalk lakes are located among the spatial reference lakes and served as our temporal reference lakes. They are dimictic, oligotrophic (total phosphorus (TP) 4–7 $\mu g \cdot L^{-1}$), and nonacidic (pH > 6.2; Dillon et al. 1988; Molot and Dillon 1991). Yan and Strus (1980), Yan (1986), and Yan et al. (1996) provide an overview of the zooplankton assemblages of both the temporal and spatial reference lakes.

The 36 lakes that provided our genetic, spatial reference set are located in the temperate region of central North America and include many lakes in south-central Ontario (Taylor and Hebert 1993).

Table 1. Selected morphometric and premanipulation chemical data from Middle and Hannah lakes.

	Hannah	Middle
Distance from smelters (km)	4.3	5.0
Mean depth (m)	4.0	6.2
Maximum depth (m)	8.5	15.0
Lake area (ha)	27.3	28.2
Total P ($\mu g \cdot L^{-1}$)	5.7	7.3
pH	4.29	4.40
Ca ($mg \cdot L^{-1}$)	11.4	9.8
Total Cu ($\mu g \cdot L^{-1}$)	1108	496
Total Ni ($\mu g \cdot L^{-1}$)	1865	1068

Note: Values are ice-free season averages of weekly to twice-monthly data for 1973 for Middle Lake and 1974 for Hannah Lake (from Yan and Miller 1984).

Methods

Ephippial densities in lake sediments

Yan and Miller (1984) found no *Daphnia* spp. in the plankton of Middle and Hannah lakes in the early 1970s, prior to the additions of base. To determine if Hannah Lake ever supported daphnids, we adopted a paleolimnological approach, developing profiles of deposition rates of resting egg-bearing ephippia of Daphniidae to the lake sediments. The paleolimnological workload restricted our focus to the upstream lake of the pair.

In the summer of 1993, sediment cores were collected in Hannah Lake with a parachute corer (description in preparation by J.-M.F.). This device lowers a 5.1 cm o.d. Lexan sampling tube vertically into the sediments at a constant velocity in free-fall. Cores were collected at 14 locations within a 100 m radius of the deepest spot in the lake. Each core was retrieved and sectioned into 14 slices that were 1 cm thick. Ten of the cores were subsequently examined in their entirety for ephippia. By hatching morphometrically dissimilar ephippia, we trained ourselves to distinguish cladoceran genera. We were able to identify ephippia of *Simocephalus* and *Daphnia* spp., but we were unable to identify species of *Daphnia* from their ephippial remnants.

To estimate the ephippial deposition history, we estimated the age of each core section in the remaining four cores. To do this we dried each section, defined profiles of cumulative dry mass versus depth, then compared our profiles with the sedimentation chronologies previously established by Dillon et al. (1984) using ^{210}Pb techniques. We log transformed the counts of *Daphnia* ephippia then plotted the mean and confidence interval for the 10 counted cores against estimated age of sediment deposition.

Abundance of *Daphnia galeata mendotae*

Yan et al. (1996) detail the zooplankton sampling and enumeration methods. Briefly, we visited Middle and Hannah lakes each year between 1973 and 1993 except 1979 and 1992. We collected zooplankton at the deepest spot in each lake on a weekly basis during the ice-free seasons of 1973 and 1974, every other week from 1975 to 1979, and monthly thereafter. We employed Schindler–Patalas traps or conical nets prior to 1980, and thereafter a metered, 12.5 cm diameter, 76-μm mesh, conical Dorset Research Centre (DRC) net (McQueen and Yan 1993). We hauled the net vertically to the surface from 7.5, 6, 4, and 2 m in Hannah Lake, and from 13, 10, 6, and 3 m in Middle Lake, combining the contents of the four hauls. The depths were chosen so that individual lake strata contributed aliquots to the composite sample in proportion to their volumes. All samples were preserved in the field in a 6% sugar–formalin solution. Changes in sampling gear do not affect the results of this study. All samples were collected with the DRC net after 1979, and all of the changes (see Results) in the *D. g. mendotae* populations occurred after that year.

We counted a minimum of 250 crustacean zooplankton in each sample, adjusting subsample volumes so that single taxa did not contribute more than 20% of the count. We identified *D. g. mendotae* in the preserved samples following Brooks (1957), confirming its identity on recent live isolates using genetic markers.

Bill Keller (Ontario Ministry of Environment and Energy, Sudbury, Ont.) sampled Middle and Hannah lakes on a monthly basis in 1992 and provided us with these data to complete our temporal record. He also sampled with a DRC net and processed the samples using protocols identical to ours.

The reference lakes were sampled on a monthly basis using the DRC net and depth-compositing strategy described above. Filtration efficiency averaged >80% in the reference lakes, a similar average (85%) to that recorded in Middle and Hannah lakes.

On every visit to the lakes we collected morphometrically weighted composite samples for water quality analyses. These were integrated through twice the Secchi transparency for chlorophyll *a* measurements and through all depths for water chemistry. Analytical methods followed Ontario Ministry of Environment and Energy (1984).

To determine if the abundance of *D. g. mendotae* was more variable in Middle and Hannah lakes than in the temporal reference lakes on an annual basis, we employed a Levene's test (Manly 1986). We transformed ($\log(x + 1)$) the ice-free season averages, and verified this improved normality by examining box plots. We then transformed the logged data to absolute deviations from each lake's long-term mean. Then we sought differences among means of the absolute deviations using a one-way analysis of variance (ANOVA) in SYSTAT. We followed this with several post-hoc comparisons using Tukey's honestly significant difference (HSD) test. Bonferroni adjustments were applied to protect overall error rates.

We used the spatial reference lakes to set our recovery target, i.e., a *D. g. mendotae* population size characteristic of nonacidic lakes. Havens et al. (1993) verified that the relationship between pH and the probability of presence of *D. g. mendotae* can be described by logistic regression (Hosmer and Lemershow 1989). Data presented in Havens et al. (their Fig. 1) suggest that an asymptotic variant of the logistic model could describe the relationship between pH and *D. g. mendotae* abundance in Canadian Shield lakes grouped in pH intervals. However, recovery targets for individual lakes cannot be developed using Havens' grouped data. Instead we used our 47 individual spatial reference lakes for this purpose.

Using the NONLIN routines in SYSTAT we fit an asymptotic variant of the logistic regression model to estimate average *D. g. mendotae* (Dgm) abundance from the pH of the 47 spatial reference lakes. The model is of the form

$$(1) \qquad \text{Log}\,(\text{Dgm} + 1) = \text{K}\,\frac{e^{(a + b(\text{pH}))}}{1 + e^{(a + b(\text{pH}))}}$$

where K is the fitted asymptote indicating the expected average abundance in nonacidic lakes. For our recovery target, we used the 95% confidence limits about the predicted abundance of *D. g. mendotae* at pH 7, the pH of Middle and Hannah lakes in the late 1980s (Fig. 1). Because pH 7 is actually beyond the maximum pH of the spatial reference lakes, predictions at this pH must be interpreted cautiously.

Genetics of *Daphnia galeata mendotae*

We collected zooplankton from the deep stations of Middle and Hannah lakes on June 21, 1994, with a 250-μm mesh net. *Daphnia galeata mendotae* was a dominant member of the zooplankton at the time. The animals were dispensed into large carboys filled with source lake water and returned to the University of Guelph, Guelph, Ont. Survival during the 24 h of storage and transport was complete.

Sixty-six individual *D. g. mendotae* from each lake were processed using cellulose acetate electrophoresis (Hebert and Beaton 1993). Seven loci were examined: aminoaspartate transferase (AAT; EC 2.6.1.1), glucose phosphoisomerase (GPI; EC 5.3.1.9), phosphoglucomutase (PGM; EC 5.4.2.2), proline dipeptidase (PEP-D; EC 3.4.13.9), dipeptidase (PEP-A; EC 3.4.13.11), lactate dehydrogenase (LDH; EC 1.1.1.27; one lake only) and fumarate hydratase (FUM; EC 4.2.1.2; one lake only). These loci were chosen because they are polymorphic in *D. g. mendotae* and contain alleles diagnostic of hybridization (Taylor and Hebert 1993). Individuals of a uniclonal culture of *D. g. mendotae* from Center Lake (Warsaw, Ind., U.S.A.) were used as electrophoretic standards. Alleles were identified by their relative mobilities (Taylor and Hebert 1992).

To detect deviations from H–W expectations we employed χ^2 tests with pooled genotypic classes for the triallelic GPI and exact tests for all of the remaining diallelic loci. All tests were performed using BIOSYS–1 (Swofford and Selander 1981).

To compare the Middle and Hannah populations with those in the reference lakes it was helpful to reduce the dimensionality of the data. We employed multidimensional scaling (MDS), with chord distances (Cavalli-Sforza and Edwards 1967) among Middle, Hannah, and the spatial reference populations forming the input dissimilarity matrix.

Table 2. Multilocus genotype frequencies for the five variable allozyme loci in the *D. g. mendotae* from Middle (n = 63) and Hannah (n = 61) lakes.

Frequencies (%)		Genotypic designation				
Hannah	Middle	sAAT	GPI	PEP-A	PEP-D	PGM
9.83	4.76	100/114	95/100	121/121	81/100	100/100
8.2	7.94	100/114	100/100	121/121	81/81	100/100
8.2	3.17[a]	100/114	95/100	121/121	81/81	100/100
4.92	1.59	100/100	95/100	121/121	81/81	100/100
4.91	14.29	100/100	100/100	121/121	81/81	100/100
4.91	3.17	100/100	100/107	121/121	81/81	100/100
3.28[a]	3.17	100/114	100/100	121/121	81/81	89/100
3.28	3.17	114/114	100/100	121/121	81/81	100/100
3.28	3.17	114/114	100/100	121/121	81/100	100/100
3.28	1.59	114/114	100/100	121/121	81/81	89/100
3.28	—	100/100	95/95	121/121	89/100	100/100
3.28	—	100/100	95/95	121/121	81/81	100/100
3.28	—	100/100	95/100	121/121	81/100	100/100
3.27	1.59	100/100	100/107	121/121	81/100	100/100
3.27	1.59	114/114	95/95	121/121	81/81	100/100
3.27	—	100/114	95/100	121/121	81/81	89/89
1.64	6.34	100/114	100/107	121/121	81/81	100/100
1.64	3.17	100/114	95/100	121/121	81/81	89/100
1.64	3.17	100/100	100/100	121/121	81/81	89/100
1.64	3.17	100/114	100/100	121/121	89/100	100/100
1.64	3.17	100/114	95/95	121/121	81/81	100/100
—	3.17	114/114	95/100	121/121	81/81	100/100

Note: Only those frequencies >3% in one lake are tabulated. Eleven and 18 additional clones were identified in Hannah and Middle lakes, respectively, only 2 of which were common to the lakes. Alleles are represented as relative mobility values.

[a]Genotypes used in the bioassays.

Chord distances are insensitive to differences in heterozygosity among taxa (Swofford and Olsen 1990).

Laboratory bioassays

Our second objective, to determine if metals affected the rate of recovery of *D. g. mendotae* in the lakes, was addressed in two sets of 21-day experiments; one used Hannah Lake water and neonate (<24 h) *D. g. mendotae* originating from Hannah Lake as experimental animals and the other set used Middle Lake water and neonate *D. g. mendotae* originating from Middle Lake.

The experimental animals represented a single clone from each lake. To obtain these, multiclonal cultures of *D. g. mendotae* from each lake were established in source lake waters in August of 1993. The animals were fed *Selenastrum capricornutum* grown in Bolds basal medium without metals. The cultures were maintained at 21 ± 1°C, illuminated for 18 h of each day, and refreshed with source lake water each week. A vigorous female from each culture was subsequently used to start a single line for the bioassays. Allozyme profiles confirmed that the experimental lines were single common clones of unhybridized *D. g. mendotae*; however, the clone selected differed between the lakes (Table 2).

The source water for the bioassay media was collected from Middle and Hannah lakes in August of 1993. We returned the water to the DRC, Dorset, Ont., passed it through 5-µm polypropylene filters, then sterilized it under high-energy ultraviolet light. The exposure media differed between lakes in all base cations and anions, pH, alkalinity, and Zn ($p < 0.05$) but not in Al ($p = 0.07$) or dissolved organic carbon (DOC) ($p = 0.15$) (Table 3).

Five historical levels of metal contamination were simulated by spiking the prepared source water with sulphate salts of Cu, Cd, and Ni. The filtered, sterilized lake waters provided the reference, i.e., 1993, treatments. The remaining treatments simulated the metal concentrations in the lakes in 1976, 1981, 1985, and 1989 (Fig. 2). These years were selected because they represented different stages in the colonization history of the two lakes by *D. g. mendotae* (see Results).

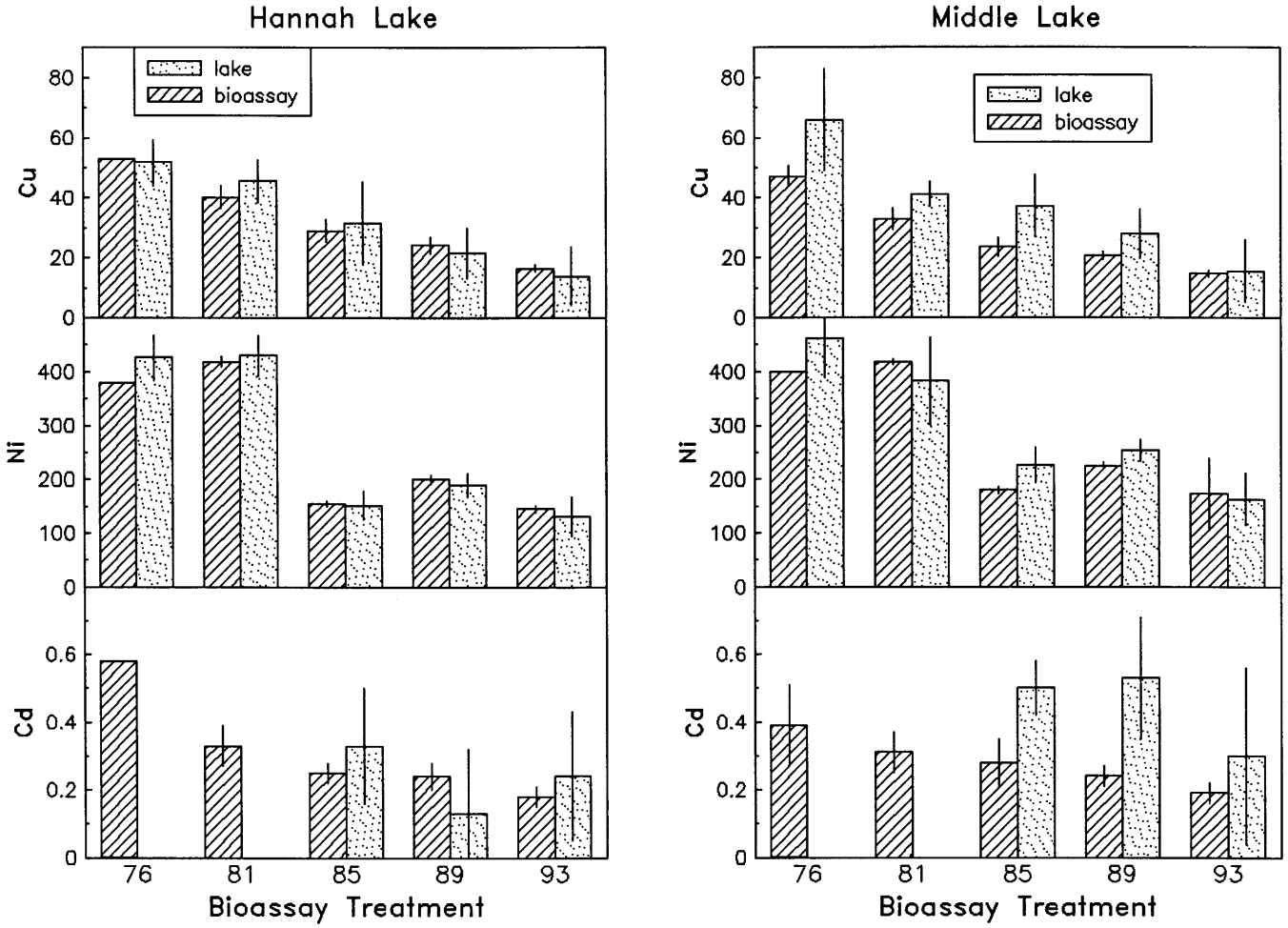
Table 3. Water quality characteristics of lake water used in the bioassays (mean ± SD, n = 5).

	Hannah Lake	Middle Lake
Zn (µg·L^{-1})	3.49±0.13	18.49±0.26
Al (µg·L^{-1})	7.33±3.79[a]	15.80±6.94
Ca (mg·L^{-1})	12.49±0.29	9.06±0.11
Mg (mg·L^{-1})	4.09±0.10	3.08±0.40
Na (mg·L^{-1})	44.10±0.35	30.00±0.25
K (mg·L^{-1})	1.71±0.02	1.56±0.11
Cl (mg·L^{-1})	82.90±3.82	55.00±0.94
SO_4-S (mg·L^{-1})	26.87±0.76	22.28±0.29
DOC (mg·L^{-1})	3.00±0.10	3.10±0.10
DIC (mg·L^{-1})	3.13±0.02	1.69±0.03
Conductivity (µS)	345.80±1.30	245.40±0.89
pH	7.31±0.03	7.03±0.01
Alkalinity (mg·L^{-1} as $CaCO_3$)	12.55±0.13	6.68±0.08

[a]n = 4.

Levels of Cu, Cd, and Ni in the test waters were monitored throughout the experiment. A composite sample was formed from daily 1-mL aliquots from each test tube. Cu and Cd analyses were performed on 3-day composites, while Ni analyses were performed on 7-day composites. Cu and Cd were analyzed using graphite furnace atomic absorption spectrophotometry (AAS), and Ni was analyzed using flame AAS. Samples were analyzed in duplicate and results were rejected if the relative standard deviation differed

Fig. 2. Comparison of total Cu, Ni, and Cd concentrations (mean ± SD; in $\mu g \cdot L^{-1}$) in the bioassays with concentrations in the lakes in the corresponding years. The SDs for the lakes reflect mainly seasonal variability.



by >5%. Regular analysis of standard water samples (National Research Council of Canada), long-term blanks, and in-run standards assured accuracy and precision.

Considering the extent of seasonal variability in metals levels in the lakes, there was a good match of historical total metal concentrations with levels in the treatments for Hannah Lake; however, the bioassay levels were a bit lower than desired for Cu and Cd in Middle Lake (Fig. 2). There were small (Fig. 2) but significant differences between the lakes within treatments for total Cu and Ni in 1993, 1989, and 1985 ($p < 0.01$) and for Cu in 1981 ($p < 0.01$). Cu levels were lower in the Middle Lake bioassays and Ni levels were lower in the Hannah bioassays; however, interyear variation greatly exceeded interlake variation.

The free divalent ion is assumed to be the toxic metal species (Morel 1983). The concentrations of divalent Cu, Ni, and Cd ions in the exposure water were calculated using the geochemical speciation model MINTEQA2 (Allison et al. 1990). The concentrations of constituents used in the model are given in Table 3 and Fig. 2. DOC was added as dissolved organic matter (DOM) using the composite ligand model (CLM) subroutine of MINTEQA2. The CLM subroutine assumes a normal distribution of equilibrium constants (log K) for DOM on the basis of carboxylic metal binding constants (Allison and Perdue 1994).

The experiments were conducted in a class–100 clean laboratory located at the DRC. Animals were individually maintained for 21 days at 22 ± 1°C in 25 mL of test water in glass test tubes ($n = 10$) under a 16 h light : 8 h dark photoperiod. Test water was renewed daily, at which time the animals were fed with *S. capricornutum* at a density of 2×10^4 cells/mL. Daily checks were performed for mortality, clutch size, production of offspring, and offspring survival through 24 h. The results were summarized using six variables: total mortality after 21 days, age at primiparity, clutch size, number of clutches produced, number of neonates produced that survived for 1 day, and number of neonates produced that died within 1 day.

The mortality data were organized into five two-way tables, one table per treatment, i.e., simulated year. Each table was analyzed using a Yates-corrected χ^2 analysis in the Tables routine of SYSTAT. We looked for differences in mortality among treatments using the Mantel–Haenszel test (Snedecor and Cochran 1967). This test is appropriate to detect differences in proportions of binary, e.g., mortality, data in sets of related two-way tables.

For the remaining variables we conducted a two-way ANOVA, with source lake and treatments as main effects. We then used Dunnett's procedure to see if any of the treatments differed significantly from the reference, i.e., the 1993, treatment.

Results

Recovery of *Daphnia galeata mendotae* in Middle and Hannah lakes

The ephippial record confirmed that Hannah Lake supported a

Fig. 3. The deposition history of daphnid ephippia in Hannah Lake. The profile represents the mean and 95% confidence interval of logged counts from 1-cm slices from 10 midlake cores.

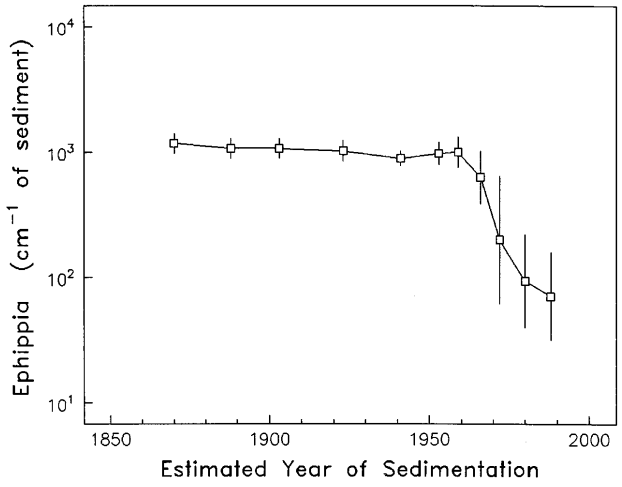
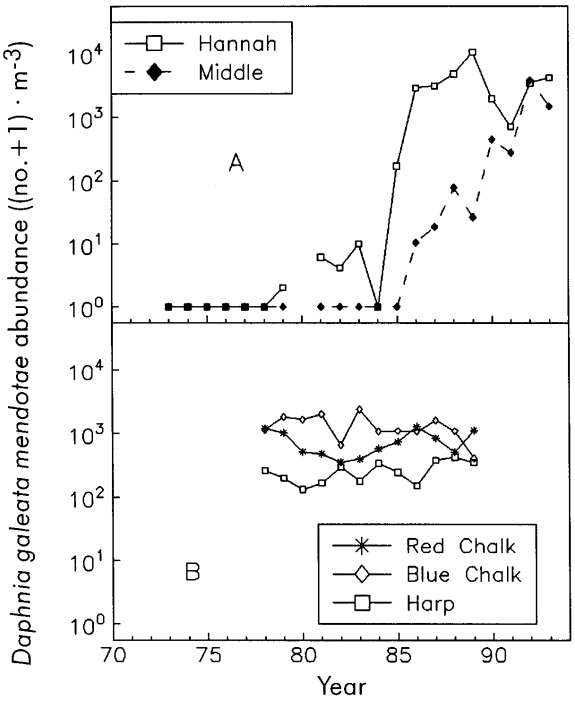


Fig. 4. Long-term changes in the abundance of *D. g. mendotae* in (A) Middle and Hannah lakes, in comparison with (B) the temporal reference lakes. Data are ice-free season averages.



large, stable *Daphnia* assemblage from 1870 to approximately 1960 (Fig. 3). Presumably, Middle Lake, being immediately downstream of Hannah Lake, also supported *Daphnia* spp. The accumulation rate of ephippia in the sediments declined markedly after 1960, associated with the acidification and metal contamination of the lake, which began about 25 years earlier (Dixit et al. 1990). However, ephippia did not disappear from the sediment record in the 1970s, while they did from the plankton (Fig. 4). While this may reflect the relatively coarse operational stratigraphy (1-cm slices), it is more probably a consequence of the blurring of the recent sediment record by mixing. Hannah Lake waters did not stratify in the early 1970s, and they stratified only weakly after the additions of base. Dillon and Smith's (1984) ^{210}Pb profiles suggest that the lake's recent sediment record was perturbed by mixing.

Table 4. Summary of post-hoc tests to determine if there were differences in variability of *D. g. mendotae* abundance among lakes.

Lake	Me	Hh	BC	RC	Hp
Me	—	<0.001	<0.001	<0.001	<0.001
Hh	0.64	—	<0.001	<0.001	<0.001
BC	0.67	1.31	—	1	1
RC	0.66	1.30	0.01	—	1
Hp	0.69	1.32	0.02	0.03	—

Note: The values below the diagonal are the mean paired difference in absolute deviations of abundance (log(x + 1) transformed) averaged over the ice-free season average. The values above the diagonal are the probability for rejecting the null hypothesis of equal absolute deviations for the pair. The lakes are Middle (Me), Hannah (Hh), Blue Chalk (BC), Red Chalk (RC), and Harp (Hp).

Daphnia galeata mendotae appeared in Hannah Lake in 1979, 4 years after the addition of base (Fig. 4A). The population remained small for the next 6 years; it grew rapidly in 1985 and 1986, and ice-free season averages approached 10 animals/L by 1989. At its peaks in 1989 and 1993, *D. g. mendotae* formed a remarkable 30% of all crustacean zooplankton in Hannah Lake, including immature copepods.

Colonization was slower in Middle Lake. *Daphnia galeata mendotae* first appeared in 1986 (Fig. 4A), 13 years after the neutralization of the lake, and 7 years after they appeared in Hannah Lake, immediately upstream. The Middle Lake population increased rapidly in the late 1980s; however, it remained smaller than the Hannah Lake population until the 1990s (Fig. 4).

The interannual variability in abundance of *D. g. mendotae* in Middle and Hannah lakes (Fig. 4A) was unusually large in comparison with the temporal reference lakes (Fig. 4B). The variability in abundance differed among the five lakes (Levene's test, $F = 33.07$, $p < 0.001$; Table 4). Post hoc comparisons indicated that the temporal variation in abundance declined in the order Hannah > Middle > Blue Chalk = Harp = Red Chalk lakes.

The average abundance of *D. g. mendotae* also ranged widely among the 47 spatial reference lakes, particularly for lakes in the pH 5.5–6 range (Fig. 5). In an exploratory stepwise multiple regression, the abundance of *D. g. mendotae* was strongly correlated with pH, but consideration of lake area, depth, and chlorophyll and P concentrations did not improve predictions. In consequence we developed our recovery target from pH alone.

The abundance of *D. g. mendotae* in the spatial reference lakes could be described by a three-parameter logistic regression (eq. 1; Fig. 5, Table 5). As a consequence of the wide variation in *D. g. mendotae* abundance among the lakes our recovery target is necessarily broad. At pH 7, the predicted 95% confidence interval stretched from $10^{1.39}$ to $10^{5.15}$, or from 24.5 to 142 000 animals/m^3. By this criterion, the *D. g. mendotae* population in Hannah lake had recovered by 1985. The population in Middle Lake had recovered by 1988 (Fig. 4A).

Fig. 5. A scattergram of log(x + 1) transformed abundances of *D. g. mendotae* (averaged over the ice-free season) versus pH for the spatial reference lakes. An asymptotic, logistic curve (with 95% confidence interval) is fit to the data.

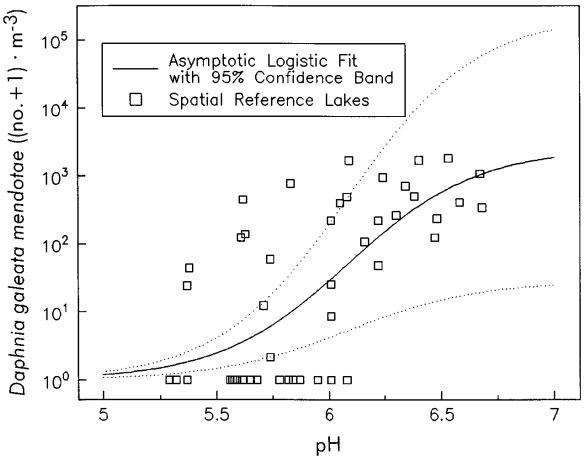


Table 5. Summary of results of fit of asymptotic logistic regression predicting the log of abundance of *D. g. mendotae* from lake water pH in the spatial reference lakes.

Parameter	Lower 95% CI	Estimate	Upper 95% CI	SE
K	1.446	3.403	5.361	0.971
a	–39.65	–21.31	–2.96	9.102
b	0.293	3.502	6.712	2.592

Note: The parameters are described in eq. 1.

The population of *D. g. mendotae* in Hannah Lake was actually unusually large in the 1990s. In the six spatial reference lakes with pH ≥ 6.5, the average abundance of *D. g. mendotae* ranged from about 200 to 2000/m^3 (Fig. 5). After 1985, the average abundance of *D. g. mendotae* in Hannah Lake exceeded all those recorded in both the spatial (Fig. 5) and temporal reference lakes (Fig. 4B).

Genetics

Hannah and Middle lakes currently support genetically diverse populations of *D. g. mendotae* that are not hybridized with other daphnid species. The lack of hybrids is evidenced by the absence or rarity of diagnostic alleles (Table 2) of the potentially hybridizing taxa *Daphnia rosea* (Pep-A) and *Daphnia galeata galeata* (Pep-D) (Taylor and Hebert 1992, 1993). As a consequence, a multivariate summarization of the genotypic frequencies locates the Hannah and Middle lake populations among their North American conspecifics, rather than with other daphnid taxa or hybrids (Fig. 6).

Of the 66 animals examined in each lake we observed 32 clones in Hannah Lake and 36 clones in Middle Lake (Table 2). The absence of an asymptote in clonal dominance–diversity curves (Fig. 7) indicates that the true clonal richness of the populations is much greater. The near concordance of the curves suggests that genetic evenness is similar in each population, even though only 20 genotypes were common to both (Table 2).

No deviations from H–W expectations were found for the loci examined (Table 6), after Bonferroni correction. The absence of deviations from H–W expectations is inconsistent with patterns in lakes that contain swarms of *Daphnia* hybrids (Taylor and Hebert 1993).

Fig. 6. Scattergrams of first two axes of a nonmetric multidimensional scaling ordination of pooled genotypic frequencies of *D. galeata mendotae* populations from Middle and Hannah lakes and the reference lakes. The populations and hybrids indicated are *D. g. mendotae* from Middle and Hannah lakes (*), *D. rosea* (○), the Eurasian *D. galeata* (□), *D. g. mendotae* from Ontario (△) and Indiana (▲), *D. mendotae* × *D. rosea* hybrids (●), and *D. g. mendotae* × *D. galeata* hybrids (■).

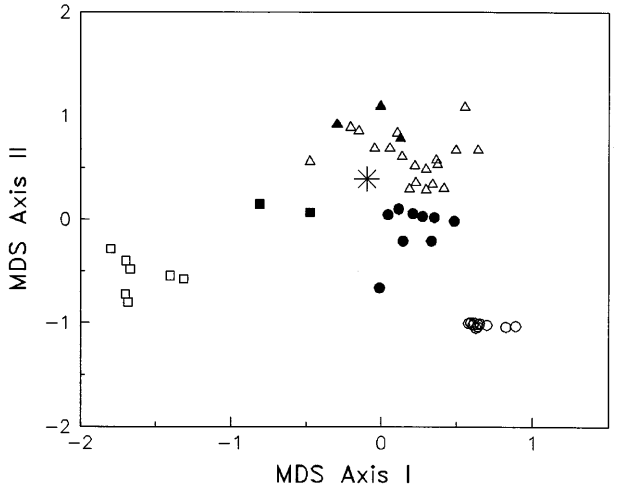
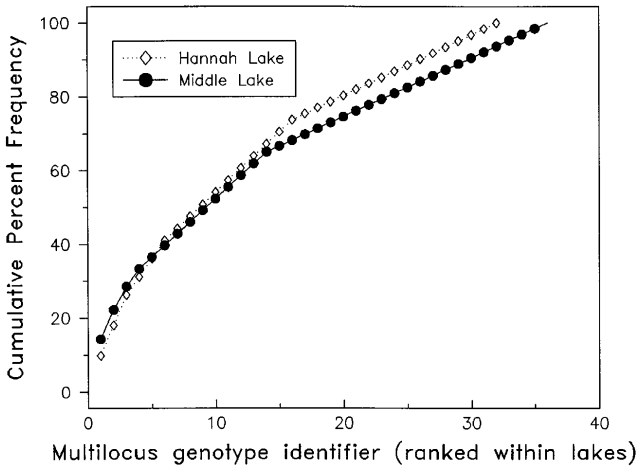


Fig. 7. A clonal dominance–diversity curve for the *D. g. mendotae* from Middle and Hannah lakes. The cumulative frequency of multilocus genotypes is plotted against the ranked frequency of that genotype within each population.



Results of bioassays

The treatments simulated total Cu, Ni, and Cd concentrations in Middle and Hannah lakes in 1976, 1981, 1985, 1989, and 1993. The 1976 treatment represented the period after liming but before the appearance of *D. g. mendotae* in either lake (Fig. 4A). The 1981 treatment was positioned at the midpoint of the period of low *D. g. mendotae* density in Hannah Lake.

Table 6. Allelic frequencies for the five loci in the *D. g. mendotae* populations of Middle and Hannah lakes.

		Middle		Hannah	
Locus	Allele	Frequency	*p*	Frequency	*p*
GPI[a]	95	0.242	0.27	0.348	0.22
	100	0.641		0.606	
	107	0.117		0.045	
PGM[b,c]	89	0.154	1.0	0.164	0.041
	100	0.846		0.836	
AAT-S[b]	100	0.516	1.00	0.583	0.80
	114	0.484		0.417	
PEP-A[b]	100	0.015	1.00	0.015	1.00
	121	0.985		0.985	
PEP-D[b]	81	0.838	1.00	0.811	1.00
	100	0.162		0.189	

Note: Frequencies were developed for between 61 and 66 animals for each locus. Probabilities for detecting departures from Hardy–Weinberg expectations are indicated.

[a]χ^2 test.

[b]Exact probability test.

[c]The large difference in *p* values between lakes is due to differing genotype frequencies. The Hannah Lake value is not significant after sequential Bonferroni correction.

The 1985 treatment immediately preceded the appearance of *D. g. mendotae* in Middle Lake. The period of rapid population expansion in Middle Lake and a large stable population in Hannah Lake was represented by the 1989 treatment. Finally, the 1993 treatment represented the current condition, with large populations in both lakes.

Modelled divalent Cu and Ni ion concentrations were consistently higher in Middle Lake than in Hannah Lake bioassays (Fig. 8), even though the total concentrations of Cu, Cd, and Ni in analogous treatments were similar (Fig. 2). Modelled free Cd concentrations were similar in analogous treatments (Fig. 8).

Conventional diagnostics indicated that the bioassays were methodologically sound. Eighty percent of the test animals survived the 21-day exposures in the reference (1993) treatments (Fig. 9). The experiments were of sufficient duration that lethal thresholds stabilized (Fig. 10). Finally, the treatments produced a wide range of responses, from complete mortality in the 1976 treatments to survivorship approaching or equalling reference levels in the 1989 treatments (Fig. 9).

The 1976 treatment was lethal to all test *D. g. mendotae* in both lakes (Fig. 9). Percent mortality did not differ from reference levels in any of the other Hannah Lake treatments (Fig. 11A). By contrast, there was a perfect rank order relationship between percent mortality in Middle Lake and treatment order (Fig. 11A), mortality declining with the simulated passage of time.

Yate's-corrected χ^2 test identified a much greater mortality in the 1981 Middle Lake treatment than in the analogous Hannah Lake treatment (Fig. 11A). These results are consistent with the absence of *D. g. mendotae* in Middle Lake in the latter 1970s and early 1980s (Fig. 4A), despite its appearance in the upstream Hannah Lake.

Those neonate *D. g. mendotae* that survived developed rapidly in the post–1976 treatments, reaching primiparity within 7–8 days (Fig. 11C). Clutch size differed among lakes ($p < 0.001$) and treatments ($p = 0.003$; Fig. 11B). The *D. g. mendotae* from Hannah Lake produced larger clutches than their Middle Lake counterparts in all treatments including the reference year. This implies either that developmental rates differed between the Middle and Hannah Lake experimental clones or that subacute toxicity is evident even at reference metal levels.

For Hannah Lake, clutch size differed from that in the reference year in the 1981 treatment but not thereafter (Fig. 11B). In contrast, the mean clutch size of the Middle Lake *D. g. mendotae* was depressed in comparison with the reference year in all treatments (Fig. 11B). The difference from the reference year was greatest for the 1981 treatment. Only one *D. g. mendotae* survived the simulated 1981 water quality in the Middle Lake assays (Fig. 11A). It was slow to mature (Fig. 11C) and produced very few neonates (Fig. 11B), most of which died within 24 h (Figs. 11E and 11F). These results are consistent

Fig. 8. Comparison of modelled divalent cation concentrations ($\mu g \cdot L^{-1}$) of metals in the Middle and Hannah lake bioassays. Years identify the treatments.

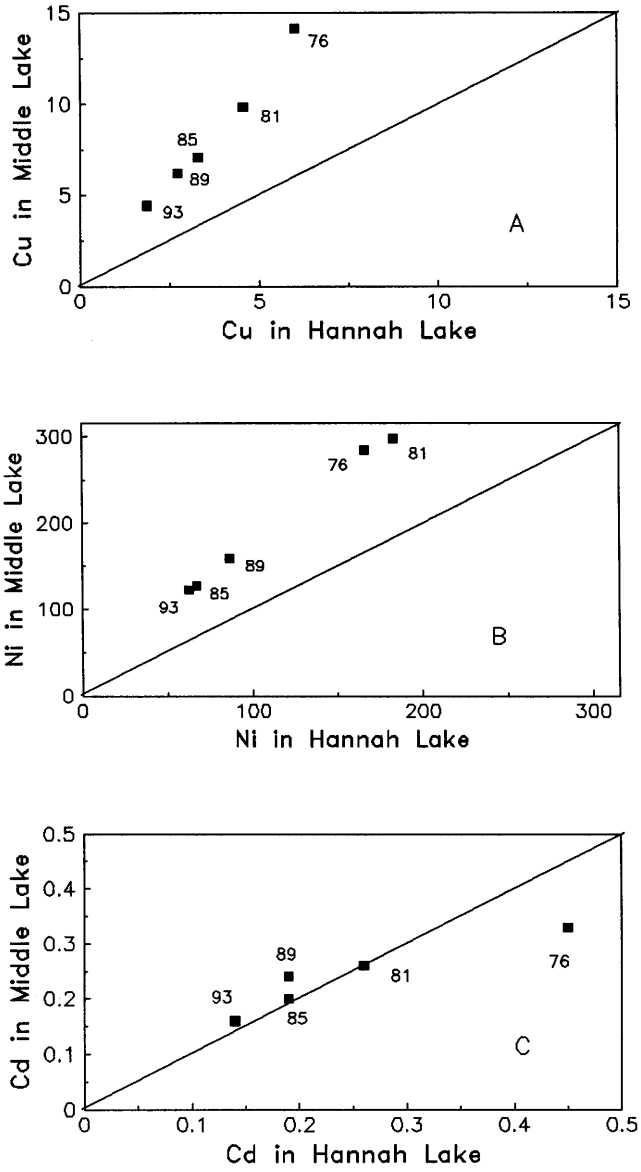


Fig. 9. Survival of *D. g. mendotae* in the five Middle and Hannah lake treatments.

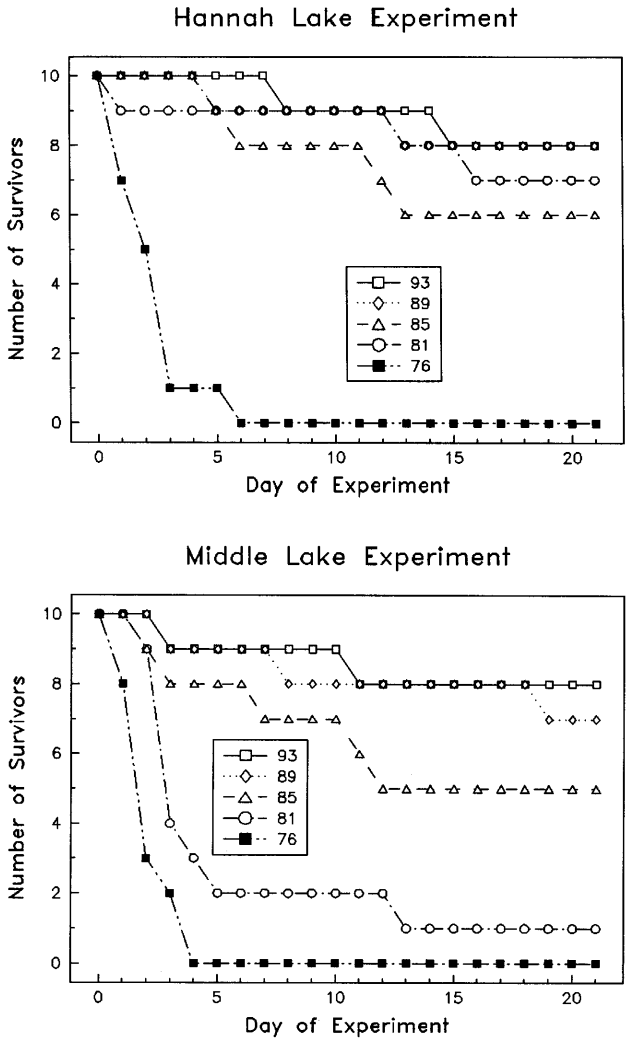
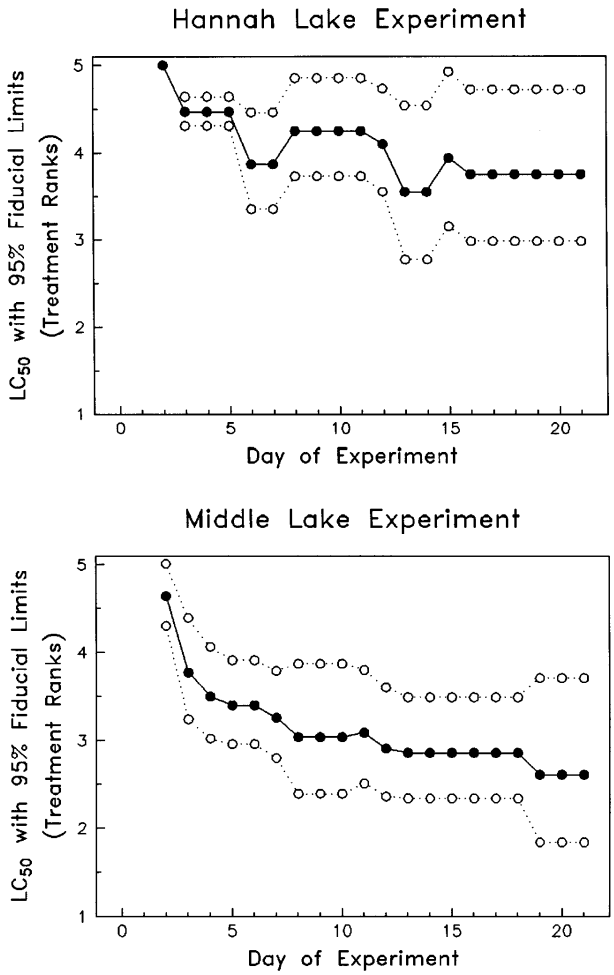


Fig. 10. The relationship between experimental duration and estimated LC_{50}, expressed as the treatment's rank: 1, 1993; 2, 1989; 3, 1985; 4, 1981; 5, 1976.



with the failure of *D. g. mendotae* to colonize Middle Lake in the latter 1970s and early 1980s despite its colonization of the upstream Hannah Lake.

The number of broods produced during the 21-day bioassays differed among lakes but not among treatments (Fig. 11D), the Hannah Lake clone producing about one extra brood. This is probably an artifact of a fixed experimental duration of 21 days and the more rapid development of the Hannah Lake clone (Fig. 11C).

The fate of the neonates produced during the experiments varied among lakes and treatments. In particular, both major effects were significant for the numbers of neonates produced that survived for 24 h (Fig. 11E). More survivors were produced by the Hannah Lake than the Middle Lake clones, although this is in part attributable to the extra brood produced by the Hannah Lake animals during the experiment. The survival of neonates was reduced in the 1981 treatment for both lake clones (Figs. 11E and 11F).

Discussion

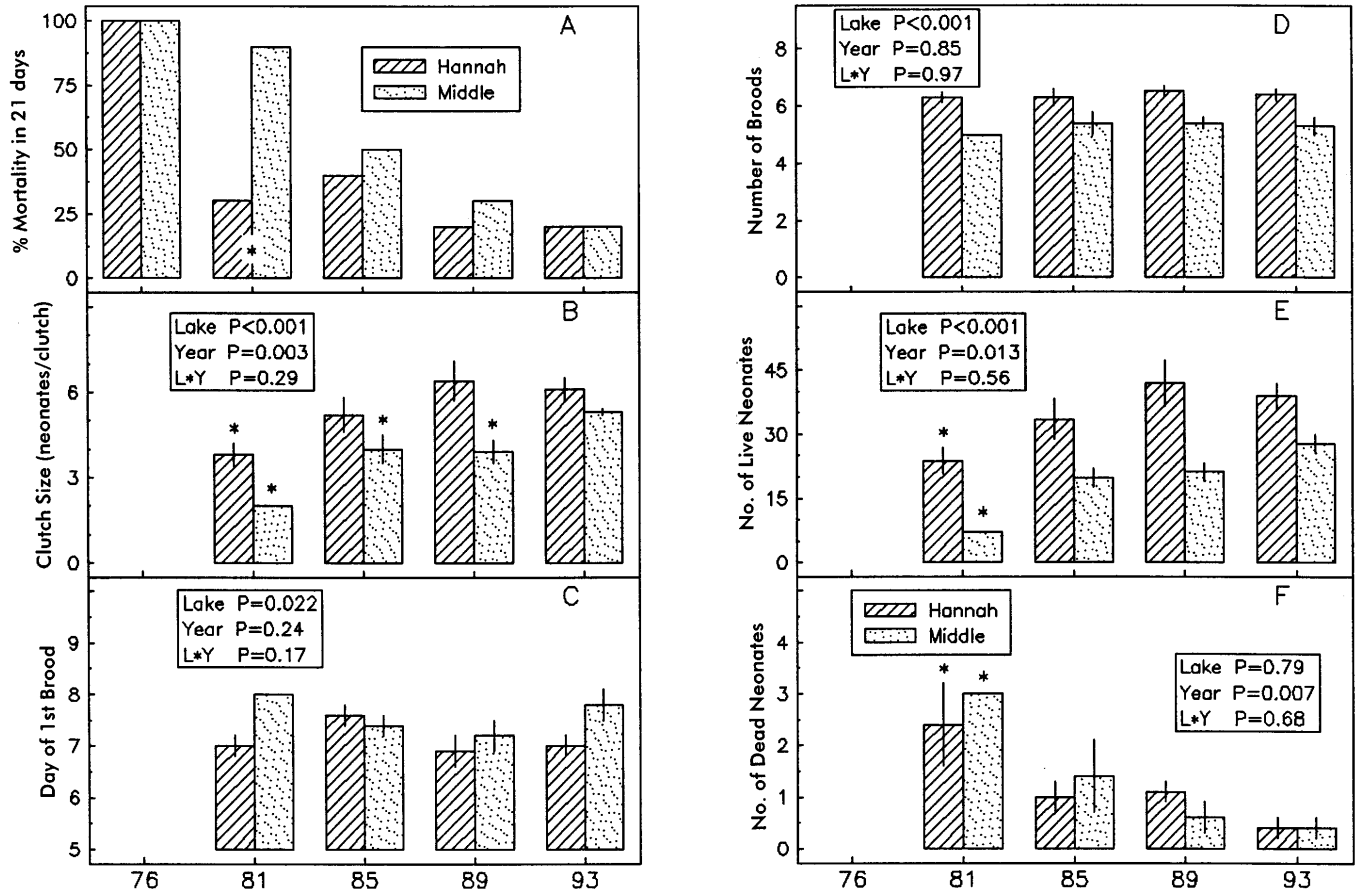
This study posed two questions. Has *D. g. mendotae* recovered in Middle and Hannah Lakes? If it has, did habitat quality, manifested as concentrations of potentially toxic metals, regulate the pace of recovery? Our answer to each question is a yes but with qualifications.

Has *Daphnia galeata mendotae* recovered?

The evidence for the recovery of *D. g. mendotae* in Middle and Hannah lakes is superficially strong. The lakes now support large populations of *D. g. mendotae* (Fig. 4), comprised of clonal mixtures that typify the taxon (Fig. 6). However, two qualifiers must be considered: the definition of recovery we employed and the significance of the unusually large *D. g. mendotae* populations. First, because of the absence of predisturbance data, recovery was defined not as a return to the prestressed condition, the conventional definition (Niemi et al. 1993), but as the attainment of a population typical of the region. Secondly, the exceptionally large size of the *D. g. mendotae* populations of Middle and Hannah lakes may not represent a stable state.

We can be reasonably confident that Middle and Hannah lakes did actually support *D. g. mendotae* in the past. The sediment record proves that they supported Daphniidae (Fig. 3), and *D. g. mendotae* is the most common daphnid in the region

Fig. 11. Summary of the bioassay results after 21 days. The bar height represents the mean value for the response metric (±SD, $n = 10$). Probabilities for rejecting the null hypothesis associated with the two main effects and their interaction are indicated. For A, an asterisk identifies a treatment with a significant lake effect detected by the Mantel–Haenszel test. For B–F, asterisks highlight treatments differing ($p < 0.05$) from the reference treatment (1993) for the lake using the Dunnett's procedure.



(Keller and Pitblado 1989). Because *D. g. mendotae* were recorded in all 17 of our intensively studied, spatial reference lakes with pH > 6.1 (Fig. 5) the actual frequency of occurrence of the taxon may exceed Keller and Pitblado's already substantial figure of 80% occurrence. Therefore, there is a high probability that Middle and Hannah lakes supported *D. g. mendotae* in their uncontaminated past. Hence, there is also a high probability that the recent establishment of *D. g. mendotae* in Middle and Hannah lakes represents recovery in the conventional sense of the term, not novel invasions.

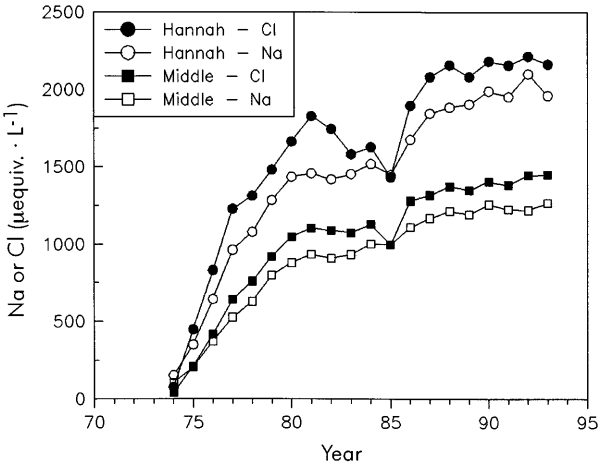
The colonization of Middle and Hannah lakes by *D. g. mendotae* is impressive given the number of barriers potential colonists had to overcome. The contamination of the lakes was chronic, severe, and multifaceted, involving toxic levels of acidity and several trace metals (Table 1). The stresses have been reduced but not eliminated. Metal concentrations are still one (Cu) to two (Ni) orders of magnitude above levels in lakes remote from Sudbury (Fig. 1). Finally, the production rate of potential colonists must be comparatively low near Sudbury. Middle and Hannah lakes are in a chronically contaminated region. Further, because the lakes have been contaminated for so long, any endogenous recruitment from long-lived ephippia must also have been curtailed both by ongoing sediment burial and by unsuccessful hatching into historically inhospitable waters.

Despite all of these hurdles, *D. g. mendotae* has colonized Middle and Hannah lakes. This augurs well for the recovery of this important zooplankton species in other acidified lakes in eastern North America that face far fewer obstacles to biological recovery.

The recovery of *D. g. mendotae* in Middle and Hannah lakes has precedents. Chang and Malley (1989) noted the reappearance of the species in the experimentally acidified Lake 223 in northwestern Ontario after the lake pH was allowed to rise from 5.0 to 5.6. In the Sudbury area, Keller and Yan (1991) noted that *D. g. mendotae* reappeared in small numbers in Joe Lake after the pH increased from 5.6 in the mid–1970s to 6.3 a decade later. Yan et al. (1996) documented the reappearance of the taxon in Nelson Lake near Sudbury after the pH was increased from 5.6 to >6 by additions of base. Finally, Locke et al. (1994) noted a general pattern of increases in *D. g. mendotae* abundances following water quality improvements in those Sudbury lakes whose pH levels had risen from below to above 6.0. It appears that *D. g. mendotae* may be as good an indicator of the onset of recovery of zooplankton from acidification as it was of damage attributable to acidification (Keller et al. 1990).

The second qualifier concerns the unprecedented size of the *D. g. mendotae* populations in Middle and Hannah lakes. Re-

Fig. 12. Long-term changes in ice-free season averages of Na and Cl in Middle and Hannah lakes.



covery carries connotations of populations that are neither too large nor too small. By the end of this study, the populations in Middle and Hannah lakes were larger than in any of the temporal or spatial reference lakes.

This situation will probably not last. Currently, *D. g. mendotae* is the only large cladoceran in Middle and Hannah lakes (Yan et al. 1996). It has no competitors for the large grazer niche (e.g., Tessier 1986), which perhaps accounts for its unusually large populations. In acidified lakes distant enough from Sudbury that metal levels are low, recent water quality improvements have been accompanied by the appearance of several large cladoceran species once pH rises above 6 (Locke et al. 1994). Therefore, we predict that the *D. g. mendotae* populations of Middle and Hannah lakes will fall to more typical levels if species richness continues to increase.

Can metals account for the recovery?

Our second objective was to determine if metals regulated the pace of recovery of *D. g. mendotae* in the study lakes. The pattern of survival and brood production of *D. g. mendotae* in the bioassays was consistent with the long-term changes in abundance of the species in the lakes (Figs. 4 and 11). However, several difficulties must be overcome en route to bridging the gap between correlation and cause.

The comparison of field and laboratory ecotoxicological data is always difficult (Cairns 1986). We cannot yet predict the size of populations in nature given their survival and reproduction in laboratory assays. There are too many physical, chemical, and biological factors to consider. Zooplankton community composition may be influenced by the weather (George and Harris 1985), water quality (Keller et al. 1990), inherent genetic tendencies (Vanni 1987), competition for resources (Neill 1984), vertebrate (Brooks and Dodson 1965) or invertebrate predators (Lynch 1979), epibiontic burden (Threlkeld et al. 1993), and parasites (Yan and Larsson 1988). Of relevance to this study, there may also be clonal differences in metal tolerance (Baird et al. 1990, 1991) and innumerable clones (e.g., Fig. 7) in nature. These factors may act directly and singly, but they more commonly act indirectly and in combinations (e.g., Neill 1978; Arnott and Vanni 1993; Yan et al. 1991). Such complexity confounds prediction and experimental verification. Hence, while our bioassay results support one logically possible explanation for the pace of recovery, they cannot exclude other untested (but hopefully less probable) possibilities (Peters 1990).

Similar difficulties were faced in attempting to attribute the disappearance of taxa from acidifying lakes to their acidity. Holtze and Hutchinson (1989), Keller et al. (1990), and Havens et al. (1993) adopted a pragmatic approach. They asserted that they could ascribe the absence of regionally common taxa in acidic lake to acidification, if sound experiments of sufficient duration in appropriate test waters using the most sensitive life stages of the taxa produced data on the probability of individual survival that were in accord with the probability of the presence of those taxa in nature. We can use a similar approach to determine if metals were the probable regulators of recovery. To do so we must demonstrate that (*i*) the assays were sound, (*ii*) the most sensitive life stages were included, (*iii*) the test waters and experimental conditions were appropriate, and (*iv*) the results are consistent with the published literature on metal toxicity to *Daphnia* spp.

The first two conditions are readily met. Conventional diagnostics indicated that the experiments were sound. There was good survival in the reference treatments and a monotonic relationship between contaminant concentration and mortality (Fig. 8). The experiments were of sufficient duration that lethal thresholds were reached (Fig. 10). Neonates are the most sensitive life stage of Cladocera (Adema 1978) and these were included in the design. In fact, given the 21-day duration of the exposures, the only daphnid life stages that were not examined were senescent animals and ephippial embryos.

Condition three is more difficult to satisfy. The daphnid cultures were established from the actual populations of interest. The exposure media were developed from water taken from the test lakes, and the metal levels used in the bioassays were within ranges of metals observed in the simulated years (Fig. 2). These are the key attributes of appropriate test media, organisms, and experimental design. However, our simulation of past conditions was imperfect in two potentially important ways: concentrations of salt and Al.

Road salt concentrations have increased dramatically in Middle and Hannah lakes mainly because of de-icing operations on a highway bypass constructed through the lakes' catchments (Yan and Dillon 1984). Because our bioassay media used 1993 water as a base, the Na and Cl concentrations of our bioassays exceeded those in the simulated years (Fig. 12). However, the salt levels of the bioassays were only 50 and 14%, respectively, in excess of levels observed in the year that *D. g. mendotae* colonized Hannah and Middle lakes (1979 and 1986, respectively). Such modest changes from already substantial salt levels should have had little influence on the latter treatments. Na and Cl form weak bonds with most ligands; hence, they have little impact on metal speciation (Buffle and Stumm 1994). If the lethal mechanism of metals is disruption in ionoregulation (Havens 1992), the elevated salt levels in the 1976 simulations might have been expected to increase survival. Nonetheless, complete mortality was observed early in this treatment (Fig. 9).

Other than Cu, Ni, and Cd, Al is the most important metal we excluded from the assays. Al levels are commonly elevated in acidic lakes in Ontario (Spry and Wiener 1991), and Middle and Hannah lakes were no exception in the early 1970s

(Fig. 1). However, after additions of base, Al levels were always less than 100 $\mu g \cdot L^{-1}$ in the lakes (Fig. 1). At circumneutral pH levels, such Al concentrations have no influence on the survival or the physiology of *D. g. mendotae* (Havens 1992). Therefore, the exclusion of Al did not bias our experimental results, and the third condition is satisfied.

Finally, the toxicological literature supports our contention that metals, Ni and especially Cu, are probable regulators of the recovery of *D. g. mendotae* in Middle and Hannah lakes. Extreme sensitivity of daphnids to Cu has been demonstrated by Ingersoll and Winner (1982) and Koivisto et al. (1992). They observed reductions in longevity at Cu levels as low as 5–10 $\mu g \cdot L^{-1}$. More typically, Biesinger and Christensen (1972) recorded 16% reproductive impairment of *Daphnia magna* at 22 $\mu g \cdot L^{-1}$ in 3-week assays. Winner (1984) recorded a 72-h LC_{50} for *Daphnia* sp. of 28 $\mu g \cdot L^{-1}$ without added organic carbon and 53 $\mu g \cdot L^{-1}$ with added humate. Winner and Farrell (1976) recorded 72-h LC_{50}s for Cu to four *Daphnia* species (*D. magna, D. pulex, D. parvula*, and *D. ambigua*) of 54–87 $\mu g \cdot L^{-1}$ in hard water and suggested that long-term survival would be possible at about half these levels, or 27–44 $\mu g \cdot L^{-1}$.

These laboratory-based lethal thresholds for Cu levels bridge the range recorded over the time series in Middle and Hannah lakes. Concentrations averaged >50 μg $Cu \cdot L^{-1}$ in 1976 (Fig. 2), levels that the literature indicates are undoubtedly lethal to daphnids. No animals survived in our 1976 treatments (Fig. 11A), and none were observed in the lakes (Fig. 4). By the late 1980s, Cu levels in Hannah and Middle lake had fallen to 21 and 28 $\mu g \cdot L^{-1}$, respectively, levels low enough to permit daphnid survival according to Winner and Farrell (1976). Hence, Cu toxicity alone may account for our bioassay results and the temporal pattern of natural recovery.

Nickel toxicity may also have contributed to the pattern of recovery. Münzinger (1990, 1994) noted that Ni levels in excess of 40 $\mu g \cdot L^{-1}$ lower the longevity of *Daphnia magna* in hard waters. Clutch size is also reduced at Ni levels >80 $\mu g \cdot L^{-1}$. While Ni levels declined steadily in the lakes, they still exceeded 100 $\mu g \cdot L^{-1}$ in Middle and Hannah lakes in 1993 (Fig. 1).

We are less certain about Cd. The inter- and intra-annual variability in the Cd time series (Fig. 1) suggests sample contamination problems (Yan et al. 1990); hence, field exposures can only be approximated, especially in the first half of the time series. Furthermore, there is some disagreement in the published literature about chronically lethal levels. Concentrations were <0.6 $\mu g \cdot L^{-1}$ in the lakes and bioassays after 1985 (Fig. 2). Most studies of chronic Cd toxicity to *Daphnia* sp. suggest that such concentrations pose no threat to daphnid health (Marshall 1978; Lawrence and Holoka 1987; Borgmann et al. 1989; Baird et al. 1990). However, Biesinger and Christensen (1972) found that exposure to 0.17 μg $Cd \cdot L^{-1}$ reduced the reproductive output of daphnids. Because we do not have reliable Cd data from the late 1970s and early 1980s, we cannot assess the influence of Cd during the early stages of recovery. However, the main body of literature suggests that Cd would not have restricted recovery in the late 1980s.

In both the field and the laboratory, the animals were exposed to the metals, not singly but in mixtures. The toxic effects of metals are additive for daphnids (Biesinger et al. 1986; Enserink et al. 1991). Hence, the pace of recovery of *D. g. mendotae* in Middle and Hannah lakes was likely regulated by the joint action of the metals, not by their individual toxicity.

Metal toxicity may also account for the differences in *D. g. mendotae* performance in analogous (i.e., same year) Middle and Hannah lake treatments. The free, divalent species of Cu and Ni is conventionally assumed to be the most toxic species (Morel 1983). Free Cu and Ni levels were higher in the Middle Lake than in the analogous Hannah Lake treatments, particularly in the earliest simulations (Fig. 8), perhaps accounting for differences in survival and brood production between the bioassays.

Yellow perch (*Perca flavascens*) invaded Hannah Lake in 1986 and contributed >99% of the numbers and mass of fish captured in both lakes after 1989 (Wright 1995). However, there is no evidence that fish influenced the rate of recovery of *D. g. mendotae* in either lake. The biomass of yellow perch averaged only about 10 $kg \cdot ha^{-1}$, less than in reference lakes (Wright 1995) and much below the biomasses required to depress zooplankton standing stocks (McQueen and Post 1988). While fish may also reduce the body size of zooplankton (Carpenter et al. 1995; Ramcharan et al. 1995), the body lengths of *D. g. mendotae* did not exhibit any long-term trends in Middle and Hannah lakes, averaging 0.87 and 0.90 mm, respectively, in the lakes.

In summary, while we cannot unequivocally ascribe the recovery of *D. g. mendotae* in Middle and Hannah lakes to reductions in their metal levels, this is the most likely explanation. The bioassays were methodologically sound and appropriately designed to test this question. The assays produced results that matched the 20-year time series of in situ recovery. Finally, this explanation is consistent with a sizeable body of metal toxicity data for daphnids.

General discussion

There have been very few studies of recovery of zooplankton from long-term stresses. Niemi et al. (1990) cite only three in their review, noting that recovery from such stressors can take several years at temperate latitudes. Despite the paucity of previous work, ours is not the first example of recovery of zooplankton from metal stress. The zooplankton community in Buttle Lake in British Columbia recovered 4 years after the metal loading to the lake from a mine site was significantly reduced (Deniseger et al. 1990).

There are other signs of biological recovery in Middle and Hannah lakes in addition to the recovery of *D. g. mendotae*. The diatom communities have recovered (Dixit et al. 1987, 1990). Yan et al. (1996) record improvements in the richness, diversity, and several multivariate community metrics of the zooplankton. Wright (1995) tracked the progress of the yellow perch that invaded the lakes in the mid–1980s. Nevertheless, the lakes remain biologically impoverished. For example, crustacean zooplankton richness is only half what it should be (Yan et al. 1996).

This is the first study to document the recovery of zooplankton from acidification at both the genetic and population levels of organization. Research at the interface of zooplankton ecology and genetics is burgeoning (Mort 1991). In Middle and Hannah lakes, the genetic component provides a clue to the probable colonist source: the ephippial egg bank. The resting stages of zooplankton may remain viable in bottom sediments

for at least several decades (Carvalho and Wolf 1989; Marcus et al. 1994). Hebert et al. (1989) associated H–W deviations either to hybridization or to lack of recruitment from an ephippial egg bank. Because the *D. g. mendotae* populations in the study lakes were not hybridized (Fig. 6), the absence of H–W deviations indicates either recovery of the ephippial egg bank or recruitment from an old egg bank established before population extinction. Additional research on the source of the colonists is warranted, because there is a higher probability that water quality improvements will be followed by biological recovery in particular lakes if the colonist source is an internal egg pool rather than an external pool of propagules.

The bioassay detected differences between the experimental clones in rates of development, brood production, and clutch size in the reference treatments (Fig. 11). Without performing reciprocal transplant experiments (each isolate in media from each lake), we cannot prove that these results have a genetic cause. However, a genetic basis for the results would not be surprising. Vanni (1987) recorded differences among *D. pulex* genotypes in size and age at primiparity, size of offspring, and clutch size after the first brood. Baird et al. (1990, 1991) observed large differences in Cd sensitivity among clones of *D. magna*.

The impact of metal exposure was clearly discernable from the clonal effect in the experiment (Fig. 11). This is the most significant result of the experiment. The good match of the bioassay and field data indicates that metal concentrations regulated the pace of recovery of *D. g. mendotae* in Middle and Hannah lakes. These lakes represent an extreme test for recovery. They were severely contaminated for many years with both acid and metals. Production rates of potential colonists probably were curtailed by the broad spatial and long temporal attributes of the problem. Under such trying conditions, it was habitat quality, not dispersal, that regulated recovery of *D. g. mendotae*. This augurs well for the probability of recovery from acidification of this and other ephippium-producing zooplankton in North America, should water quality improve in response to reductions in continental emissions of SO_2.

Acknowledgements

We thank Peter Dillon, Neil Hutchinson, Keith Somers, Karl Havens, and Martyn Futter for advice; Kim Miller, Bob Girard, Peter Grauds, John Colborne, Anne Neary, Sharon Cushing, and Julie Desjardins for technical assistance; Bill Keller for his unpublished 1992 data; and Paul Hebert for access to his genetics laboratory. Funding for H.L.'s stay at the Dorset Research Centre was provided to George Dixon at the University of Waterloo by the World University Service of Canada's Canada–China Human Development Training Programme.

References

Adema, D.M.M. 1978. *Daphnia magna* as a test animal in acute and chronic toxicity tests. Hydrobiologia, **59**: 125–134.

Allison, J.D., and Perdue, E.M. 1994. Modelling metal–humic interactions with MINTEQA2. Allison Geoscience Consultants, 3920 Perry Lane, Flowery Branch, GA 30542, U.S.A.

Allison, J.D., Brown, D.S., and Novo-Gradac, K.J. 1990. MINTEQA2/PRODEFA2, a geochemical assessment model for environmental systems: user's manual, version 3.0 edition. EPA/600/3–91/021. U.S. Environmental Protection Agency, Athens, Ga.

Arnott, S.E., and Vanni, M.J. 1993. Zooplankton assemblages in fishless bog lakes: influence of biotic and abiotic factors. Ecology, **74**: 2361–2380.

Baird, D.J., Barber, I., and Calow, P. 1990. Clonal variation in general responses of *Daphnia magna* Straus to toxic stress. 1. Chronic life-history effects. Funct. Ecol. **4**: 399–408.

Baird, D.J., Barber, I., Bradley, M., Soares, A.M.V.M., and Calow, P. 1991. A comparative study of genotype sensitivity to acute toxic stress using clones of *Daphnia magna* Straus. Ecotoxicol. Environ. Saf. **21**: 257–265.

Biesinger, K.E., and Christensen, G.M. 1972. Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*. J. Fish. Res. Board Can. **29**: 1691–1700.

Biesinger, K.E., Christensen, G.M., and Fiandt, J.T. 1986. Effects of metal salt mixtures on *Daphnia magna* reproduction. Ecotoxicol. Environ. Saf. **11**: 9–14.

Blomqvist, P., Bell, T.T., Olofsson, H., Stensdotter, U., and Vrede, K. 1993. Pelagic ecosystem responses to nutrient additions in acidified and limed lakes in Sweden. Ambio, **22**: 283–289.

Borgmann, U., Millard, E.S., and Charlton, C.C. 1989. Effect of cadmium on a stable, large volume laboratory ecosystem containing *Daphnia* and phytoplankton. Can. J. Fish. Aquat. Sci. **46**: 399–405.

Brooks, J.L. 1957. The systematics of North America *Daphnia*. Mem. Conn. Acad. Arts Lett. **13**: 1–180.

Brooks, J.L., and Dodson, S.I. 1965. Predation, body size, and composition of plankton. Science (Washington, D.C.), **150**: 28–35.

Buffle, J., and Stumm, W. 1994. General chemistry of aquatic systems. *In* Chemical and biological regulation of aquatic systems. *Edited by* J. Buffle and R.R. DeVitre. Lewis Publishers, Boca Raton, Fla. pp. 1–42.

Cairns, J., Jr. 1986. What is meant by validation of predictions based on laboratory toxicity tests? Hydrobiologia, **137**: 271–278.

Cairns, J., Jr. 1990. Lack of a theoretical basis for predicting rate and pathways of recovery. Environ. Manage. **14**: 517–526.

Carpenter, S.R., Christensen, D.L., Cole, J.J., Cottingham, K.L., He, X., Hodgson, J.R., Kitchell, J.F., Knight, S.E., Pace, M.L., Post, D.M., Schindler, D.E., and Voichick, N. 1995. Biological control of eutrophication in lakes. Environ. Sci. Technol. **29**: 784–786.

Carter, J.C.H., Dadswell, M.J., Roff, J.C., and Sprules, W.G. 1980. Distribution and zoogeography of planktonic crustaceans and dipterans in glaciated eastern North America. Can. J. Zool. **58**: 1355–1387.

Carter, J.C.H., Taylor, W.D., Chengalath, R., and Scruton, D.A. 1986. Limnetic zooplankton assemblages in Atlantic Canada with special reference to acidification. Can. J. Fish. Aquat. Sci. **43**: 444–456.

Carvalho, G.R., and H.G. Wolf. 1989. Resting eggs of lake *Daphnia*. I. Distribution, abundance, and hatching of eggs from various depths in the lake sediments. Freshwater Biol. **22**: 459–470.

Cavalli-Sforza, L.L., and Edwards, A.W.F.. 1967. Phylogeny analysis and estimation procedures. Evolution, **21**: 550–570.

Chang, P.S.S., and Malley, D.F. 1989. Partial recovery of the zooplankton community in a small Precambrian Shield lake as experimental acidification is reduced. Symp. Biol. Hung. **38**: 203–208.

Deniseger, J., Erickson, L.J., Austin, A., Roch, M., and. Clark, M.J.R. 1990. The effects of decreasing heavy metal concentrations on the biota of Buttle Lake, Vancouver Island, British Columbia. Water Res. **24**: 403–416.

Detenbeck, N.E., DeVore, P.W., Niemi, G.J., and Lima, A. 1992. Recovery of temperate-stream fish communities from disturbance: a review of case studies and synthesis of theory. Environ. Manage. **16**: 33–53.

Dillon, P.J., and Smith, P.J. 1984. Trace metal and nutrient accumulation in the sediments of lakes near Sudbury, Ontario. *In* Environmental impacts of smelters. *Edited by* J. Nriagu. J. Wiley and Sons, New York. pp. 375–416.

Dillon, P.J., Yan, N.D., Scheider, W.A., and Conroy, N. 1979. Acidic lakes in Ontario, Canada: characterization, extent and responses to base and nutrient additions. Ergeb. Limnol. **13**: 317–336.

Dillon, P.J., Yan, N.D., and Harvey, H.H. 1984. Acidic deposition: effects on aquatic ecosystems. Crit. Rev. Environ. Control, **13**: 167–194.

Dillon, P.J., Reid, R.A., and Girard, R. 1986. Changes in the chemistry of lakes near Sudbury, Ontario, following reductions of SO_2 emissions. Water Air Soil Pollut. **31**: 59–65.

Dillon, P.J., Nicholls, K.H., Locke, B.A., de Grosbois, E., and Yan, N.D. 1988. Phosphorus–phytoplankton relationships in nutrient-poor soft-water lakes in Canada. Verh. Int. Ver. Theor. Angew. Limnol. **23**: 258–264.

Dixit, S.S., Dixit, A.S., and Evans, R.D. 1987. Paleolimnological evidence of recent acidification in two Sudbury (Canada) lakes. Sci. Total Environ. **67**: 53–67.

Dixit, S.S., Dixit, A.S., and Smol, J.P. 1990. Paleolimnological investigation of three manipulated lakes from Sudbury, Canada. Hydrobiologia, **214**: 245–252.

Enserink, E.L., Mass-Diepeveen, J.L., and Van Leeuwen, C.J. 1991. Combined effects of metals; an ecotoxicological evaluation. Water Res. **25**: 679–687.

George, D.G., and Harris, G.P. 1985. The effect of climate on long-term changes in the crustacean zooplankton biomass of Lake Windemere, UK. Nature (London), **316**: 536–539.

Havens, K.E. 1992. Acid and aluminum effects on sodium homeostasis and survival of acid-sensitive and acid-tolerant Cladocera. Can. J. Fish. Aquat. Sci. **49**: 2392–2398.

Havens, K.E., Yan, N.D., and Keller, W. 1993. Lake acidification: effects on crustacean zooplankton populations. Environ. Sci. Technol. **27**: 1621–1624.

Hebert, P.D.N., and Beaton , M.J. 1993. Methodologies for allozyme analysis using cellulose acetate electrophoresis. Helenea Laboratories, Beaumont, Tex.

Hebert, P.D.N., Schwartz, S.S., and Hrbáček, J. 1989. Patterns of genetic diversity in Czechslovakian *Daphnia*. Heredity, **62**: 207–216.

Holtze, K.E., and Hutchinson, N.J. 1989. Lethality of low pH and Al to early life stages of six fish species inhabiting Precambrian Shield waters in Ontario. Can. J. Fish. Aquat. Sci. **46**: 1188–1202.

Hosmer, D.W., and Lemershow, S. 1989. Applied logistic regression. John Wiley & Sons, New York.

Ingersoll, C.G., and Winner, R.W. 1982. Effect on *Daphnia pulex* (de Geer) of daily pulse exposures to copper or cadmium. Environ. Toxicol. Chem. **1**: 321–327.

Jeffries, D.S., Lam, D.C.L., Wong, I., and Bloxam, R.M. 1992. The predicted effect of SO_2 emission controls on the water quality of eastern Canadian lakes. Environ. Monit. Assess. **23**: 99–113.

Keller, W., and Conlon, M. 1995. Crustacean zooplankton communities and lake morphometry in Precambrian Shield lakes. Can. J. Fish. Aquat. Sci. **51**: 2424–2434.

Keller, W., and Pitblado, J.R. 1986. Water quality changes in Sudbury area lakes: a comparison of synoptic surveys in 1974–1976 and 1981–1983. Water Air Soil Pollut. **29**: 285–296.

Keller, W., and Pitblado, J.R. 1989. The distribution of crustacean zooplankton in northern Ontario, Canada. J. Biogeogr. **16**: 249–259.

Keller, W., and Yan, N.D. 1991. Recovery of crustacean zooplankton species richness in Sudbury area lakes following water quality improvements. Can. J. Fish. Aquat. Sci. **48**: 1635–1644.

Keller, W., Yan, N.D., Holtze, K.E., and Pitblado, J.R. 1990. Inferred effects of lake acidification on *Daphnia galeata mendotae*. Environ. Sci. Technol. **24**: 1259–1261.

Keller, W., Pitblado, J.R., and Carbone, J. 1992*a*. Chemical responses of acidic lakes in the Sudbury, Ontario, area to reduced smelter emissions, 1981–89. Can. J. Fish. Aquat. Sci. **49**(Suppl. 1): 25–32.

Keller, W., Gunn, J.M., and Yan, N.D. 1992*b*. Evidence of biological recovery of acid stressed lakes near Sudbury, Canada. Environ. Pollut. **78**: 79–85.

Koivisto, S., Ketola, M., and Walls, M. 1992. Comparison of 5 cladoceran species in short-term and long-term copper exposure. Hydrobiologia, **248**: 125–136.

Lautenbach, W.E.,Miller, J., Becket, P.J.,Negusanti, J.J., and Winterhalder, K. 1995. Municipal land restoration program: the regreening process. *In* Restoration and recovery of an industrial region. *Edited by* J.M. Gunn. Springer-Verlag, New York. pp. 109–122.

Lawrence, S.G., and Holoka, M.H. 1987. Effects of low concentrations of cadmium on the crustacean zooplankton community of an artificially acidified lake. Can. J. Fish. Aquat. Sci. **44**: 163–172.

LaZerte, B., Evans, D., and Grauds, P. 1989. Deposition and transport of trace metals in an acidified catchment of central Ontario. Sci. Total Environ. **87–88**: 209–221.

Locke, A., and Sprules, W.G. 1993. Effects of experimental acidification on zooplankton population and community dynamics. Can. J. Fish. Aquat. Sci. **50**: 1238–1247.

Locke, A., Sprules, W.G., Keller, W., and Pitblado, J.R. 1994. Zooplankton communities and water chemistry of Sudbury area lakes: changes related to pH recovery. Can. J. Fish. Aquat. Sci. **51**: 151–160.

Lynch, M. 1979. Predation, competition, and zooplankton community structure: an experimental study. Limnol. Oceanogr. **24**: 253–272.

Manly, B.F.J. 1986. Multivariate statistical methods: a primer. Chapman & Hall, London and New York.

Marcus, N.H., Lutz, R., Burnett, W., and Cable, P. 1994. Age, viability, and vertical distribution of zooplankton resting eggs from an anoxic basin: evidence of an egg bank. Limnol. Oceanogr. **39**: 154–158.

Marmorek, D.R., and Korman, J. 1993. The use of zooplankton in a biomonitoring program to detect lake acidification and recovery. Water Air Soil Pollut. **69**: 223–241.

Marshall, J.S. 1978. Population dynamics of *Daphnia galeata mendotae* as modified by chronic cadmium stress. J. Fish. Res. Board Can. **35**: 461–469.

Molot, L.A., and Dillon, P.J. 1991. Nitrogen/phosphorus ratios and the prediction of chlorophyll in phosphorus-limited lakes in central Ontario. Can. J. Fish. Aquat. Sci. **48**: 140–145.

McQueen, D.J., and Post, J.R. 1988. Cascading trophic interactions: uncoupling at the zooplankton–phytoplankton link. Hydrobiologia, **159**: 277–296.

McQueen, D.J., and Yan, N.D. 1993. Metering filtration efficiency of fresh water zooplankton hauls: reminders from the past. J. Plankton Res. **15**: 57–65.

Morel, F.M.M. 1983. Principles of aquatic chemistry. John Wiley & Sons, New York.

Mort, M.A. 1991. Bridging the gap between ecology and genetics: the case of freshwater zooplankton. Trends Ecol. Evol. **6**: 41–44.

Mort, M.A., and Wolf, H.G. 1986. The genetic structure of large lake *Daphnia* populations. Evolution, **40**: 756–766.

Münzinger, A. 1990. Effects of nickel on *Daphnia magna* during chronic exposure and alterations in the toxicity to generations pre-exposed to nickel. Water Res. **24**: 845–852.

Münzinger, A. 1994. The influence of nickel on population dynamics and on some demographic parameters of *Daphnia magna*. Hydrobiologia, **277**: 107–120.

Neill, W.E. 1978. Experimental studies on factors limiting colonization by *Daphnia pulex* Leydig of coastal montane lakes in British Columbia. Can. J. Zool. **56**: 2498–2507.

Neill, W.E. 1984. Regulation of rotifer densities by crustacean zooplankton in an oligotrophic montane lake in British Columbia. Oecologia, **61**: 175–181.

Niemi, G.J., DeVore, P., Detenbeck, N., Taylor, D., Lima, A., Pastor, J., Yount, J.D., and Naiman, R.J. 1990. Overview of case studies on recovery of aquatic systems from disturbance. Environ. Manage. **14**: 571–588.

Niemi, G.J., Detenbeck, N.E., and Perry, J.A. 1993. Comparative analysis of variables to measure recovery rates in streams. Environ. Toxicol. Chem. **12**: 1541–1547.

Noss, R.F. 1990. Indicators for monitoring biodiversity: a hierarchical approach. Conserv. Biol. **4**: 355–364.

Ontario Ministry of Environment and Energy. 1984. Handbook of analytical methods. Laboratory Services Branch, Ontario Ministry of Environment and Energy, Toronto, Ont.

Peters, R.H. 1990. Pathologies in limnology. Mem. Ist. Ital. Idrobiol. Dott Marco Marchi, **47**: 181–217.

Pinel-Alloul, B., Methot, G., Verreault, G., and Vigneault, Y. 1989. Zooplankton species associations in Quebec lakes: variation with abiotic factors, including natural and anthropogenic acidification. Can. J. Fish. Aquat. Sci. **47**: 110–121.

Ramcharan, C.W., D.J. McQueen, E. Demers, S.A. Popiel, A.M. Rocchi, N.D. Yan, and K.D. Hughes. 1995. A comparative approach to determining the role of fish predation in structuring limnetic ecosystems. Arch. Hydrobiol. **133**: 389–416.

Scheider, W.A., Jeffries, D.S. , and Dillon, P.J. 1981. Bulk-deposition in the Sudbury and Muskoka–Haliburton areas of Ontario during the shutdown of Inco Ltd. in Sudbury. Atmos. Environ. **15**: 945–956.

Schindler, D.W., Mills, K.H. , Malley, D.F., Findlay, D.L., Shearer, J.A., Davies, I.J., Turner, M.A., Linsey, G.A., and Cruikshank, D.R. 1985. Long-term ecosystem stress: the effects of years of experimental acidification on a small lake. Science (Washington, D.C.), **228**: 1395–1401.

Schindler, D.W., Frost, T.M., Mills, K.H., Chang, P.S.S., Davies, I.J., Findlay, D.L., Malley, D.F., Shearer, J.A., Turner, M.A., Garrison, P.G., Watras, C.J., Webster, K., Gunn, J.M., Brezonik, P.L., and Swenson, W.A. 1991. Comparisons between experimentally- and atmospherically-acidified lakes during stress and recovery. Proc. R. Soc. Edinb. Sect. B Biol. Sci. **97**: 193–227.

Siegfried, C.A., and Sutherland, J.W. 1992. Zooplankton communities of Adirondack lakes: changes in community structure associated with acidification. J. Freshwater Ecol. **7**: 97–112.

Snedecor, G.W., and Cochran, W.G. 1967. Statistical methods. Iowa State University Press, Ames, Iowa.

Sprules, W.G. 1975. Midsummer crustacean zooplankton communities in acid-stressed lakes. J. Fish. Res. Board Can. **32**: 389–395.

Sprules, W.G. 1977. Crustacean zooplankton communities as indicators of limnological conditions: an approach using principal component analysis. J. Fish. Res. Board Can. **34**: 962–975.

Spry, D.J., and Wiener, J.G. 1991. Metal bioavailability and toxicity to fish in low alkalinity lakes: a critical review. Environ. Pollut. **71**: 243–304.

Swofford, D.L., and Olsen, G.J. 1990. Phylogeny reconstruction. *In* Molecular systematics. *Edited by* D.M. Hillis and C. Moritz. Sinauer, Sunderland, Mass. pp. 411–501.

Swofford, D.L., and Selander, R.K. 1981. BIOSYS–1. A Fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. **72**: 281–283.

Taylor, D.J., and Hebert, P.D.N. 1992. *Daphnia galeata mendotae* as a cryptic species complex with interspecific hybrids. Limnol. Oceanogr. **37**: 658–665.

Taylor, D.J., and Hebert, P.D.N. 1993. Cryptic intercontinental hybridization in *Daphnia* (Crustacea): the ghost of introductions past. Proc. R. Soc. Lond. Ser. B Biol. Sci. **254**: 163–168.

Tessier, A.J. 1986. Comparative population regulation of two planktonic Cladocera (*Holopedium gibberum* and *Daphnia catawba*). Ecology, **67**: 285–302.

Threlkeld, S.T., Chaivelli, D.A., and Willey, R.L. 1993. The organization of zooplankton epibiont communities. Trends Ecol. Evol. **8**: 317–321.

Vanni, M.J. 1987. Colonization dynamics and life history traits of seven *Daphnia pulex* genotypes. Oecologia, **72**: 263–271.

Winner, R.W. 1984. The toxicity and bioaccumulation of cadmium and copper as affected by humic acid. Aquat. Toxicol. **5**: 267–274.

Winner, R.W., and Farrell, M.P. 1976. Acute and chronic toxicity of copper to four species of *Daphnia*. J. Fish. Res. Board Can. **33**: 1685–1691.

Wolf, H.G., and Carvalho, G.R. 1989. Resting eggs of lake *Daphnia*. II. In situ observations on the hatching of eggs and their contribution to population and community structure. Freshwater Biol. **22**: 471–478.

Wright, M.E. 1995. Assessment of temporal changes in yellow perch (*Perca flavascens*, Mitchill) biomass as evidence of recovery of acid and metal stressed lakes near Sudbury, Ontario. M.Sc. thesis, Laurentian University, Sudbury, Ont.

Yan, N.D. 1986. Empirical prediction of crustacean zooplankton biomass in nutrient-poor Canadian Shield lakes. Can. J. Fish. Aquat. Sci. **43**: 788–796.

Yan, N.D., and Dillon, P.J. 1984. Experimental neutralization of lakes near Sudbury, Ontario. *In* Environmental impacts of smelters. *Edited by* J. Nriagu. J. Wiley & Sons, New York. pp. 417–456.

Yan, N.D., and LaFrance, C.J. 1984. Responses of acidic and neutralized lakes near Sudbury, Ontario, to nutrient enrichment. *In* Environmental impacts of smelters. *Edited by* J. Nriagu. J. Wiley & Sons, New York. pp. 457–521.

Yan, N.D., and Larsson, J.I.R. 1988. Prevalence and inferred effects of Microsporidia of *Holopedium gibberum* (Crustacea: Cladocera) in a Canadian Shield Lake. J. Plankton Res. **10**: 875–886.

Yan, N.D., and Miller, G.E. 1984. Effects of deposition of acids and metals on chemistry and biology of lakes near Sudbury, Ontario. *In* Environmental impacts of smelters. *Edited by* J. Nriagu. J. Wiley & Sons, New York. pp. 244–282.

Yan, N.D., and Strus, R. 1980. Crustacean zooplankton communities of acidic, metal-contaminated lakes near Sudbury, Ontario. Can. J. Fish. Aquat. Sci. **37**: 2282–2293.

Yan, N.D., and Welbourn, P.M. 1990. The impoverishment of aquatic communities by smelter activities near Sudbury, Canada. *In* The Earth in transition: patterns and processes of biotic impoverishment. *Edited by* G.M. Woodwell. Cambridge University Press, Cambridge, U.K. pp. 477–494.

Yan, N.D., Girard, R.E., and LaFrance, C.J. 1979. Survival of rainbow trout, *Salmo gairdneri*, in submerged enclosures in lakes treated with neutralizing agents near Sudbury, Ontario. Technical report No. LTS 79–2. Ontario Ministry of the Environment, Dorset, Ont.

Yan, N.D., Keller, W., Pitblado, J.R. , and Mackie, G.L. 1988. *Daphnia–Holopedium* relationships in Canadian Shield lakes ranging in acidity. Verh. Int. Ver. Theor Angew. Limnol. **23**: 252–257.

Yan, N.D., Mackie, G.L. and Grauds, P. 1990. Control of cadmium levels in *Holopedium gibberum* (Crustacea: Cladocera) in Canadian Shield lakes. Environ. Toxicol. Chem. **9**: 895–908.

Yan, N.D., Keller, W., MacIsaac, H.J., and McEachern, L.J. 1991. Regulation of zooplankton community structure of an acidified lake by *Chaoborus*. Ecol. Appl. **1**: 52–65.

Yan, N.D., Keller, W., and Gunn, J.M. 1995. Liming of Sudbury lakes: lessons for recovery of aquatic biota from acidification. *In* Environmental restoration and recovery of an industrial region. *Edited by* J.M. Gunn. Springer-Verlag, New York. pp. 195–204.

Yan, N.D., Keller, W., Somers, K.M., Pawson, T.W., and Girard, R.E. 1996. The recovery of crustacean zooplankton commu-

nities from acidification: comparing manipulated and reference lakes. Can. J. Fish. Aquat. Sci. **53**. This issue.

Zeng, Y., and Hopke, P.K. 1994. Comparison of the source locations and seasonal patterns for acidic species in precipitation and ambient particles in Southern Ontario, Canada. Sci. Total Environ. **143**: 245–260.