

## A new approach to historical reconstruction: Combining descriptive and experimental paleolimnology

W. Charles Kerfoot

Lake Superior Ecosystem Research Center and Department of Biological Sciences, Michigan Technological University, Houghton, Michigan 49931

John A. Robbins

NOAA Great Lakes Environmental Research Laboratory, 2205 Commonwealth Blvd., Ann Arbor, Michigan 48105

Lawrence J. Weider<sup>1</sup>

Max-Planck-Institut für Limnologie, Postfach 165, D-24302 Plön, Germany

### Abstract

Here we introduce a combined experimental and descriptive approach (termed resurrection ecology) to reconstructing historical perturbations, pointing out how direct tests with sediments and hatched resting eggs complement the traditional descriptive calculation of microfossil fluxes. In the Keweenaw Waterway, a freshwater estuary off Lake Superior, turn-of-the-century copper mining impacted the resident biota. Remain fluxes document that diatom, rhizopod, and *Bosmina* production all declined during stamp sand discharges but recovered rapidly after World War II, moving above background levels due to developing eutrophication. In addition to biogenic silica, we discovered that bromine flux holds promise as an indicator of diatom production and confirmed that this element is present in several genera. Fluxes of *Daphnia* resting eggs also increased dramatically since the 1940s, dominated by a hybrid apparently produced from crosses between offshore and interior Waterway species, after channeling promoted greater mixing of water masses. Toxicity studies with sediments and *Daphnia* clones directly tested recovery of environments after cessation of mining activities. The studies document that increased concentrations and fluxes of copper in the Waterway during mining discharges were toxic to invertebrates. Once stamp sand discharges ceased, the biota recovered rapidly due to a combination of decreased copper cycling and organic complexation. Although sedimentation has returned to near-background conditions and surficial sediments in much of Portage Lake are no longer toxic, eutrophication and faunal exchange with Lake Superior make it unlikely that the original zooplankton community composition will return to the Waterway system.

Because they are strongly coupled to biotic production, fluxes of certain pelagic microfossils (diatom frustules, cladoceran exuviae, and resting eggs) offer insights into the magnitude of historic environmental perturbations and the time scales of population responses to ecosystem recovery. A recent attempt to improve the ecological relevance of paleolimnological reconstructions includes comparing expect-

ed production of remains with representation in sediment traps and sediments (Kerfoot 1981, 1995a; Hall and Yan 1997). Following microfossil remains from production through deposition and deep burial allows experimental evaluation of deposition dynamics. However, identifying new elemental signatures and retrieving entombed resting eggs for biological tests extends experimental approaches over greater time scales. In particular, the idea of resurrecting viable resting eggs or retrieving DNA from spent resting eggs for assaying past environmental conditions opens a new field with great promise. Bringing back ancestors or ancestral genetic material for transgenerational tests represents a new experimental approach to paleolimnology (resurrection ecology) and answers the vexing question of how to test paleolimnological inferences directly. Here we document chemical and remain fluxes from accurately dated lake sediments and then conduct experiments with past sediments, *Daphnia*, and resting eggs to clarify cause and effect relationships. This approach goes beyond mere description of historical genetic changes (Weider et al. 1997).

The Keweenaw Peninsula juts out like a gigantic thumb along the southern margin of Lake Superior, making shoreline travel difficult (Fig. 1). Explorers traversed the base of the Peninsula, taking the name Keweenaw from the Chipewewa word for “place where we make a shortcut on foot.” Travel was made even easier by an inland natural waterway

<sup>1</sup> Present address: The University of Oklahoma Biological Station, HC-71, Box 205, Kingston, Oklahoma 73439.

### Acknowledgements

This research was supported by NSF OCE 97-12827 to W.C.K. and by Michigan Great Lakes Protection Fund grants 910417 and 090192 to W.C.K. and J.A.R. Gary Ankley (Environmental Protection Agency-Duluth) and Jim Mihelsic (Michigan Technological Univ.) assisted on the copper effects portion of the discussion. Kevin Kirk carried out initial *Ceriodaphnia* clay toxicity tests. Special thanks go to George Lauster, who helped with pond experiments; Scot Hagerthey and Lucille Zelazny, who aided diatom identification and did the SEM bromine analyses; Susan Savage, for many of the sediment and copper flux calculations and graphics; and to Nancy Moorehead, for carbon, biogenic silica, and radionuclide analysis at the NOAA Great Lakes Research Laboratory, Ann Arbor, Michigan. W.C.K. thanks Winfried Lampert, Director, Max Planck Institute for Limnology, for sabbatical support during manuscript revisions. Carla Caceres, University of Illinois Natural History Survey, read an early draft and offered helpful advice.

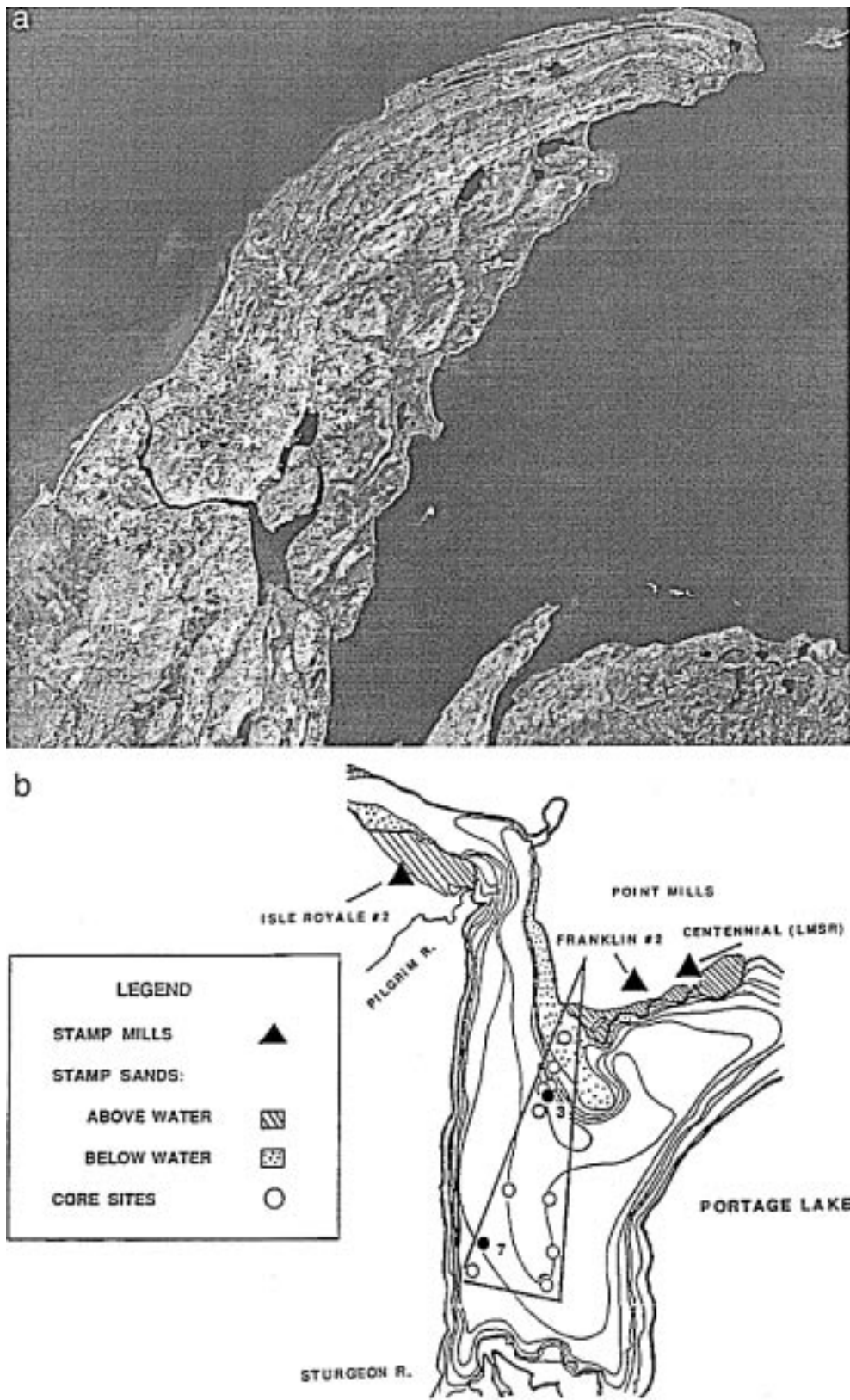


Fig. 1. The Keweenaw Peninsula stretching into Lake Superior, showing (a) the location of the Keweenaw Waterway, connecting Torch (northern) and Portage Lakes (Landsat, false color composite), and (b) the region immediately around Portage Lake, indicating the location of coring sites PL3 and PL7.

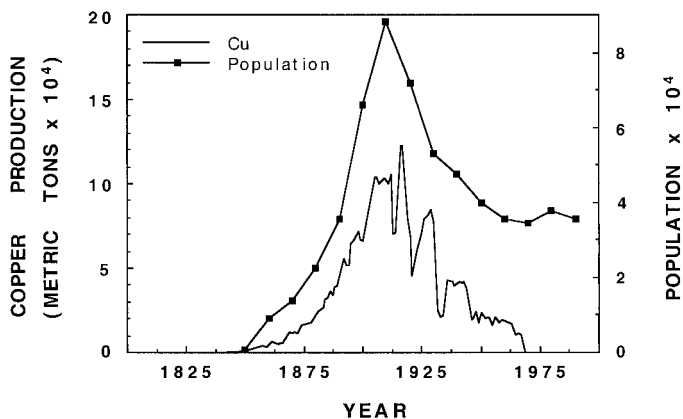


Fig. 2. Total copper production from the Keweenaw Peninsula plotted along with the combined population size of Keweenaw and Houghton Counties.

that received waters from Torch Lake and that drained via the meandering Portage River into Keweenaw Bay. One narrow arm of the inland lake, a flooded late glacial channel, extended westward nearly to the shoreline of Lake Superior. The lake created an almost complete portage across the base of the Peninsula and was named Portage Lake. To facilitate ship passage during mining, the southeastern and northwestern ends of the portage were channeled beginning in fall 1859 and improved circa 1862–1873 (Swineford 1876), creating the Keweenaw Waterway. Opened to navigation in 1890, the entries to the Waterway were expanded and more deeply channeled in the 1920s, late 1930s, and early 1940s, creating an extensive freshwater estuary open to Lake Superior at both ends.

A key regional ecosystem perturbation on the Keweenaw Peninsula was turn-of-the-century native copper and silver mining. As one of the first great North American metal mining rushes (Murdoch 1943), copper mining began in the late 1840s, reached peaks between 1890 to 1925, and ended in 1968 (Fig. 2). Whereas mines stretched for 106 km along the Portage Lake Volcanic Series, over 95% of the district's total native copper production came from deposits that ran 23 km north and 15 km south of Portage Lake (Fig. 1). Early sources of metal were vein copper or mass copper (large chunks of pure metal). As rich veins played out, industry shifted to extracting native copper from poorer ore lodes by crushing the parent rock with gravity stamps. The location and production histories of stamp mills are discussed in Kerfoot et al. (1994). The actual concentration of copper in the rock processed at stamp mills averaged between 0.5 and 6.1%, producing vast amounts of discarded copper-rich stamp sands as a tailings byproduct. The parent rock material closely resembled naturally outcropping bedrock formations, but contained high concentrations of metals (particularly copper). Between 1850 and 1968, about half a billion metric tons of stamp sands were sluiced directly into rivers, interior waterways (Torch Lake, Portage Lake portions of the Keweenaw Waterway), and coastline waters of Lake Superior. The coarse sand fraction ended up as long-shore bars or beach deposits, subject to further reworking by waves and currents. Clay-sized particles created during the milling pro-

cess, the so-called slime fraction (Lankton and Hyde 1982), dispersed much further than the sands, moving across broad reaches of inland lakes and sheltered bays. The slime fraction averaged two to four times higher in copper concentration than the coarse fraction (Kerfoot and Robbins 1999), because copper adsorbed onto the surface of clay particles during processing.

Between 1850 and 1929, the Keweenaw district was the second largest producer of copper in the world. In this one-industry environment, the population expanded hand-in-hand with copper production. Despite population build-up, construction of a centralized sewage trunk line system and a treatment plant that discharged into the Waterway did not begin until the late 1940s. The system was expanded in the 1950–1970s, following a pattern common to many locations in North America.

Our initial investigations focused on the use of varve chronology and radiotracer techniques to measure sediment and elemental fluxes precisely. Here we use changes in the flux of microfossils and associated elemental tracers to examine the impacts of turn-of-the-century industrial buildup and postwar eutrophication on biological productivity. In addition to descriptive measures, toxicity tests with both sediments and *Daphnia* complement the flux studies by more directly addressing cause and effect relationships.

## Materials and Methods

*Core studies: Dating accuracy and precision*—Early work emphasized dating techniques, largely to insure accurate and precise flux calculations. Nineteen sediment cores were taken from Portage Lake in the fall of 1991 from 12 to 14 m depth, using a 2-in. Phleger-type KB gravity corer (site locations in Kerfoot et al. [1994]). This type of corer allows water to pass through the 1-m core barrel during free fall, minimizing disturbance to sampled strata. X-rays of cores revealed a rich sequence of varve-like clay bands that could be correlated closely with the reconstructed mill discharge history. The distinctive bands in the cores offered outstanding opportunities for accurately determining time horizons and deposition rates down to 1860. Radiotracer studies complemented the varve counts. Both the  $^{137}\text{Cs}$  and  $^{210}\text{Pb}$  profiles are now completed and cross-correlated with banding patterns (Kerfoot et al. 1994; Kerfoot and Robbins 1999).

*Chemical and microfossil analyses*—Whereas all cores were used to determine lakewide sedimentation patterns and sieved (350 and 75  $\mu\text{m}$  Nitex) for scarce microfossils, two cores (PL3 and PL7) were selected for detailed temporal microfossil analyses. Core PL3 was close to the deep, central basin of Portage Lake, whereas core PL7 was further removed from slime-clay discharge sources and was near the southeastern portion of the lake (Fig. 1b). Initial steps involved slicing cores into 1-cm intervals and removing subsamples for analysis of algal and animal remains. Sediment subsamples used for  $^{137}\text{Cs}$ ,  $^{210}\text{Pb}$ , and neutron activation studies were dried at 90°C. Portions of dried, ground sediments were placed in plastic vials of standardized geometry (Robbins and Edgington 1975) and analyzed for  $\gamma$ -emitting radionuclides ( $^{137}\text{Cs}$  and  $^{40}\text{K}$ ) using a high-resolution, planar



GE(Li)  $\gamma$ -detection/multichannel analyzer system. Detector efficiency was determined by doping sediments with precisely known (1%) amounts of an National Bureau of Standards-traceable mixed-radionuclide standard solution (Amersham QCY46.1) counted in the same standard geometry.  $^{137}\text{Cs}$  was determined, from its 661.6 keV  $\gamma$  emission, with a precision generally better than 5% for counting times of  $\sim 1$  d. Concentrations of trace elements were analyzed by atomic absorption spectrophotometry (AAS) (Kerfoot et al. 1994) and instrumental neutron activation analysis (INAA) (see Kerfoot et al. 1994; Kerfoot and Robbins 1999). AAS determinations for copper values are used here because these data extend the entire length of the core. The plotted values for Cu are the means of three subsamples from the digested slice (see Kerfoot et al. [1994] for standard errors).

Depth-specific concentrations and fluxes are reported for the cores. Concentration is expressed as  $\mu\text{g g}^{-1}$  for elements and numbers  $\text{cm}^{-3}$  (ml) for microfossils. Division of concentration ( $\mu\text{g cm}^{-3}$ , numbers  $\text{cm}^{-3}$ ) by deposition time ( $\text{yr cm}^{-1}$ ) gives the deposition flux per year ( $\mu\text{g cm}^{-2} \text{yr}^{-1}$ , numbers  $\text{cm}^{-2} \text{yr}^{-1}$ ).

Organic matter and carbon flux: General methods followed Dean (1974) and Wetzel and Likens (1991). Wet slices of sediment were weighed and then dried in a Blue M drying oven at  $105^\circ\text{C}$  until they attained a constant weight. The samples were then heated to  $550^\circ\text{C}$  in a Thermolyne 1400 oven, cooled under desiccation, and reweighed. Percent organic matter was determined by loss on ignition.

Concentrations of total carbon were analyzed by conventional gasometric methods (Kolpack and Bell 1968) using a LECO carbon analyzer. Organic carbon ( $\text{C}_o$ ) was calculated as the difference between the total and inorganic carbon content. The uncorrected organic matter and carbon fluxes were determined as the product of the mass sedimentation rate ( $\text{g cm}^{-2} \text{yr}^{-1}$ ) and the organic matter or carbon content (grams of organic matter or carbon per gram of dry sediment). These values underestimate the actual flux if there is significant decomposition of organic carbon. Some attempted corrections for burial decomposition are treated in Robbins et al. (1989) but were not applied here.

Biogenic silica and bromine profiles: Samples for biogenic silica (BSi) were leached with 1.0%  $\text{Na}_2\text{CO}_3$  at  $85^\circ\text{C}$  and analyzed following Schelske et al. (1985), including a correction for silicate mineral dissolution (DeMaster 1981). Bromine (Br) was determined as part of an extensive (32 element) INAA (Dams and Robbins 1970; Kerfoot and Robbins 1999). Small amounts of sample were irradiated with neutrons using the Ford Nuclear Reactor, University of Michigan, Ann Arbor, at fluxes up to  $10^{13}$  neutrons  $\text{cm}^{-2} \text{s}^{-1}$  for as much as 20 h. Standards for calibration, coirradiated with samples, included U.S. Geological Survey rock (Andesite, AGV-1, and Granite, G-2) and National Institute of Standards and Technology (NIST) fly ash (SRM 1633A). These determinations augmented normal Phoenix Laboratory standards. An extensive table of calibrations is available from the authors on request.

In the elemental profile study (Kerfoot and Robbins 1999), bromine stood out as the only element negatively correlated with stamp sand production ( $N = 55$ ,  $r = -0.600$ ,  $P < 0.01$ ). Because this element had previously been associated

with organic production in Lake Superior waters (Nussman 1965), and similar patterns emerged from Lake George sediment cores in the St. Marys discharge of the lake, the findings prompted us to explore bromine's relationship with the biotic component. Periphyton was collected from littoral sediments. Initial preparation followed Van Der Werff (1955): addition of  $\text{K}_2\text{Cr}_2\text{O}_7$ , dilution with distilled water, and placement of diatoms on a coverslip, followed by air drying. The sample was then mounted on a stub and coated with carbon using a vacuum evaporator. Individual diatom frustules were examined under a Jeol JSM-35U scanning electron microscope (SEM) with an EDS/MCA system (energy-dispersive X-ray spectrometer/multichannel analyzer).

Microfossil remains: In addition to determining percent organic matter and biogenic silica, we surveyed what algal and animal microfossils were well-preserved throughout the cores, then selected certain categories for recording impacts on aquatic food webs: diatom valves, rhizopod cases, and the cladoceran *Bosmina* head shields and carapaces. The first category was selected to register impacts on pelagic and benthic primary producers, the second for impacts on the production of benthic protozoans, and the third for impacts on secondary production of herbivorous and detritivorous pelagic zooplankton (Kerfoot and Kirk 1991, 1993). Within the three groups, emphasis was placed on cladoceran counts. Confidence intervals were determined from replicated 20- $\mu\text{l}$  subsamples drawn from stirred 1-ml samples, mounted on glass slides, and covered by coverslip. Significance between mean counts was tested using *t*-tests (SYSTAT, Wilkinson 1989).

Diatom preparations followed Van Der Werff (1955). Generally four to seven slides were prepared from each centimeter slice, two transect counts across each slide. In pre-mining and postmining strata, between 200 and 1,000 diatoms were identified, whereas in the mining strata, because cells were much more scarce, counts were based on 30–90 frustules.

Rhizopods are small amoeboid protozoans that form protective houses from chitinous membranes or cemented minute sand grains, flattened silicious plates, and rods. Genera include species of *Diffugia*, *Centropyxis*, and *Cucurbitella*. Minimal chemical pretreatment was used for rhizopod counts, because a 10% KOH treatment might disarticulate cases. In the latter counts, 1 ml of sediment was placed in 10 ml of distilled water within a 50-ml Erlenmeyer flask. The sample was stirred gently with a plastic stirring bar for 20–40 min, until the relatively inorganic sediment was finely divided into small particles of clay, translucent organic sediment, and cases. Then 20- $\mu\text{l}$  subsamples were mounted on glass slides in a 50% glycerin/water mixture and covered with a coverslip. Counts were made at 500 $\times$  under a Zeiss Universal compound microscope. During initial counts, we decided that total counts, rather than individual species tallies, provided a better indicator of overall mining impacts.

Cladoceran procedures utilized a 10% KOH pretreatment (Kerfoot 1981). Ten 10- $\mu\text{l}$  subsamples were mounted on glass slides in a 50% glycerin/water mixture and covered with a coverslip. Counts were made at 500 $\times$  under a Zeiss Universal compound microscope. Head shields and carapac-

es often disarticulate after molting or death, so these were tabulated separately. To indicate if perturbations indirectly influenced predator-prey relationships, or if historical modifications had altered population structure, we measured the length of *Bosmina* carapaces, tail spines, and antennules. The length of these features in small-bodied *Bosmina* species often indirectly indicates the strength of invertebrate versus vertebrate predation (Kerfoot 1975, 1987). Significance between mean lengths was tested using a *t*-test (SYSTAT, Wilkinson 1989).

Certain potentially valuable microfossils (e.g., cladoceran ephippia, copepod resting eggs, *Leptodora* tail spines) are too scarce for conventional analysis by 1-ml subsamples. Many cores from the original 1991 sampling were sieved (350, 75  $\mu\text{m}$  Nitex) for medium-sized microfossils. These studies revealed abundant resting eggs. Three new replicate cores, collected in 1997 from the deep site (PL3) also were X-rayed, sliced into 2-cm intervals, then sieved through 75- $\mu\text{m}$  Nitex netting to quantify cladoceran (*Daphnia* and *Bosmina*) ephippia and copepod resting eggs. Position in the chronological sequence was derived from clay banding patterns and the laminae used to assign stratum dates. Hatching success was directly tested for *Daphnia* ephippia, by placing individual resting eggs in petri dishes at 10–20°C under long daylength illumination. Hatched *Daphnia* were cloned and identified to species, using a combination of allozyme (phosphoglucosmutase, PGM; glucose-6-phosphate isomerase, PGI; aldehyde oxidase, AO; glutamate-oxaloacetate transaminase, GOT) and mitochondrial (mt)DNA 12S/16S data. We discovered that PGI could also be run directly on unhatched resting eggs. To clarify the status of potential hybrid populations, we employed the two diagnostic restriction enzymes used by Taylor and Hebert (1993).

Sediment and field toxicity tests: *Daphnia*: If the varve-like clay layers sealed sediments, could sediments and entombed *Daphnia* be used to test historic patterns of slime-clay toxicity? Two types of tests were performed. The first directly examined historical sediment toxicity by using lab clones of *Daphnia* exposed to stratigraphic sediment samples and different modern-day spatial environments. To our knowledge, this is the first time that such sediment tests have been used to test toxicity systematically in paleolimnological studies. The second type of test quantified hatching of resting eggs and toxicity tests of present and past populations of *Daphnia*, checking tolerance to dissolved copper concentrations.

Sediments were examined first. Preliminary laboratory tests utilized an Environmental Protection Agency clone of *Ceriodaphnia dubia* and mid-core pink clays (1900–1920 interval) to test for sediment toxicity. Tests (Kerfoot 1995b) checked pore water and solid-phase sediment toxicity in addition to mass-dependent effects (kaolinite clay controls, to check for clay effects alone). These early tests showed acute toxicity from both pore waters (liquid phase) and suspended bulk sediments (solid phase). Whereas *Ceriodaphnia* is present at low concentrations in Portage Lake, the native species do not include *C. dubia*. To counter the argument that laboratory *Ceriodaphnia* are ill adapted to Keweenaw waters and hence inappropriate for field tests, we purposely selected and maintained native *Daphnia pulex* clones for use in sub-

sequent extensive sediment and translocation experiments. We determined  $\text{LC}_{50}$  values for copper, using American Society for Testing and Materials (ASTM 1991) methods outlined below.

The *Daphnia* stock came from a roadside pool, 2 km south of Gay, Michigan. For each test, *Daphnia* were reared in 20-liter aquaria provided with 12 liters of filtered (Milipore, 0.45  $\mu\text{m}$ ) Lake Superior water (total copper 2.5  $\mu\text{g liter}^{-1}$ ). *Daphnia* cultures were fed twice a week with *Chlamydomonas* and YT (a mixture of trout chow and yeast; ASTM 1991) for approximately 2 weeks before testing. Three days prior to toxicity tests, *Daphnia* were fed every day in order to insure good lipid reserves.

A replicate core, taken near core PL3 (Kerfoot et al. 1994), was first X-rayed to determine clay band chronology and then cut into approximately 1-cm slices that were stored at 4°C in whirl pak bags until assayed. For toxicity tests, we used sediment samples from every third to fourth slice between 4 and 43 cm depth. Solid-phase sediments were prepared by initially centrifuging sediment slurries at 3,600 rpm for 15 min, decanting the supernatant, then centrifuging for an additional 1 h, decanting the second supernatant. Prior to experiments, 10 ml of millipore-filtered Lake Superior water were added to sediment samples, which were then sonicated to produce a slurry.

The laboratory experiments followed standard acute toxicity testing procedures (ASTM 1991). Thirty 250-ml Pyrex beakers were used in each bioassay test. Each beaker was filled with 200 ml millipore-filtered Lake Superior water. Ten beakers served as controls (medium only), 10 received a 40-mg reagent-grade kaolinite clay slurry (clay control), and 10 received the 40-mg core sediment slurry (sediment), producing a  $\sim 200\text{-}\mu\text{g g}^{-1}$  concentration. Beakers were set up in a systematic block design and all were stirred periodically to resuspend particles. Healthy adult *Daphnia* were added individually to the 30 beakers in round-robin fashion moving from control, kaolinite clay, and sediment treatments, to achieve a final number of 10 individuals per beaker. Beakers were occasionally stirred to resuspend any settled clay. *Daphnia* were counted at approximately 2, 5, 10, 20, and 24 h, and all dead individuals were removed. Surviving *Daphnia* were preserved in 10% formalin-sucrose. Percentage survival was calculated for each treatment and time interval. Generally, medium- and kaolinite clay controls showed good survivorship over the 24-h period, whereas the sediment slurry treatment showed decreased survivorship in slime-clay strata. We used repeated-measures ANOVA (SYSTAT, Wilkinson 1989) on arc-sine-transformed survivorship values to evaluate the significance of these departures.

Laboratory toxicity tests on *Daphnia* clones and on hatched ephippia were conducted using standardized copper ion concentrations with copper chloride ( $\text{CuCl}_2$ ) in a 96-h test. Fifty beakers each contained 10 *Daphnia*. Ten beakers were run with filtered Lake Superior water as a control. The remaining 40 beakers were subdivided into four sets of 10 beakers, representing a dilution sequence (5, 25, 50, 250  $\mu\text{g Cu liter}^{-1}$ ). A graph of survivorship versus the dilution concentration gave the 50% lethal concentration ( $\text{LC}_{50}$ ) value, with confidence intervals determined by fitting a linear regression equation ( $Y = mX + b$ ) to the dilution results.

*Daphnia* occur locally in lakes, ponds, and beach pools near the shoreline of the Keweenaw Peninsula. However, no cladocerans occur in the 30+ ponds or lakes situated adjacent to or on stamp sands, we presume because pore water and slime-clay-bound copper flushes into ponds after spring snow melt and during rain events. To test directly the unsuitability of these environments to cladocerans, we carried out transfer experiments.

*Daphnia* were obtained in late May to early June from the Gay source pond. At the control and stamp sand sites, 50-ml shell vials were filled with water from the site pond. A single adult female from the control site was then placed in each vial. Field incubation experiments in a stamp sand pond (Gay Pond no. 3), fed by groundwater seepage through slime-clay-rich substrata, were run on 15–25 May 1992 and 28 May–12 June 1992. The first set had 58 vials initially in the control (Coal Dock Road Pond) and experimental (Gay no. 3) sets, whereas the second had 83 in the control and 86 in the experimental. The vials were covered with 125- $\mu\text{m}$  Nitex netting secured by rubber bands and placed in a plastic-coated test tube rack in groups of 50 vials. The racks were submerged at a depth of 0.5 m. Every 2–3 days, we scored surviving adults and counted any young born. At the end of the experiment, all surviving animals were preserved in formalin-sucrose. Standard chi-square contingency tables (Pyke and Thompson 1986) were used to determine significance. Dissolved copper concentrations in pond waters were determined following millipore (Gellman 0.45  $\mu\text{m}$ ) filtration with a graphite furnace AAS (Perkin Elmer model 5100, analytical error, 1–2%; detection limits, 0.5  $\mu\text{g liter}^{-1}$ ).

## Results

*Sediment flux*—The two Portage Lake cores (central PL3 and southern PL7 sites) record a major perturbation in mass flux (Fig. 3). At the central site, early values averaged around 0.03  $\text{g cm}^{-2} \text{yr}^{-1}$  (corresponding to a sediment accumulation of 1  $\text{mm yr}^{-1}$ ). This value is probably conservative, based on basal thin bands. The rate more than tripled to 0.11  $\text{g cm}^{-2} \text{yr}^{-1}$  (1.3–2.0  $\text{mm yr}^{-1}$ ) during the early copper mining years (1850–1890). With the opening of large-volume stamp mills that discharged directly into the northern and central basin of Portage Lake, there was a severalfold increase, again coming principally from discharged mill slime clays. Some of the details involve local events. Mass flux peaked between 1909 and 1917 at 0.99  $\text{g cm}^{-2} \text{yr}^{-1}$  (12.1  $\text{mm yr}^{-1}$ ) during the interval that the two large-volume Point Mills and single Isle Royale stamp mills operated simultaneously. Sedimentation decreased dramatically after the Point Mills operations closed in 1919 and after the last mill, the Isle Royale Mill, reduced operations in 1920 and finished discharging in 1947 (Kerfoot et al. 1994). At present, mass flux has declined to around 0.05  $\text{g cm}^{-2} \text{yr}^{-1}$  (3.0  $\text{mm yr}^{-1}$ ).

Premining mass flux at the southern core site was slightly more ( $\sim 0.04 \text{ g cm}^{-2} \text{yr}^{-1}$ ; 1.2  $\text{mm yr}^{-1}$ ) than at the central site, probably because the Sturgeon and Pike Rivers discharge substantial sediment loads nearby. Although the pattern of mass flux resembled the historical pattern at the cen-

tral site, values were lower. The maximum mass flux peaked at 0.35  $\text{g cm}^{-2} \text{yr}^{-1}$  (5.3  $\text{mm yr}^{-1}$ ), less than one third the maximum value at the mid-lake site (Fig. 3). We presume that lower fluxes of the slime-clay fraction were due simply to greater distances from the mill discharge sources. Since 1920, mass flux declined progressively, approaching presettlement values (0.04–0.07  $\text{g cm}^{-2} \text{yr}^{-1}$ ; 1.2–2.8  $\text{mm yr}^{-1}$ ).

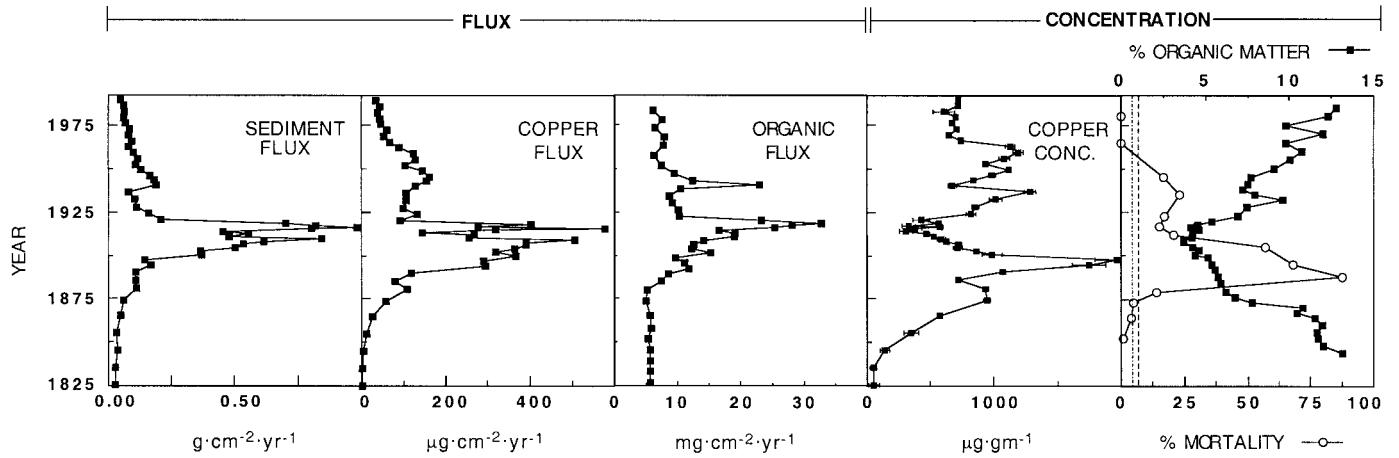
*Copper concentration and fluxes*—At the central site, copper concentrations were relatively low in early strata and increased rapidly during the initial copper mining period, correlated with slime-clay discharges, achieving a maximum of 1,978  $\mu\text{g g}^{-1}$  between 1888 and 1896. The maximum peak resulted from a combination of two factors: processing of high-grade ore and low efficiency of copper extraction from the slime fraction. Copper concentrations declined to 320–860  $\mu\text{g g}^{-1}$  after the opening of the high-volume steam stamp mill operations, because these operations included methods for extracting copper from the slime fraction (Wilfley Table, introduced in 1898; Benedict 1955). Despite cessation of clay discharges in 1947 and the return of sedimentation rates to near-background values, copper concentrations continued to remain as high in modern-day surface sediments as at the peak of slime-clay discharges, ranging between 500 and 1,000  $\mu\text{g g}^{-1}$  over the past 30 yr.

Flux calculations revealed a distinctly different pattern, more closely tied to discharge history (Kerfoot et al. 1994). Copper flux was relatively low in premining strata (1.7–2.3  $\mu\text{g cm}^{-2} \text{yr}^{-1}$ ), increased gradually during the early gravity mill operations, then climbed dramatically with the onset of steam stamp mill operations. Maxima (505–579  $\mu\text{g cm}^{-2} \text{yr}^{-1}$ , 220–340 $\times$  background) occurred during 1900–1920. Flux then declined (90–162  $\mu\text{g cm}^{-2} \text{yr}^{-1}$ , 40–95 $\times$  background) after the two main mills at Point Mills (Franklin no. 2, Centennial) closed in 1920 and after the final mill (Isle Royale) suspended activity in 1947. At present, fluxes are lower still, yet far above background, ranging between 34 and 60  $\mu\text{g cm}^{-2} \text{yr}^{-1}$  (15–35 $\times$  presettlement values).

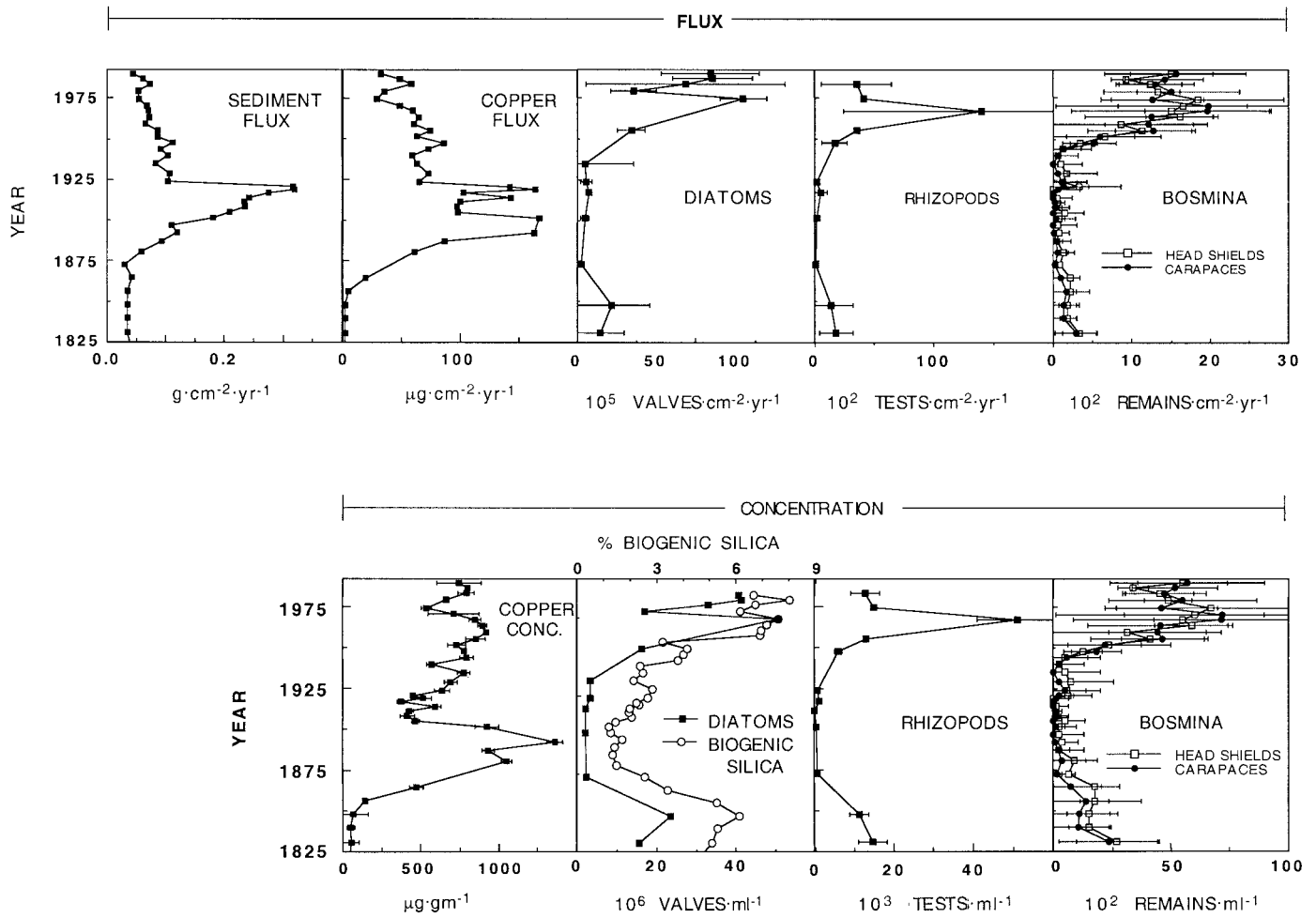
At the southern site (PL7), peak historical copper concentrations were lower (1,360  $\mu\text{g g}^{-1}$ ) than at the central site, although values for recent strata are similar to current mid-lake values (last 30 yr, 536–851  $\mu\text{g g}^{-1}$ ), probably because of greater bioturbation and resuspension. Peak copper fluxes (168  $\mu\text{g cm}^{-2} \text{yr}^{-1}$ ) also were lower than at the central site, presumably again because less of the slime clays reached the site. As at the central site, modern fluxes are considerably above presettlement levels, ranging between 29 and 64  $\mu\text{g cm}^{-2} \text{yr}^{-1}$ . Overall, the relative profile patterns are similar to the central site.

*Percent organic matter, percent carbon, and carbon flux*—Percent organic matter in the central basin core (Fig. 3) was very sensitive to mining activities. Values originally averaged 12–13% in presettlement strata. Initial phases of mining depressed values only slightly, although by 1880, stamp sand discharges from numerous small operations and erosion inputs decreased percent organic matter to 5–7%, largely through dilution from inorganic slime-clay inputs. Lows were reached between 1900 and 1920, at 3–4%. From 1920 until the present, percent organic matter gradually re-

### CENTRAL (16 meters)



### SOUTHERN (13 meters)





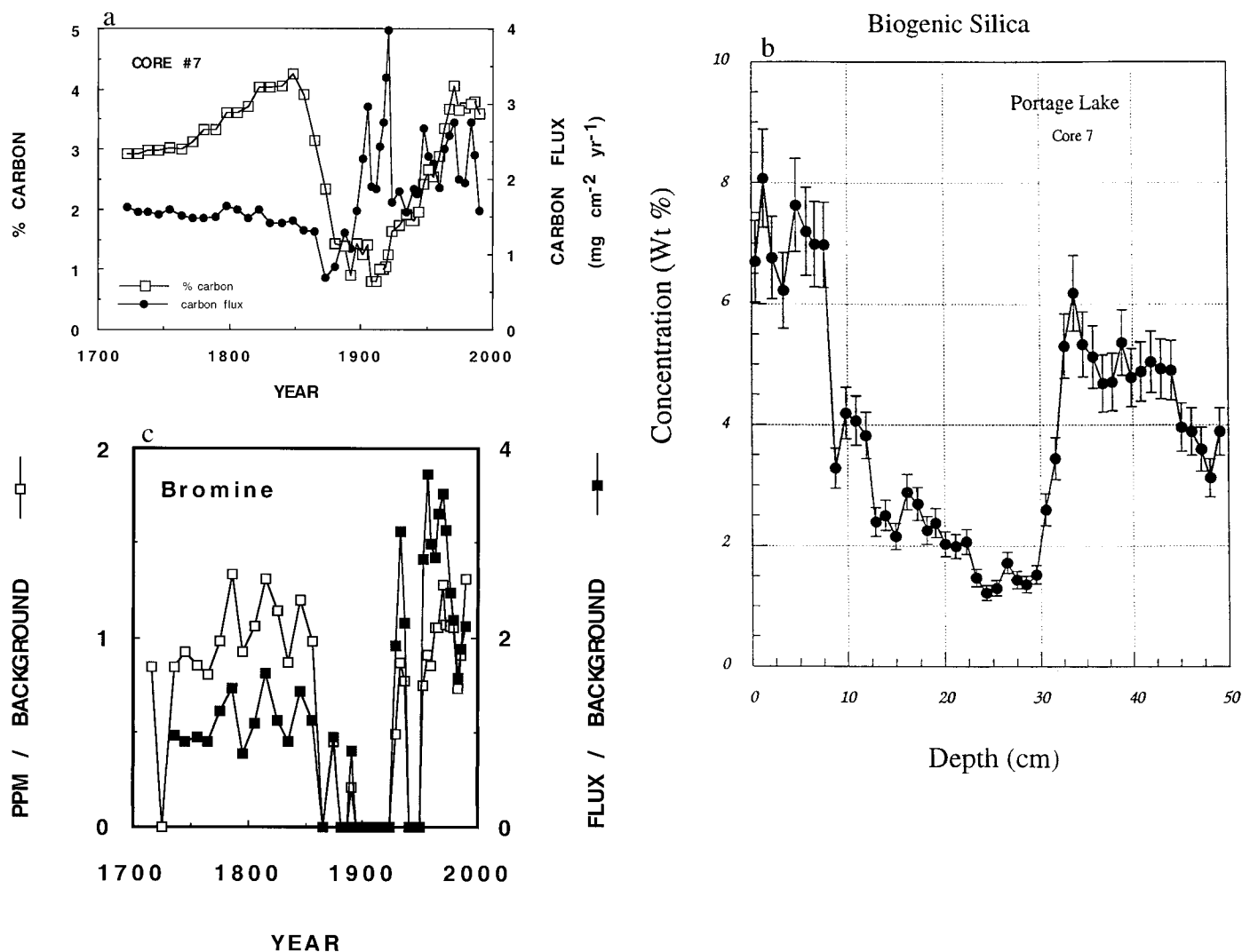


Fig. 4. Carbon and bromine fluxes: (a) percentage carbon and carbon flux at the southern site (PL7), (b) biogenic silica (BSi) concentration at PL7; with plus or minus 1 SD plotted about the means, and (c) bromine concentration and flux ( $\mu\text{g cm}^{-2} \text{yr}^{-1}$ ) at the central (PL3) site, expressed as enrichments above background (i.e., concentration or flux/premining value).

bounded fully to initial conditions, currently residing around 13%.

Although percent organic matter was diluted by inorganic slime-clay discharges, the absolute organic flux was positively associated with mining activity at the central site (Fig. 3). Increased flux is expected because increased allochthonous inputs of carbon from surrounding watersheds coincided with the deforestation and accelerated erosion that accompanied mining activity. Background values were originally around  $6 \text{ mg organic matter cm}^{-2} \text{yr}^{-1}$  in presettlement strata. Fluxes increased to a maximum (six times background) of about  $33 \text{ mg organic matter cm}^{-2} \text{yr}^{-1}$  during the early 1920s

and then declined to  $9\text{--}10 \text{ mg organic matter cm}^{-2} \text{yr}^{-1}$  during the depression years. Levels increased to a second peak of about  $25 \text{ mg organic matter cm}^{-2} \text{yr}^{-1}$  during the World War II (WWII) mining years. As in the case of mass sedimentation flux, organic flux also is approaching background values, around  $6 \text{ mg organic matter cm}^{-2} \text{yr}^{-1}$ .

In this relatively soft-water lake, measures of percent carbon and carbon flux also provide additional information on organic matter sedimentation. Figure 4a shows changes in percent carbon and carbon flux at the southern core site. Percent carbon averages around 3% in presettlement horizons. There is a slight increase in values prior to 1850, then

←

Fig. 3. Concentrations and fluxes for sediment mass, copper, organic matter, biogenic silica, and various organism remains (total diatoms, total rhizopod cases, carapaces, and head shields of *Bosmina*). Organism remains are plotted with 95% confidence limits on either side of the mean. Results of sediment toxicity tests (*Daphnia* 24 h mortality) are plotted along with % organic matter in the far right panel of the central core results; dashed and broken lines indicate lake medium-only and kaolinite clay mean control values.



values decrease to around 1% during the period of maximum stamp sand discharge (1890–1920). After 1920, values gradually return to circa 1800–1850 levels. Carbon flux is lowest in pre-1860 samples, averaging around  $1.3\text{--}1.5\text{ mg cm}^{-2}\text{ yr}^{-1}$ , increases to a maximum of  $4\text{ mg cm}^{-2}\text{ yr}^{-1}$  around 1920, then fluctuates between  $1.6\text{ and }2.8\text{ mg cm}^{-2}\text{ yr}^{-1}$  until the present.

*Microfossil concentrations and fluxes*—Biogenic silica concentrations, counts of diatom valves, rhizopod tests, and *Bosmina* remains all revealed highly significant (Fig. 3; *t*-test,  $P < 0.01$ ) fluctuations in strata concentrations between premining, mining, and postmining eras. Declines from 1850 until the end of WWII produce a pattern that coincides with slime-clay discharges. For example, mean concentrations  $\pm$  95% confidence intervals at 3 cm depth (postmining era, 1982) are  $33.4 \pm 10.3 \times 10^6$  diatoms,  $12.8 \pm 3.6 \times 10^3$  rhizopod tests,  $47.2 \pm 18.0 \times 10^2$  *Bosmina* carapaces, and  $45.0 \pm 14.7 \times 10^2$  head shields per ml. In contrast, many samples between 14 and 27 cm (1940–1892) contain much lower concentrations. To illustrate, at 25 cm depth (mining era, 1901) we find  $2.04 \pm 0.35 \times 10^6$  diatoms,  $0.5 \pm 0.29 \times 10^3$  rhizopod tests,  $1.3 \pm 1.3 \times 10^2$  carapaces and  $2.5 \pm 7.1 \times 10^2$  head shields per ml. At 35 cm depth (premining era, 1831) mean concentrations are  $23.8 \pm 8.0 \times 10^2$  carapaces and  $27.0 \pm 6.5 \times 10^2$  head shields per ml. However, because all these concentration measures are potentially susceptible to dilution from enhanced inorganic sedimentation, flux calculations of remains are necessary (1) to more properly reflect temporal changes in species production of remains and (2) to resolve true historic patterns of impacts on remain production.

Fluxes of remains indicate relatively low production in premining strata, depression of all three biotic groups (small cladocerans, benthic rhizopods, pelagic, and benthic diatoms) during the slime-clay discharge period, and major increases in production after WWII. The 95% confidence intervals are plotted in Fig. 3. Total diatoms average between  $25\text{ and }30 \times 10^5$  valves  $\text{cm}^{-2}\text{ yr}^{-1}$  in premining strata, decrease below  $10 \times 10^5$  valves  $\text{cm}^{-2}\text{ yr}^{-1}$  during the slime-clay discharge interval, then increase to between  $50\text{ and }150 \times 10^5$  valves  $\text{cm}^{-2}\text{ yr}^{-1}$  in postdischarge strata. Total rhizopod tests average around  $20 \times 10^2$  tests  $\text{cm}^{-2}\text{ yr}^{-1}$  in pre-settlement strata, decrease to less than  $5 \times 10^2$  tests  $\text{cm}^{-2}\text{ yr}^{-1}$  during the slime-clay interval, then increase to between  $40\text{ and }150 \times 10^2$  tests  $\text{cm}^{-2}\text{ yr}^{-1}$  in postdischarge strata. *Bosmina* average  $1.8\text{--}3.3 \times 10^2$  remains  $\text{cm}^{-2}\text{ yr}^{-1}$  in 1830–1865 strata, decrease considerably below  $1 \times 10^2$  remains  $\text{cm}^{-2}\text{ yr}^{-1}$  (low of  $2\text{--}4$  remains  $\text{cm}^{-2}\text{ yr}^{-1}$ ) during the slime-clay discharge era (1856–1947), then increase abruptly to levels between  $10\text{ and }20 \times 10^2$  remains  $\text{cm}^{-2}\text{ yr}^{-1}$  in postdischarge strata. Comparisons between strata with a nested ANOVA confirmed highly significant differences (concentration: PM, postmining 1950–1991,  $df = 12$ ,  $MS = 0.337337E + 08$ ,  $F = 19.8$ ,  $P < 0.000$ ; LSSE, large stream stamp era 1950–1900,  $df = 15$ ,  $MS = 0.224235E + 08$ ,  $F = 13.2$ ,  $P < 0.000$ ; EGSE, early gravity stamp mill era 1899–1850,  $df = 6$ ,  $MS = 0.268501E + 08$ ,  $F = 15.8$ ,  $P < 0.000$ ; PE, premining era 1899–1850,  $df = 2$ ,  $MS = 0.159964E + 08$ ,  $F = 4.7$ ,  $P < 0.01$ ; error  $df = 152$ ,  $MS = 1704630$ . Flux:

PM,  $df = 12$ ,  $MS = 3128455$ ,  $F = 32$ ,  $P < 0.000$ ; LSSE,  $df = 15$ ,  $MS = 1200974$ ,  $F = 12$ ,  $P < 0.000$ ; EGSE,  $df = 6$ ,  $MS = 1892866$ ,  $F = 20$ ,  $P < 0.000$ ; PE,  $df = 2$ ,  $MS = 1603804$ ,  $F = 17$ ,  $P < 0.000$ ; error  $df = 152$ ,  $MS = 96489$ ).

*Biogenic silica, bromine, and algal composition*—An unexpected indicator of biological impacts emerged from elemental analysis of sediments. Although we were concerned that biogenic silica values might be corrupted by opal or other hydrated silicates associated with crushed ores, corrected values gave a strong signal (Figs. 3, 4b). Diatom counts were highly correlated with biogenic silica (linear regression  $Y = -12.83 + 6.82X$ ,  $r^2 = 0.866$ ; improving with the nonlinear fit  $Y = 4.64 - 3.74X + 1.38X^2 - 3.15e^{-2X^3}$ ,  $r^2 = 0.906$ ,  $P \ll 0.01$ ). However, both biogenic silica and diatom counts also were strongly correlated with bromine (Fig. 5). Regressions with BSi ( $N = 43$ ,  $Y = -3.22 + 3.02X$ ,  $r^2 = 0.684$ ,  $P \ll 0.01$ ) and diatom counts ( $N = 12$ ,  $Y = 4.72 + 0.288X$ ,  $r^2 = 0.508$ ,  $P < 0.01$ ) prompted us to examine the association more closely.

The pattern for Br flux suggests an association with diatom production. For example, Br concentrations were clearly diluted by slime-clay influx. The Br flux calculation (Fig. 4c), which compensates for any dilution effects from mass loading, was severely depressed during peak stamp sand discharge years. Moreover, Br flux showed trends consistent with enhanced primary production since WWII. Subsequent SEM microprobe examination of prepared frustules and sectioned whole mounts of freshwater diatoms confirmed strong Br concentrations in siliceous frustules. Bromine was found in all four taxa examined: *Fragilaria construens*, *F. c. var binodis*, *Navicula rhynchocephala*, and *Fragilaria pinnata*, which suggests a new signature worthy of further exploration.

Although fluxes of total diatoms were reduced, the composition of the pelagic diatom community showed little change in dominant genera through time, although species determinations will probably reveal differences. Premining, mining, and postmining strata had the same five dominant pelagic genera in approximately similar percentages (*Melosira* 32–40%, *Stephanodiscus* 16–28%, *Cyclotella* 7–10%, *Fragilaria* 6–18%, and *Tabellaria* 2–16%). Although further work is necessary, the small degree of change may relate to periodic exchange between Portage and Lake Superior waters via the Portage River connection. The conservative response also was seen in *Bosmina* morphology, but not in overall cladoceran species composition.

*Bosmina morphology and Daphnia ehippial record*—Although remains and preliminary electrophoresis tests suggest a mixture of two sibling species (presumably *Bosmina freyi* and *Bosmina liedereri*, DeMelo and Hebert 1994a,b), *Bosmina* morphology changed only slightly over the slime-clay discharge interval (Table 1). The morphology indicates moderate to high densities of invertebrate predators, i.e., intermediate to long mucro and antennule lengths (Kerfoot 1987, 1995a). Moderate invertebrate predation is consistent with the modern-day presence of predatory copepods (*Mesocyclops*, *Epischura*, *Limnocalanus*), some predatory cladocerans (*Leptodora*, *Polyphemus*, recent invasion by *Bythotre-*

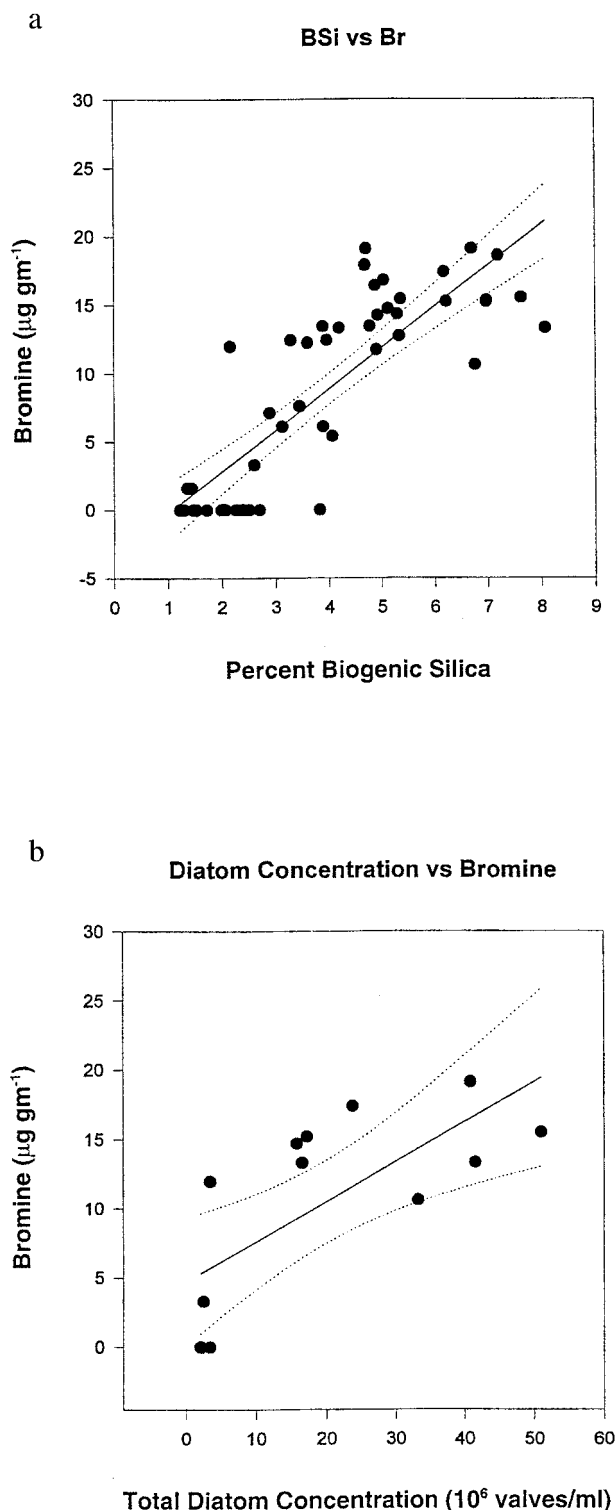


Fig. 5. Correlation between (a) biogenic silica and bromine concentration and (b) diatom counts and bromine concentration.

phes), phantom midge larvae (*Chaoborus*), and predatory rotifers (*Asplanchna*). The similarity in body size, mucro, and antennule spine lengths throughout the mining interval suggests that the importance of invertebrate versus fish pre-

ation did not change radically. There were, however, some subtle changes. Carapace length shows a significant increase during the mining interval, coinciding with population declines. We interpret this to reflect a shift in the age distribution of the population, corresponding to reduced production and a smaller fraction of juveniles. There are also indications that the present-day *Bosmina* populations possess slightly shorter mucrones and antennules than earlier populations, reflective of recent late-season population shifts correlated with increasing eutrophication of Portage Lake.

In contrast, the *Daphnia* species composition was very sensitive to the creation of the Waterway, which permitted increased mixture of inland and Lake Superior plankton (Table 2). Originally, a relatively large-bodied *Daphnia rosea* (= *D. dentifera*; Hebert 1995) dominated Portage Lake assemblages. After the creation of the Waterway, a hybrid between *Daphnia galeata mendotae* (= *D. mendotae*; Hebert 1995) and *D. rosea* became abundant, along with *Daphnia retrocurva*. Plankton samples from the past 10 yr indicate numerical superiority of the hybrid and *D. retrocurva*. The hybrid, characterized by a smaller, roundish, and more transparent ephippium, has dominated the ephippial egg bank for the last 60 yr. On a milliliter basis, concentrations of total *Daphnia* ephippia are low, so entire counts for 2-cm slices (volume = 40.6 cm<sup>3</sup>) are given in Table 2. Counts of sieved *Bosmina* exuviae are highly correlated with total *Daphnia* ephippia ( $r = 0.84$ ), again probably reflecting increased eutrophication. Expressed in concentrations per milliliter, premining (1825–1848) concentrations of *Daphnia* resting eggs are low (0.3–0.7 ml<sup>-1</sup>). Mining era (1848–1920) values increase to 0.4–1.8 ml<sup>-1</sup>. There is a brief burst beginning during the Great Depression, when mills closed, which extends through WWII (5.3–10.2 ml<sup>-1</sup>), then a sustained increase since the early 1950s (2.2–13.4 ml<sup>-1</sup>). Fluxes of *Daphnia* ephippia suggest low production in premining strata (0.03–0.06 ephippia cm<sup>-2</sup> yr<sup>-1</sup>), gradually increasing until the present, peak fluxes of 4.3–6.3 ephippia cm<sup>-2</sup> yr<sup>-1</sup>, two orders of magnitude above background values. The increased *Daphnia* ephippial concentrations and flux in upper strata, considerably above background rates, again seems to suggest increased eutrophication (productivity) of the Waterway relative to premining strata.

Although viable resting eggs of *Daphnia* extended the deepest (18–20 cm) in core strata, down 60–70 yr, viable copepod (12–14 cm) and *Bosmina* eggs (14–16 cm) also were common. We utilized 211 hatchlings to establish clonal populations from three replicate cores (Table 3). Almost all hatched individuals were assignable to the hybrid cross. The allozyme data support a more *dentifera*-like than *mendotae*-like assignment for the hybrid, whereas the mtDNA evidence confirms some *mendotae* influence. Employing the two diagnostic restriction enzymes used by Taylor and Hebert (1993), every isolate analyzed ( $n = 42$ ) was fixed for the B and very rare E patterns of *Rsa*-I and *Taq*-I, respectively. Taylor and Hebert (1993) found only three individuals from three different Indiana populations that expressed the E pattern at *Taq*-I, and all three were *mendotae* × *dentifera* hybrids. Hence the distinctive Portage Lake population over the past 60 yr is most likely a *D. mendotae* × *dentifera* hybrid.

Table 1. Morphology of *Bosmina* from various layers in core PL7. Entries give mean and standard deviation (SD). Core samples arranged by depth in cm and assigned a midslice date. Lengths rounded to nearest  $\mu\text{m}$ .

Age	Depth (cm)	N	Carapace length	N	Mucro length	N	Antennule length	N	Sutures	N	Segments
1990	0–1	106	237 (53) 156–404	106	47 (12) 24–70	61	94 (23) 57–148	106	2.9 (1.2) 1.0–6.0	61	10.6 (1.7) 8.0–14.0
1987	2	85	232 (47) 143–344	85	42 (14) 15–70	42	104 (24) 64–143	85	2.7 (1.1) 1.0–5.0	42	1.8 (1.5) 9.0–14.0
1982	3	44	234 (50) 148–370	44	48 (13) 26–77	44	89 (21) 51–143	44	3.0 (1.4) 0.0–6.0	24	10.4 (1.9) 7.0–15.0
1977	4	27	243 (53) 156–426	27	44 (11) 22–68	27	112 (23) 59–148	27	2.9 (1.4) 0.0–5.0	18	11.9 (1.4) 9.0–15.0
1972	5	34	240 (70) 159–425	34	46 (15) 22–77	34	97 (17) 66–123	34	2.9 (1.4) 0.0–5.0	28	11.0 (1.1) 9.0–13.0
1966	7	28	229 (52) 152–362	28	47 (12) 13–66	28	112 (25) 62–150	28	3.5 (1.0) 0.0–5.0	28	11.9 (1.5) 10.0–16.0
1954	10	57	255 (75) 159–490	57	53 (14) 20–81	57	117 (26) 46–176	57	3.8 (1.3) 1.0–6.0	28	11.6 (2.0) 8.0–15.0
1932	15	57	270 (63) 167–415	57	56 (9) 20–79	57	107 (23) 73–157	57	3.9 (1.3) 0.0–6.0	30	11.5 (1.8) 8.0–15.0
1873	30	50	257 (51) 163–371	50	57 (17) 22–84	24	104 (15) 77–135	50	2.4 (1.0) 0.0–4.0	24	9.5 (1.1) 8.0–11.0
1831	35	30	224 (53) 135–368	48	49 (10) 22–70	33	104 (22) 59–176	28	3.5 (1.3) 1.0–6.0	24	11.6 (2.0) 7.0–16.0
1655	56	58	231 (52) 167–450	58	52 (6) 24–66	34	105 (17) 73–161	58	3.5 (0.7) 1.0–5.0	34	12.1 (1.3) 9.0–15.0

*Sediment and field toxicity tests*—To illustrate the historical bioavailability of copper, we tested the toxicity of sediment suspensions taken from various horizons of the sediment core, using a native clone of the microcrustacean *Daphnia pulex* in standard test protocols (see Materials and methods). To measure relative toxicity, we used a constant mass (40 mg/200 ml medium) of solid-phase sediments in bioassays. Identical concentrations of a nontoxic clay (kaolinite) were used to test for clay effects only.

We checked exposure values for slime clays against observed historic settling rates and reconstructed concentrations. The 250-ml beakers had a volume of 200 ml and an approximate bottom surface area of 539 cm<sup>2</sup>. Hence, settling of clay during a 24-h experiment (40 mg day<sup>-1</sup>/539 cm<sup>2</sup>) = 0.074 mg cm<sup>-2</sup> day<sup>-1</sup>. Recall that the annual mass flux for the southern site (PL7) ranged from 0.100 to 0.319 g cm<sup>-2</sup> yr<sup>-1</sup> during 1880–1947, giving a daily range of 0.274–0.874 mg cm<sup>-2</sup> day<sup>-1</sup>, whereas that for PL3 ranged between 0.110 and 0.985 g cm<sup>-2</sup> yr<sup>-1</sup>, giving 0.301–2.699 mg cm<sup>-2</sup> d<sup>-1</sup>. Thus, in terms of settling mass on a daily basis, the experiments were conservative. They fell below observed ranges and were usually less than the mean mass flux experienced by benthic organisms. Because the water depth and daily sediment accumulation amount also is known, we can back-calculate concentration exposure. Solid-phase concentrations were set during experiments at 200 mg ml<sup>-1</sup>, whereas reconstructed daily values at PL7 ranged between 249 and 795 mg ml<sup>-1</sup> and at PL3 between 201 and 1,799 mg ml<sup>-1</sup>. Again the experimental exposures were conservative, falling at the low end of historic exposure levels.

Selected survivorship curves from different strata are illustrated in Fig. 6. Above and below clay horizons, repeated-measures ANOVA *F* scores are low and treatment effects

are nonsignificant or marginally significant (e.g., slices at 11, 17, and 20 cm, corresponding to 1945, 1923, and 1917 time horizons). Within the clay-discharge interval, *F* scores are high and treatment effects are highly significant (e.g., slices at 33, 35, and 37 cm, corresponding to 1888, 1879, and 1873 time horizons). For example, at 33 cm, a repeated-measures ANOVA that compared Lake Superior water control, kaolinite clay control, and suspended solid-phase sediment treatments gave a treatment *F* of 119.9 (df = 2, *P* < 0.000).

Figure 3 shows 24 h mortality plotted along with the average medium-only and kaolinite clay control mortality levels. Twenty-four-hour mortality in medium-only (Lake Superior water, LSW) and kaolinite clay (kaolinite + LSW) controls ranged between 0 and 15% (mean, LSW only = 7.6%; kaolinite control = 4.2%). In contrast, 24-h mortality increased to as high as 87% at peak copper concentrations of suspended sediment. That is, solid-phase sediments (slime-clay layers) from the mining era were highly toxic. Highest levels of mortality came from slices deposited between 1890 and 1910. High mortality is not surprising, because dissolved Cu concentrations in pore waters (discarded supernatant) from slime-clay strata averaged 1,360  $\mu\text{g liter}^{-1}$ , orders of magnitude above levels potentially lethal to *Daphnia* (ca. 7–90  $\mu\text{g liter}^{-1}$ ; Hodson et al. 1979). Toxicity tests gave an estimated LC<sub>50</sub> value of 10.4  $\mu\text{g Cu liter}^{-1}$  for the *D. pulex* clone used in sediment tests. Organic-rich top and bottom solid-phase sediments showed noticeably lower levels of toxicity. Mortality declined to negligible values in the upper 7 cm of organic-rich strata, despite high sediment copper concentrations, and this corresponded to a decrease in pore-water copper. Overall, percent organic matter in sediments was highly correlated with survivorship (*r* = 0.82).

Table 2. Sieved (75- $\mu\text{m}$  Nitex netting) remains from 2-cm slices, Portage Lake sediment cores. Dates are midslice values. Depths are in cm. Count totals include large *Bosmina* carapaces and total *Daphnia* ephippia per slice (volume = 40.6 cm<sup>3</sup>). Total ephippial concentrations and fluxes are given, then ephippia broken into hybrid (*mendotae*  $\times$  *dentifera*) and *dentifera* subtotals. Concentration is expressed as ephippia cm<sup>-1</sup>, whereas flux is number of ephippia cm<sup>2</sup> yr<sup>-1</sup>.

Date	Depth	<i>Bosmina</i> carapace	<i>Daphnia</i> ephippia				
			Total count	Concen- tration	Flux	Hybrid	<i>denti- fera</i>
1991	1	455	133	3.3	1.3	130	3
1986	3	2,719	389	9.6	2.7	382	7
1979	5	1,751	429	10.6	3.5	429	0
1973	7	1,179	544	13.4	3.8	544	0
1966	9	332	278	6.8	2.0	278	0
1959	11	1,115	99	2.2	0.7	87	12
1953	13	209	54	1.3	0.4	12	42
1946	15	568	415	10.2	4.1	95	320
1941	17	211	216	5.3	1.2	53	163
1932	19	4	19	0.5	0.1	6	13
1924	21	10	60	1.5	1.0	4	56
1921	23	15	38	0.9	0.5	1	37
1917	25	21	46	1.1	1.1	2	44
1915	27	3	73	1.8	1.2	9	64
1912	29	20	73	1.8	1.8	2	71
1910	31	9	55	1.4	0.9	12	43
1907	33	13	52	1.3	0.5	5	47
1902	35	11	18	0.4	0.2	2	17
1898	37	10	21	0.5	0.1	0	21
1890	39	33	41	1.0	0.2	3	38
1880	41	18	73	1.8	0.2	2	71
1865	43	25	66	1.6	0.2	4	62
1848	45	3	12	0.3	0.03	0	12
1825	47	12	28	0.7	0.06	1	27

That is, although copper was present in organic-rich sediments, it was not bioavailable.

*Daphnia* ephippia were removed from core slices and hatched. Over 211 clones from the *D. rosea*  $\times$  *mendotae* hybrid were established from surface to 18-cm depth slices, corresponding to a maximum of 70 yr of age (Table 3). Toxicity tests with clones from various strata suggest inherent differences in Cu resistance (Kerfoot unpubl.). We wish to point out that *Daphnia* resting eggs are known to retain viability over much longer durations than 60 yr (C. Caceres pers. comm.), suggesting that high pore-water copper concentrations may have killed resting eggs in deeper strata, eliminating seed bank components during heavy slime-clay discharge periods.

Results from both series of field incubation experiments were similar (Fig. 7). Around 80% of mature fecund females survived 12–14 d in their natural source pond (Coal Dock Road Pond), whereas all females were dead by the 11th day in the first series and by the 13th day in the second series in the stamp sand pond. Chi-square tests of homogeneity show significant differences ( $P < 0.05$ ) between controls and experimentals by the 4th–6th day of the experiment. The number of young that survived to the end of the experiment also showed significant differences between treatments. For

Table 3. Mean number of viable resting eggs retrieved from sieved, 2-ml thick slices of a Portage Lake sediment core. The number of isolated *Daphnia* hatchlings (isolates) used to establish clonal cultures from three sliced cores are tabulated and matched with the stratum date.

Date	Depth (cm)	<i>Daphnia</i> isolates		<i>Bosmina</i>	<i>Ceriodaphnia</i>	Copepod
1993	1	32	37	1	6	15
1986	3	43	29	1	0	10
1979	5	53	37	15	0	117
1973	7	60	12	15	0	70
1966	9	93	27	16	2	64
1959	11	78	14	5	2	16
1953	13	98	41	1	4	7
1946	15	159	13	1	4	7
1932	17	54	1	0	0	0
1924	19	7	0	0	0	0
1921	21	0	0	0	0	0
1917	23	0	0	0	0	0

example, in the 15–26 May experiment,  $14.1 \pm 1.7$  (mean  $\pm$  95% confidence limit; standard deviation [SD] = 5.9) young per vial survived in the natural pond environment, whereas only  $7.8 \pm 1.5$  (SD = 5.4) young per vial survived in the stamp sand pond ( $t$ -test;  $P < 0.001$ ). Extensive follow-up experiments at several sites in 1993–1995 gave similar results and showed that none of the second-generation offspring could survive to maturity (Lytle 1997).

The inability of stamp sand environments to sustain viable populations of cladocerans is underscored by the lack of cladoceran remains and viable resting eggs in sediments of 34 ponds surveyed by short cores, despite the persistence of some ponds for more than 42 yr (durations based on 1954–1992 U.S. Geological Aerial Survey photographs). In contrast, *Daphnia*, *Ceriodaphnia*, *Bosmina*, and their resting eggs occur quite commonly in nearby coastal ponds spatially removed from stamp sand contamination. One obvious explanation for poor survivorship in the stamp sand ponds is dissolved copper concentrations of 50–2,000  $\mu\text{g liter}^{-1}$  (Kerfoot and Harting pers. comm.; Sarah Green pers. comm.), greatly in excess of the normal LC<sub>50</sub> values for *Daphnia*. The much higher levels of dissolved copper are beyond tolerance levels, precluding successful colonization and the accumulation of a viable resting egg pool.

## Discussion

Paleolimnological studies are usually ex post facto reconstructions, i.e., explanations of patterns based on descriptive, not experimental, studies. Recent attempts to quantify the relationship of remain fluxes to population production (Kerfoot 1981, 1995a) provide experimental and predictive approaches to deposition dynamics. The use of multiple indicators, i.e., species from different trophic levels or niches, and structurally associated elements (e.g., biogenic Si, Br), broaden the efforts to community and elemental cycling inferences. Here we encourage additional novel direct experimental approaches, using buried sediments and resting eggs to test inferences directly.



The events in Portage Lake provide an important example of how depositional environments and sediment types alter biologically available metal at given concentrations, illustrating the interplay between ecosystem processes and biotic interactions. As mining began around Portage Lake, several factors contributed to increase sediment flux, some more than others. Intensive deforestation associated with early mining activities increased runoff. Period photographs and drawings document barren and badly eroded hillsides surrounding the Keweenaw Waterway (Lankton and Hyde 1982; Lankton 1991). Shoreline erosion also probably increased after channeling opened the Waterway to large ship traffic. However, the bulk of the increased mass flux came from early stamp mill discharges. A close correspondence exists between the thickness of laminae and yearly stamp mill discharges (Kerfoot et al. 1994). The correlation between observed mass flux in sediment cores and recorded total stamp sand discharge is strong ( $N = 55$ ,  $r = 0.831$ ,  $P < 0.01$ ; Kerfoot and Robbins 1999).

Among the heavy metals, copper does not bioaccumulate in vertebrates and is generally not a threat to human health. However, copper may severely impact pelagic and benthic algae, aquatic macrophytes, invertebrates, and larval fish (Hites and Eisenreich 1987). Apparently, there are previously unrecognized sensitive elemental indicators. For example, the strong relationship of Br to BSi and diatom productivity suggests that the flux of the element Br is closely associated with freshwater diatom production and burial. The physiological requirement for bromine is poorly documented in diatoms, with most reports confined to the red and brown algae (Fries 1966; McLachlan and Craigie 1967; Wolk 1968). However, Johnson et al. (1995) recently demonstrated a nutritional requirement of bromine in the stalked marine diatom *Achnanthes*. Extracellular polymeric substance (EPS) secretion in this species apparently exhibits an absolute requirement for bromide as both stalk formation and adhesion require this compound. Moreover, bromoform and bromoalkane emissions have been documented for the ice diatoms *Nitzschia stella* and *Porosira pseudodenticulata* (Sturges et al. 1992, 1993) and a brominated phenolic benzaldehyde synthesized by *Navicula* sp. was reported by Manley and Chapman (1978). We have verified that Br is found in silicious frustules and extracellular exudates of several freshwater diatom genera, and we suspect that the incidence is widespread.

Fluxes of remains (diatom valves, rhizopod tests, *Bosmina* exuviae) and bromine indicate moderate depression of productivity during the period of active stamp mill discharge, despite increased total organic flux. Because the taxa selected are representative of radically different trophic levels, we conclude that the food web of Portage Lake was adversely affected by active slime-clay discharge. There are also

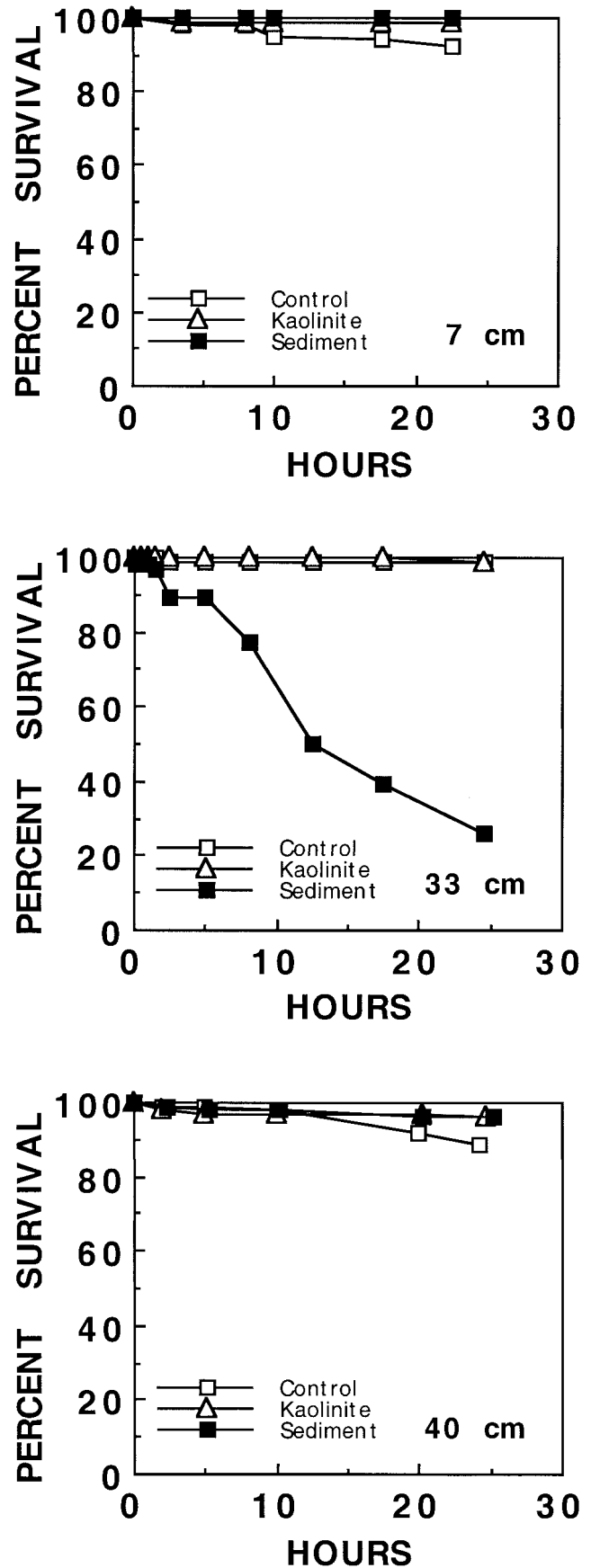


Fig. 6. Selected examples of survivorship patterns during clay toxicity tests. *Daphnia pulex* responses to sediments at three different levels: (a) premining, mining, and postmining. Dashed and broken lines indicate lake medium-only and kaolinite clay-only controls.

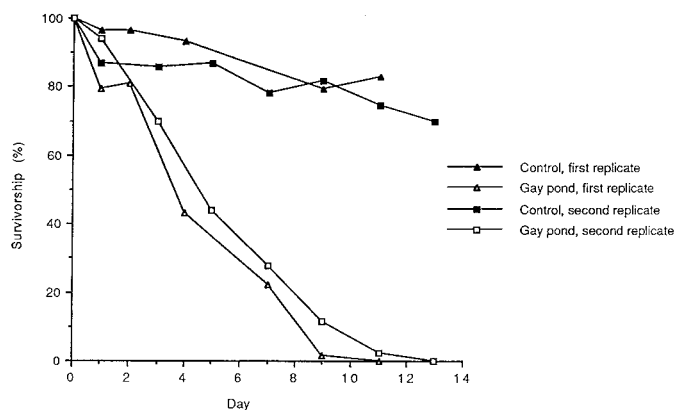


Fig. 7. Survivorship curves for in situ *Daphnia* incubation experiments. Curves contrast *Daphnia* survivorship in a Gay stamp sand pond (experimental) with survivorship in their native habitat (Coal Dock road pond; control). Results from two replicate experiments run between 15 and 25 May (58 vials, experimental and control) and 28 May and 12 June 1992 (86 experimental vials, 83 control vials).

indications that the pool of resting eggs, the so-called seed bank, was severely damaged, although the extent of this effect is difficult to assess because the highest copper fluxes were so long ago. The sediments from the mining era remain toxic to cladocerans (our studies) and to bacteria (Microtox tests; Cusack 1995; Cusack and Mihelcic 1999). Given the increasing human population density along the margins of the Waterway (Fig. 2), we also expected an increased productivity due to increased eutrophication aided recovery. However, all three biotic categories did not show major increases in the upper sediment layers until after cessation of active slime-clay discharge in 1947. The recovery ensued despite continued high copper concentrations in sediment strata and was associated with a return of mass sedimentation and percent organic matter to nearly background (pre-settlement) levels. During the recovery, we suspect that soluble copper interactions shifted from particle-bound dominance to much more organically mediated processes.

Some caution is necessary, for historical impacts of slime clays could be due to several causes, operating alone or in concert: systemwide physical disturbances, turbidity effects of suspended slime-clay particles on primary production, general effects of slime-clay ingestion on zooplankton, or direct toxicity caused by the copper associated with the slime clay. However, laboratory and field results suggest direct effects of slime-clay discharge, as sediment horizons that corresponded to slime-clay layers were found highly toxic. Sediments that contained higher organic matter concentrations were much less toxic, and the organically rich top 7 cm of sediment showed no toxicity. Moreover, laboratory clones and hatched resting eggs document a progressive decline in copper resistance that coincides with decreasing copper availability. An additional bioassay technique for resting eggs, presently explored in our laboratory, is to monitor the metallothionein activity of eggs and hatched clones. The field and laboratory observations suggest that there are marked differences in the bioavailability of copper among

the Portage Lake sediment horizons that ostensibly appear related to the organic matter content of the sediments.

The exact details of biogeochemical transformations are under investigation. Present shoreline stamp sand piles are porous. The slime fraction settles within the piles, is rinsed out after rains, and is remobilized by shoreline erosion. Whereas local rainfall is slightly acidic, groundwater that percolates through shoreline piles is basic (pH 8–10, the buffering capacity originating from calcite found with basalt in ore rocks) and receives Cu from the coarse fraction or from Fe-tenorite (CuO) complexes on the surface of the slime-clay fraction and then reprecipitates the copper as malachite (CuCO<sub>3</sub>), which is more soluble, around the edges of piles (Jeong et al. 1999). Regions immediately around near-shore stamp sand piles are characterized by reduced diversity of benthic macroinvertebrates (Kraft 1979; Kraft and Sypniewski 1981). The freshly reworked stamp sand sediments are toxic to *Daphnia* and *Hexagenia* because they release Cu across the pore-water gradient (Malueg et al. 1984). Additional laboratory toxicity experiments with slime-clay-rich sediments from the Keweenaw Waterway and Torch Lake showed that solid-phase sediments and aqueous fractions (e.g., interstitial water) associated with the slime-clay sediments were lethal to several taxa of freshwater macroinvertebrates: chironomids (*Chironomus tentans*), oligochaetes (*Lumbriculus variegatus*), amphipods (*Hyalella azteca*), and cladocerans (*Ceriodaphnia dubia*). Moreover, the observed toxicity was due to copper, as opposed to other metals (principally lead and zinc) present in the sediments (Schubauer-Berigan et al. 1993; West et al. 1993). Toxicity to benthic organisms was not predictable based on total copper concentrations in the sediments; rather it appeared that the bioavailable copper, i.e., the fraction that caused toxicity, was freely dissolved in the sediment interstitial water (Ankley et al. 1993).

Key factors that control interstitial concentrations of divalent metals are often sediment and system specific. For example, in anoxic sediments, labile sulfide complexes can be critical in dictating metal bioavailability, whereas in more aerobic systems, interstitial water concentrations of cationic metals are often controlled by complexation with iron and manganese oxides or organic carbon. The Keweenaw Waterway system is oligotrophic–mesotrophic, slightly basic (pH 6.5–9.1), and like much of the Lake Superior basin, surficial sediments are relatively aerobic. Recent studies indicate that sulfide does not appear to be an important binding phase that controls the bioavailability of copper in the Keweenaw Waterway sediments. Rather, in these aerobic freshwater sediments, organic carbon constitutes a major binding phase for cationic copper (Ankley et al. 1993). Although total organic carbon flux increased with mining activity, the increase was only 6× background as compared to the 220–340× increase in Cu flux. At present, aerated sediments allow recolonized benthos to work organic matter through a well-mixed layer 2–4 cm deep. Concentrations of pore-water copper show major decreases in the top organic-rich upper sediments of Portage and Torch Lake, due to organic complexation (Cusack 1995; Cusack and Mihelcic 1999). In the Waterway, at least 50% of the dissolved copper in pelagic waters is bound in an organic fraction (Brandt 1973). These

findings are consistent with the observation that in mesocosm experiments with *Daphnia*, dissolved organic matter (DOM) binding of copper is often the chief means of detoxification (Meador et al. 1993). Although the specific nature of the organic complexing agents is unclear, currently there is a substantial influx of humic acids from the Sturgeon, Trap, Pike, and Pilgrim Rivers (Spain et al. 1969) and moderate fulvic acid production from increased summer pelagic algal populations associated with historic eutrophication.

The full extent of recovery is still under investigation. *Daphnia* incubation experiments in stamp sand ponds indicate that most contain dissolved Cu concentrations too high to support cladoceran populations and that many existing shoreline stamp sand piles produce a lethal halo zone along the shoreline (Lytle 1997). However, zooplankton assemblages are returning to Torch Lake, the most disturbed local environment, and viable resting eggs can be found in surficial sediments. In the late 1960s to early 1970s, dissolved copper concentrations in Torch Lake were as high as 100  $\mu\text{g liter}^{-1}$  (Brandt 1973), yet have now declined below 20–30  $\mu\text{g liter}^{-1}$  (Lytle 1997).

Thus, despite its present pristine appearance, the Keweenaw Peninsular region appears to be an ecosystem in recovery from turn-of-the-century mining operations. The long-term legacy of regional mining operations is persistent, enhanced flux of metals, particularly copper. Our investigations underscore the valuable historical information provided by inorganic and organic flux calculations from sediment cores and the value of associated sediment and organism tests. The Keweenaw Waterway scenario suggests a natural ecosystem-scale self-healing process overridden by a trend of nearshore eutrophication, one that couples enhanced copper cycling from mining disturbances with organic complexation from nearshore wetland discharges and increased algae exudates to detoxify the circulating copper. Unfortunately, increased exchange of Lake Superior and Keweenaw Waterway waters has promoted hybridization and allowed elements of the large lake fauna to colonize inland waters successfully, suggesting that recovery from mining impacts will not mean return of zooplankton communities to their previous condition.

## References

- ANKLEY, G. T., V. R. MATTSON, E. N. LEONARD, C. W. WEST, AND J. L. BENNETT. 1993. Predicting the acute toxicity of copper in freshwater sediments: Evaluation of the role of acid volatile sulfide. *Environ. Toxicol. Chem.* **11**: 315–320.
- AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1991. New standard guide for conducting solid-phase sediment toxicity tests with freshwater invertebrates. ASTM Document E1383. American Society for Testing and Materials, Philadelphia.
- BENEDICT, C. H. 1955. Lake Superior milling practice. Michigan College of Mining and Technology, Houghton.
- BRANDT, D. J. 1973. Factors controlling soluble copper (II) levels in the Keweenaw Waterway. M.S. thesis, Michigan Technological University.
- CUSACK, C. C. 1995. Sediment toxicity from copper in the Torch Lake (MI) Great Lakes area of concern. M.S. thesis, Michigan Technological University.
- , AND J. R. MIHELICIC. 1999. Sediment toxicity from copper in the Torch Lake (MI) Great Lakes Area of Concern. *J. Great Lakes Res.* In press.
- DAMS, R., AND J. A. ROBBINS. 1970. Nondestructive neutron activation analysis of environmental samples. Great Lakes Research Division, Special Report 48. University of Michigan, Ann Arbor.
- DEAN, W. E., JR. 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition: Comparison with other methods. *J. Sediment. Petrol.* **44**: 242–248.
- DEMASTER, D. J. 1981. The supply and accumulation of silica in the marine environment. *Geochim. Cosmochim. Acta* **45**: 1715–1732.
- DE MELO, R., AND P. D. N. HEBERT. 1994a. A taxonomic reevaluation of North American Bosminidae. *Can. J. Zool.* **72**: 1808–1825.
- , AND ———. 1994b. Allozymic variation and species diversity in North American Bosminidae. *Can. J. Fish. Aquat. Sci.* **51**: 873–880.
- FRIES, L. 1966. Influence of iodine and bromine on growth of some red algae in axenic culture. *Physiol. Plant.* **19**: 800–808.
- HALL, D. J., AND N. D. YAN. 1997. Comparing the annual population growth estimates of the exotic invader *Bythotrephes* using sediment and plankton records. *Limnol. Oceanogr.* **42**: 112–120.
- HEBERT, P. N. 1995. The *Daphnia* of North America—an illustrated fauna. CD Rom, Macromedia.
- HITES, R. A., AND S. J. EISENREICH. 1987. Sources and fates of aquatic pollutants. American Chemical Society, Washington, D.C.
- HODSON, P. V., U. BORGMANN, AND H. SHEAR. 1979. Toxicity of copper to aquatic biota, p. 307–372. In J. O. Nriagu [ed.], *Copper in the environment, part II. Health effects*. Wiley-Interscience.
- JEONG, J., N. R. URBAN, AND S. GREEN. 1999. Release of copper from mine tailings on the Keweenaw Peninsula. *J. Great Lakes Res.* In press.
- JOHNSON, L. M., K. D. HOAGLAND, AND M. R. GRETZ. 1995. Effects of bromide and iodide on stalk secretion in the biofouling diatom *Achnanthes longipes* (Bacillariophyceae). *J. Phycol.* **31**: 401–412.
- KERFOOT, W. C. 1975. The divergence of adjacent populations. *Ecology* **56**: 1298–1313.
- . 1981. Long-term replacement cycles in cladoceran communities: A history of predation. *Ecology* **62**: 216–233.
- . 1987. Translocation experiments: *Bosmina* responses to copepod predation. *Ecology* **68**: 596–610.
- . 1995a. *Bosmina* remains in Lake Washington sediments: Qualitative heterogeneity of bay environments and quantitative correspondence to production. *Limnol. Oceanogr.* **40**: 211–225.
- . 1995b. The legacy of mining around Lake Superior: Ecosystem effects of copper mining. Final Report. Michigan Great Lakes Protection Fund, Office of the Great Lakes, Department of Natural Resources, Lansing.
- , AND K. L. KIRK. 1991. Degree of taste discrimination among suspension-feeding cladocerans and copepods: Implications for detritivory and herbivory. *Limnol. Oceanogr.* **36**: 1107–1123.
- , AND ———. 1993. Why the hypolimnion is not a desert: The taste discrimination–temperature hypothesis. *Verh. Int. Verein. Limnol.* **25**: 335–343.
- , G. LAUSTER, AND J. A. ROBBINS. 1994. Paleolimnological study of copper mining around Lake Superior: Artificial varves from Portage Lake provide a high resolution record. *Limnol. Oceanogr.* **39**: 649–669.
- , AND J. A. ROBBINS. 1999. Nearshore regions of Lake Su-

- perior: Multi-element signatures of mining discharges and a test of Pb-210 deposition under conditions of variable sediment mass flux. *J. Great Lakes Res.* In press.
- KOLPACK, R. L., AND S. A. BELL. 1968. Gasometric determination of carbon in sediments by hydroxide absorption. *J. Sediment. Petrol.* **38**: 139–145.
- KRAFT, K. J. 1979. *Pontoporeia* distribution along the Keweenaw shore of Lake Superior affected by copper tailings. *J. Great Lakes Res.* **5**: 28–35.
- , AND R. H. SYPNIEWSKI. 1981. Effect of sediment copper on the distribution of benthic macroinvertebrates in the Keweenaw Waterway. *J. Great Lakes Res.* **7**: 258–263.
- LANKTON, L. D. 1991. Cradle to grave. Oxford Univ. Press.
- , AND C. K. HYDE. 1982. Old reliable: An illustrated history of the Quincy Mining Company. Hancock, Michigan.
- LYTLE, R. 1997. The survival of *Daphnia pulex* relative to copper toxicity in the natural waters of the Keweenaw Peninsula. M.S. thesis, Michigan Technological University, Houghton.
- MALUEG, K. W., G. S. SCHUYTEMA, D. F. KRAWCZYK, AND J. H. GAKSTATTER. 1984. Laboratory sediment toxicity tests, sediment chemistry and distribution of benthic macroinvertebrates in sediments from the Keweenaw Waterway, Michigan. *Environ. Toxicol. Chem.* **3**: 233–242.
- MANLEY, J. A., AND D. J. CHAPMAN. 1978. Formation of 3-bromo-4-hydroxybenzaldehyde from L-tyrosine in cell-free homogenates of *Odonthalia floccosa* (Rhodophyceae): A proposed biosynthetic pathway for brominated phenols. *FEBS Lett.* **93**: 97–101.
- MCLACHLAN, J., AND J. S. CRAIGIE. 1967. Bromide, a substitute for chloride in a marine algal medium. *Nature (Lond.)* **214**: 604–605.
- MEADOR, J. P., F. B. TAUB, AND T. H. SIBLEY. 1993. Copper dynamics and the mechanism of ecosystem level recovery in a standardized aquatic microcosm. *Ecol. Appl.* **3**: 139–155.
- MURDOCH, W. A. 1943. Boom copper: The story of the first United States mining boom. MacMillan.
- NUSSMAN, D. G. 1965. Trace elements in the sediments of Lake Superior. Ph.D. thesis. Univ. Michigan.
- PYKE, D. A., AND J. N. THOMPSON. 1986. Statistical analysis of survival and removal rate experiments. *Ecology* **67**: 240–245.
- ROBBINS, J. A., AND D. N. EDGINGTON. 1975. Determination of recent sedimentation rates in Lake Michigan using <sup>210</sup>Pb and <sup>137</sup>Cs. *Geochim. Cosmochim. Acta* **39**: 285–304.
- , T. J. KEILTY, D. S. WHITE, AND D. N. EDGINGTON. 1989. Relationships between tibificid abundances, sediment composition, and accumulation rates in Lake Erie. *Can. J. Fish. Aquat. Sci.* **46**: 223–231.
- SCHELSKE, C. L., D. L. CONLEY, AND W. F. WARWICK. 1985. Historical relationships between phosphorus loading and biogenic silica accumulation in Bay of Quinte sediments. *Can. J. Fish. Aquat. Sci.* **42**: 1401–1409.
- SCHUBAUER-BERIGAN, M. K., J. R. DIERKES, P. D. MONSON, G. T. ANKLEY. 1993. pH-dependent toxicity of Cd, Cu, Ni, Pb, and Zn to *Ceriodaphnia dubia*, *Pimephales promelas*, *Hyalella azteca* and *Lumbriculus variegatus*. *Environ. Toxicol. Chem.* **12**: 1261–1266.
- SPAIN, J. D., D. B. DROWN, AND J. M. YANKO. 1969. The use of concentrations of electrolytes and naturally fluorescent materials to study water mass movements in a freshwater “estuary.” *Proc. 12th Conf. Great Lakes Res.* **72**: 723–733.
- STURGES, W. T., G. F. COTA, AND P. T. BUCKLEY. 1992. Bromoform emission from Arctic ice algae. *Nature (Lond.)* **358**: 660–662.
- , C. W. SULLIVAN, R. C. SCHNELL, L. E. HEIDT, AND W. H. POLLOCK. 1993. Bromoalkane production by Antarctic ice algae. *Tellus* **45B**: 120–126.
- SWINEFORD, A. P. 1876. History and review of the copper, iron, silver, slate and other material interests of the south shore of Lake Superior. The Mining Journal. Marquette, Michigan.
- TAYLOR, D. J., AND P. D. N. HEBERT. 1993. A reappraisal of phenotypic variation in *Daphnia galeata mendotae*: The role of interspecific hybridization. *Can. J. Fish. Aquat. Sci.* **50**: 2137–2146.
- VAN DER WERFF, A. 1955. A new method of concentrating and cleaning diatoms and other organisms. *Assoc. Theor. Appl. Limnol.* **12**: 276–277.
- WEIDER, L. J., W. LAMPERT, M. WESSELS, J. K. COLBOURNE, AND P. LIMBURG. 1997. Long-term genetic shifts in a microcrustacean egg bank associated with anthropogenic changes in the Lake Constance ecosystem. *Proc. R. Soc. Lond. Ser. B* **264**: 1613–1618.
- WEST, C. W., V. R. MATTSON, E. N. LEONARD, G. L. PHIPPS, AND G. T. ANKLEY. 1993. Comparison of the relative sensitivity of three benthic invertebrates to copper contaminated sediments from the Keweenaw Waterway. *Hydrobiologia* **262**: 57–68.
- WETZEL, R. G., AND G. E. LIKENS. 1991. *Limnological analyses*, 2nd ed. Springer-Verlag.
- WILKINSON, L. 1989. SYSTAT: The system for statistics. SYSTAT.
- WOLK, C. P. 1968. Role of bromine in the formation of refractile inclusions of the vesicle cells of the Bonnemaisoniaceae (Rhodophyta). *Planta* **78**: 371–378.

Received: 6 November 1998

Accepted: 18 February 1999

Amended: 13 April 1999