

## Experimental paleoecology (resurrection ecology): Chasing Van Valen's Red Queen hypothesis

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### Abstract

Taking an experimental approach to paleoecology, we evaluated the prey portion of Van Valen's Red Queen hypothesis by retrieving diapausing eggs from lake sediments for laboratory tests of evolutionary responses. The sediments consisted of core samples from Portage Lake (Michigan), which were dated by a combination of varve counts and radioisotope (<sup>137</sup>Cs and <sup>210</sup>Pb) techniques. The sedimentary record of Portage Lake documented several major environmental changes (mining, channeling, post-1950s eutrophication) over the past century. Remains of *Leptodora*, *Chaoborus*, and *Polyphemus* from sediments and historic fish surveys suggested a changing balance of invertebrate and vertebrate (fish) predators. *Daphnia retrocurva* was present in Portage Lake for over 80 yr and replaced *Daphnia dentifera* during the eutrophication period. Against the background of environmental change, we tested a part of the Red Queen hypothesis (i.e., that the prey species *D. retrocurva* must be continually evolving relative to its primary invertebrate predator, just to remain in place). We examined *D. retrocurva* morphology and genetics through time by retrieving and hatching diapausing eggs from different sediment strata for genetic characterization and by common garden experiments (i.e., isolates reared under the same environmental conditions). Mitochondrial DNA 12S/16S analyses and allozyme electrophoresis were used to characterize hatchlings from various levels. Allele frequencies at one allozyme (*Pgi*) locus in *D. retrocurva* differed over the eutrophication phase, suggesting founder effects, natural selection, or both. Common garden experiments with *D. retrocurva* hatchlings documented significant microevolutionary adjustments in both helmet and spine lengths, supporting the hypothesis of continual evolution in prey against a changing balance of predation.

Because they are tied to seasonal production, fluxes of well-preserved pelagic microfossils offer insights into the timing and magnitude of population responses to ecosystem perturbations. Paleoecology begins to merge with modern population ecology when the discipline follows production of zooplankton microfossils into sediment traps and sediments (Kerfoot 1974, 1981, 1995; Hall and Yan 1997; Kerfoot et al. 2000, 2004; Jarnagin et al. 2004) and then retrieves living categories of remains (e.g., zooplankton diapausing eggs) for laboratory ecological tests. In particular, the idea of removing viable propagule stages for assaying

past population responses opens up a new field with great promise (Hairston et al. 1999a; Kerfoot et al. 1999). Retrieving diapausing eggs for DNA analysis (e.g., sequencing), allozyme characterization, and simultaneous experimental testing can (1) provide an "in situ" historical bioassay for gauging the importance of ecosystem perturbations, (2) directly document evolution in time and space, and (3) allow experimental evaluation of alternative paleoecological and evolutionary conjectures. One can address the paradox of how to test historical events without altering history by retrieving ancestral lineages from routinely collected sediment cores for evaluation of alternative, plausible hypotheses. The approach has been termed resurrection ecology (Kerfoot et al. 1999) to underscore its novelty.

The first attempts to test historical hypotheses used diapausing eggs of cladocerans and sediment chemistry, complementing traditional descriptions from microfossil morphology, concentration, and flux (Hairston et al. 1999b, 2001; Kerfoot et al. 1999). Allozyme characterization and sequencing of *Daphnia* diapausing eggs from sediment cores (Weider et al. 1997; Hairston et al. 1999b; Kerfoot et al. 1999; Duffy et al. 2000; Cousyn et al. 2001; Limburg and Weider 2002; Kerfoot et al. in press) demonstrated the feasibility of the approach. Both Kerfoot et al. (1999) and Jepsen et al. (2001) have stressed the implications for paleoecology.

Certain aspects of egg bank retrieval studies still require attention, yet recent developments are encouraging. A fre-

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quently expressed concern is that many of the originally produced propagules hatch during the natural seasonal cycle, leaving only a small fraction of the original annual cohort in the egg bank (e.g., <5–10% of produced eggs end up in the long-term egg bank; De Stasio 1989; Kerfoot et al. 2004). A simple test for surficial egg bank bias is to check gene frequencies of living populations against gene frequencies of diapausing eggs in the top sediments (0–4 cm; Jankowski and Straile 2003; Keller and Spaak 2004). Bias could also enter during laboratory hatching (Limburg and Weider 2002). Standard protocols involve hatching a randomly selected subset of *Daphnia* eggs from core strata and growing these individuals as clonal stocks for experimental tests. However, if laboratory hatching success is low, one might ask whether the established clones are a true subsample of the diapausing egg pool. The discovery that certain allozymes (e.g., *Pgi*; Kerfoot et al. 1999) can be run directly on cladoceran diapausing eggs helps to circumvent this problem, complementing previous hatching and cloning protocols. The ability to genetically characterize all viable cladoceran eggs with polymerase chain reaction and DNA analyses (Duffy et al. 2000; Cousyn et al. 2001; Limburg and Weider 2002) also minimizes concerns about hatching bias (Gomez and Carvalho 2000). Finally, retrieved individuals are heat-killed after experiments, ensuring that past genetic material does not get into modern-day populations. Both direct (genetic) and indirect (i.e., rearing of hatchlings) approaches are used here.

Paleoecological investigations of propagules can provide valuable regional and local historical perspectives. For example, decomposition-resistant dormant or diapausing stages could answer several questions: Are the species of concern recent to the lake, or have they been residents for a lengthy time? To what degree have species evolved since colonizing a lake? Are there temporal differences in behavior, morphology, competitive ability, or resistance to predators since colonization? As in the Red Queen hypothesis (Van Valen 1973), to what degree have species had to evolve merely to stay in place, given ecological changes in the local environment (aquatic food web)? Van Valen's (1973) Red Queen hypothesis was based on an interpretation of progressive morphological change in the fossil record, suggesting co-evolutionary responses between predator and prey pairs or two competitors. With resurrection ecology, we can begin to test evolutionary changes in prey and predator pairs to see whether both respond through time. We can measure the nature of trait responses and observe the intensity of interactions with laboratory tests. Many of the grand conjectures of evolutionary ecology now come within reach of laboratory testing because the time frame of evolutionary responses (i.e., multiple generations) conforms nicely to the span of living eggs retrieved from sediment cores.

The aims of this investigation are modest. Embayments along an irregular shoreline could be an important source of species that disperse seasonally around coastal zones, creating a metapopulation structure (i.e., a population of populations; Levins 1970). Crucial to the metapopulation argument is the degree of independence of events within embayments and the linking of individual species to the multispecies coastal assemblages. Embayment histories can be

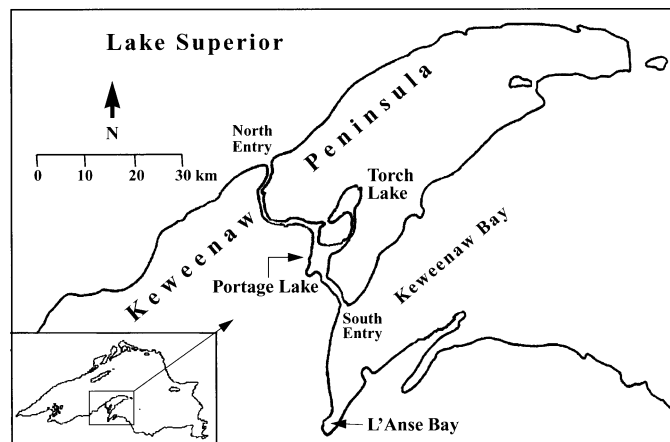


Fig. 1. The Keweenaw Peninsula region, showing the locations of Portage Lake, the North and South Entries to the Keweenaw Waterway, and the L'Anse Bay portion of Keweenaw Bay, Lake Superior.

very simple or extremely complicated, with species coming and going in time and space. Species in embayments might respond to a variety of historical site-specific selective pressures, while contributing individuals to the larger coastal pool (Kerfoot 1975, 1995; Kerfoot and Peterson 1980). Site-specific evolutionary adjustments could be important in allowing long-term persistence in the embayments. Here, we use paleoecological techniques to trace the arrival of *Daphnia retrocurva* at Portage Lake in the Keweenaw Waterway and to see how populations subsequently responded to local site conditions. Our aim is to document historical environmental changes by first observing the nature and fluxes of microfossils through time. Then we experimentally test for possible microevolutionary responses within a current dominant prey species, *D. retrocurva*, in Portage Lake as it experienced the changing predator environments.

## Materials and methods

**Keweenaw Waterway**—The Keweenaw Peninsula juts 96 km into Lake Superior from the southern coastline (Fig. 1). At its base, the Keweenaw Waterway comprises a system of lakes and canals (46°58' to 47°14'N, 88°23' to 88°39'W) in an extensive freshwater embayment. The major lake at the center is Portage Lake, with a total volume of  $2.9 \times 10^8$  m<sup>3</sup>, a maximum depth of 16.5 m, and a mean depth of 8.2 m. The lake has three arms, one of which extends 21.6 km to Lake Superior on the north side of the Peninsula (North Entry), a second which extends 7.9 km to Keweenaw Bay of Lake Superior on the south (South Entry), and a third which extends about 8.0 km in a northeasterly direction to Torch Lake.

Channeling at the turn of the century allowed increased water exchange between Lake Superior waters and the original land-locked system. Originally, the dual-basin depression (Torch Lake, Portage Lake) drained via the Portage River into the Keweenaw Bay region of Lake Superior. To facilitate ship passage during early mining activities, the southeastern and northwestern ends of the portage were

channeled in fall 1859 by private enterprise and improved ca. 1862–1873 (Swineford 1876), creating the Keweenaw Waterway. Opened to general navigation in 1890 by the Army Corps of Engineers, the entries to the Waterway were expanded and more deeply channeled in the late 1910s and early 1920s, then straightened in the 1930s to early 1940s, increasing water exchange.

*Historical background of zooplankton and fish communities*—Around the shorelines of Lake Superior are three kinds of zooplankton assemblages (Kwiatkowski 1980): a cold, deep-water copepod assemblage dominated by large-bodied calanoid copepods (e.g., *Leptodiaptomus sicilis*, *Limnocalanus macrurus*, *Scenecella calanoides*, *Eurytemora affinis*); a seasonally transient warm-water coastal assemblage with cladocerans (e.g., *Daphnia mendotae*, *Holopedium gibberum*, *Eubosmina longispina*, *Eubosmina coregoni*, *Bosmina leideri*, *Leptodora kindtii*, *Bythotrephes cederstroemi*) and some characteristic copepods (e.g., *Epischura lacustris*, *Leptodiaptomus oregonensis*); and a series of freshwater embayment communities with distinctive resident species (e.g., cladocerans: *Daphnia dentifera*, *Ceriodaphnia* sp., *Diaphanosoma* sp., *Bosmina freyi*, *Polyphemus* sp.; copepod: *Leptodiaptomus minutus*). Recruitment into the cold-water offshore assemblage is predominately by overwintering adults, whereas recruitment into both the nearshore coastal and embayment assemblages depends on hatching of diapausing eggs. At some sites, species hatch from eggs deposited along shelf regions, whereas at other sites, individuals are contributed by river and embayment discharges.

There is evidence for relative stability of deep-water offshore zooplankton communities, at least over the past 30 yr (Patalas 1972; Selgeby 1975; Watson and Wilson 1978; Gorman et al. pers. comm.). By contrast, the species composition of coastal assemblages has fluctuated over the same span, in large part because of introduced invertebrate predators (*Bythotrephes cederstroemi*) and planktivorous fishes (rainbow smelt, *Osmerus mordax*; yellow perch, *Perca flavescens*). The Fisheries and Wildlife Laboratory, Ashland, Wisconsin (now U.S. Biological Survey [USBS]-BRD), has surveyed nearshore fish for decades. Their research has documented how endemic (lake herring, *Coregonus artedii*) and introduced (rainbow smelt) planktivores have fluctuated in density by more than an order of magnitude over decadal scales (Selgeby 1982; Hansen 1990; Conner et al. 1993). From the 1950s until the 1990s, smelt became very abundant in Keweenaw Bay and the Waterway, with major smelt runs in the Pilgrim and Sturgeon Rivers.

By contrast, fish changes in the Waterway are less well documented through time. There are scattered regional collections of embayment fish communities, along with occasional fish scales in sediments. The impression from early regional surveys was that humic-stained stream and river waters that contained native trout, grayling, and indigenous stream lamprey populations discharged into the clear, cold waters of Lake Superior (Agassiz 1850; Moore and Braem 1965). By the 1970s to 1990s, the Michigan Department of Natural Resources began to conduct routine fish surveys in the Keweenaw Waterway. During the 26 July–7 August 1971 and 1973 surveys, the Waterway was sampled at 40

stations with gill, trap, and fyke (hoop) nets. In 1995, a 52-night survey used gill and trap nets in Portage Lake, the South Entry part of the Keweenaw Waterway, and the L'Anse Bay of Lake Superior. These surveys were supplemented by fyke net and electrofishing efforts in 1994, 1998, and 2000. The compiled data (Table 1) document that by the latter half of the 1900s, warmer water species such as yellow perch, centrarchids, and walleye were now common in the Keweenaw Waterway during late summer.

*Sediment cores*—Previous core work on Portage Lake sediments emphasized dating techniques, largely to ensure accurate sedimentation and microfossil flux calculations (Kerfoot et al. 1994, 1999; Kerfoot and Robbins 1999). In 1997, three (21 Sep 97) sediment cores were taken with a 5.1-cm (2-in.) diameter Phleger-type KB gravity corer from the deep, central depression of Portage Lake. The KB corer allows water to pass through the 1-m-long core barrel during free fall, minimizing disturbance to sampled strata. Initial cores in Portage Lake were used to establish sedimentation rates and fluxes of Cu and diatom and cladoceran remains. In order to provide additional samples for a detailed genetic characterization of diapausing eggs, sediments were sampled from the same Portage deep-hole site aboard the R/V *Laurentian* in the summers of 1998–1999, with the use of a large-volume (70 × 70 × 100 cm) box corer (MK-V Soutar/Van Veen). Three core tube liners from the KB corer were lowered under partial vacuum into the box core sediments, providing replicate cores. The replicate cores were X-rayed, sliced at 1- or 2-cm intervals, then sieved through 350- and 75- $\mu$ m Nitex netting.

Position in the chronological sequence was determined from annual varves and by radioisotope dating techniques ( $^{137}\text{Cs}$  and  $^{210}\text{Pb}$ ; Fig. 2). X-rays of sediment revealed a rich sequence of varvelike clay bands that derived from annual copper mill discharges (Fig. 2 insert). The distinctive bands in the cores offered outstanding opportunities for accurately determining time horizons and deposition rates down to the late 1850s, the beginning of mill operations. The X-ray-dense bands corresponded to slime clay fractions of the tailings discharged under ice cover during winter. Radiotracer studies in top strata complemented the varve counts. X-rayed replicate cores were cross-compared with  $^{137}\text{Cs}$ - and  $^{210}\text{Pb}$ -dated cores, matching varves against time horizons dated by radioisotope techniques. Initial cross-comparisons are published (Kerfoot et al. 1994; Kerfoot and Robbins 1999). Replicate cores showed close correspondence with top strata, although deeper bands (1880–1860) of the 1998–1999 cores showed some divergence of sedimentation rate that required correction with varve stratigraphy.

For flux determinations, initial steps involved slicing the original three cores (1997) into 1-cm intervals and removing subsamples for analysis of animal remains (*Bosmina* carapace and head shield counts). Sediment subsamples used for  $^{137}\text{Cs}$  and  $^{210}\text{Pb}$  analyses 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  $^{40}\text{K}$ ,  $^{210}\text{Pb}$  and  $^{226}\text{Ra}$ ) with a high-resolution, planar GE(Li)  $\gamma$ -detection/multichannel analyzer system. For  $^{137}\text{Cs}$  analysis, detector effi-

Table 1. Fish collected in the Keweenaw Waterway. Early records from 1921 to 1929 state surveys, whereas later records compiled from Baraga Michigan Department of Natural Resources census studies. Totals from replicate samples separated by commas. Numbers not strictly comparable because they come from different gear (see *Materials and Methods*). +, present.

Type	No. of fish						
	Early	1971	1973	1994	1995	1998	2000
Perch	+	1,159	358	265	92, 96	89	216
White sucker	+	308	202	124	155, 201	38	582
Walleye		21	1	38	19, 27	11	124
Rock bass	+	55	3	250	13, 0	57	1,972
B. bullhead		189		122	66, 0	45	11,224
Alewife		624		16	3, 43	16	4
Northern pike		120	42	54	10, 14		88
Trout and salmon	+	8	4	2	2, 21	2	14
Longnose sucker	+	16	77	4	6, 2		32
Rainbow smelt		83		8	0, 5		
Trout-perch	+	198	28		4, 0	50	
Burbot	+	1	7		6, 3		4
Bluegill, pumpkinseed		186		34		37	56
Smallmouth bass	+	4		4		86	76
Redhorse	+	23		100		5	308
Carp		3		2		1	4
Golden shiner	+	3					
Black crappie							70
Whitefish							4
Lake sturgeon	+					1	
Spottail shiner	+					18	
Lake herring	+			2			
Brook lamprey	+						

ciency was determined by doping sediments with precisely known (1%) amounts of a 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 emission, with a precision generally better than 5% for counting times of  $\sim 1$  d. Checks on both the  $^{137}\text{Cs}$  and  $^{210}\text{Pb}$  profiles are now completed and cross-correlated with varve banding patterns (Kerfoot and Robbins 1999).

Use of the radio-lead dating method depends on the presence of a significant signal of excess  $^{210}\text{Pb}$  above background, in which the latter results from in situ decay of parent  $^{226}\text{Ra}$ . However, under variable sediment accumulation rates, both  $^{210}\text{Pb}$  and  $^{226}\text{Ra}$  must be measured. For each sample,  $4.00 \pm 0.01$  g of dry sediment was packed into small counting vials of cylindrical cross section to a constant height. Fixing both the geometry and mean bulk density of sediments removed the major sources of variability in efficiency of gamma counting. Samples were sealed with radium-free epoxy cement, and gamma-emitting progeny were allowed to equilibrate with radium for at least 20 d prior to counting. Three photopeaks associated with  $^{214}\text{Pb}$  and  $^{214}\text{Bi}$  were used (295, 352, and 609 keV) to determine a single, weighted least-squares average value of  $^{226}\text{Ra}$ .  $^{210}\text{Pb}$  activity was assessed by the 46.5-keV photopeak. The efficiency of detection was checked with samples of the same sediment spiked with a well-determined amount of a National Institute of Standards and Technology standard solution of  $^{226}\text{Ra}$ . Uncertainties in determination of  $^{226}\text{Ra}$  were  $\leq 5\%$ . Near the top of the core, uncertainties in determination of excess  $^{210}\text{Pb}$

were  $\sim 3\%$ , but increased to  $\sim 20\%$  in the region of background below  $\sim 20$  cm depth (Kerfoot and Robbins 1999).

Sediment subsamples for Cu analysis were air dried and homogenized by grinding to a fine powder. A small aliquot ( $< 0.25$  g) of each sample was placed into a preweighed Teflon digestion vessel. Ultrapure nitric acid solution (25 ml of 10%, v/v) was added to each vessel and digested with a CEM microwave digester. Filtrate samples (0.45  $\mu\text{m}$  Gelman Supor-450) were analyzed for Cu by atomic absorption spectrophotometry (Perkin-Elmer model 5000, Kerfoot et al. 1994; Kerfoot and Robbins 1999). The plotted values for Cu represent the means of three subsamples from the digested slice.

Concentrations of *Bosmina* head shields and carapaces (numbers  $\text{cm}^{-3}$ ) were determined from replicated 20- $\mu\text{l}$  subsamples drawn from stirred 1-ml samples, mounted on glass slides, and covered by a coverslip. A 10% KOH pretreatment helped to remove flocculated organic matter and debris (Kerfoot 1981). Counts and measurements of *Bosmina* were made at  $\times 500$  under a Zeiss Universal compound microscope. Head shields and carapaces often disarticulated after molting or death, so these items were tabulated separately. Division of concentration (numbers  $\text{cm}^{-3}$ ) by deposition time ( $\text{yr cm}^{-1}$ ) gives the deposition flux per year (numbers  $\text{cm}^{-2} \text{yr}^{-1}$ ).

Certain potentially valuable microfossils (e.g., cladoceran ephippia, copepod diapausing eggs, *Leptodora* tail spines and mandibles, *Chaoborus* mandibles) are too scarce for conventional analysis by 1-ml subsamples. We sieved (350, 75- $\mu\text{m}$  Nitex) entire core slices to retrieve these microfossils.

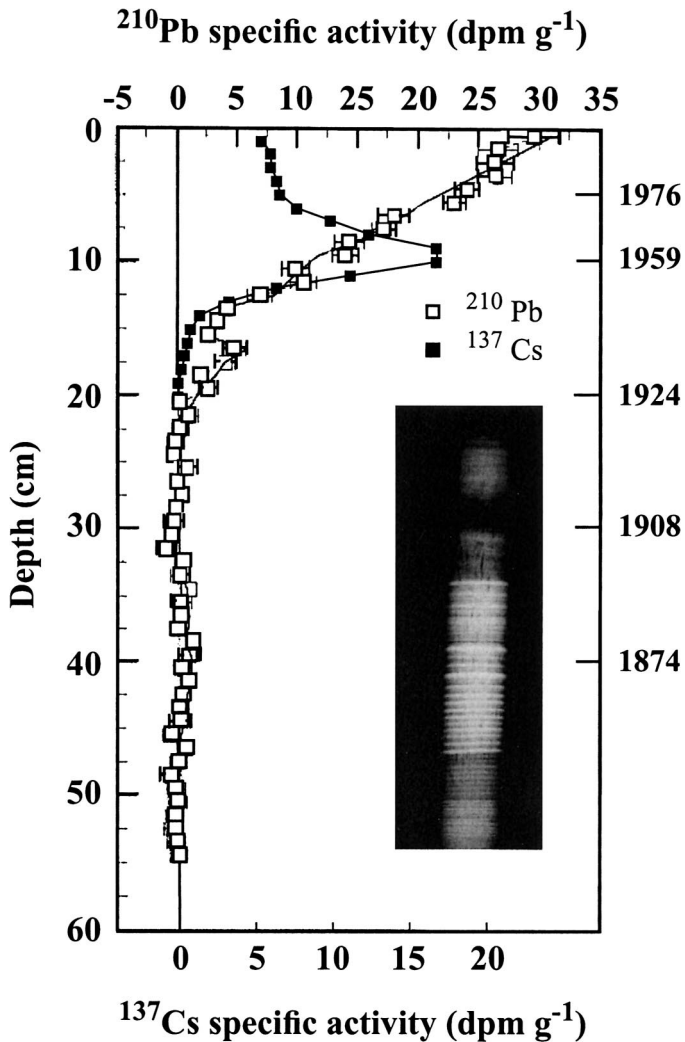


Fig. 2. Radioisotope ( $^{137}\text{Cs}$ ,  $^{210}\text{Pb}$ ) profiles from Portage Lake sediments (deep hole) and X-ray of slime clay varves (see Kerfoot et al. 1994, 1999; Kerfoot and Robbins 1999 for more details on varves and radioisotope profiles). The slime clay bands cover the interval of stamp sand discharges from 1857 to 1957, whereas the radioisotope techniques cover the interval from 1925 to the present. The excess (unsupported) activity of  $^{210}\text{Pb}$  declines from a maximum of about  $30 \text{ dpm g}^{-1}$  at the surface to levels supported through decay of radium at about 20 cm depth.

To assay for frequency dependence, we counted the incidence of *Leptodora* tail spines and mandibles relative to *Bosmina* carapaces. We also measured the length of *Leptodora* spines, quantifying size changes through time. However, the size differences largely reflect the incidence of various instars (age structure), rather than changes in adult size.

*Characterization of Daphnia from plankton samples*—Plankton samples were collected sporadically from several sites around the Keweenaw Peninsula over the past 10 yr and systematically at approximately monthly intervals from April through October 1997–2000 as part of the National Oceanic and Atmospheric Administration/National Science Foundation Keweenaw Interdisciplinary Transport Experi-

ment in Lake Superior (NOAA/NSF KITES) project, which investigated nearshore currents and Waterway exchanges. In addition, samples were taken from the Keweenaw Waterway at the deep (18 m), central depression of Portage Lake. A 0.5-m-diameter, 125- $\mu\text{m}$ -mesh Nitex net (Puget Sound model) was hauled vertically, although additional small net tows in Portage Lake supplemented the larger monthly tows. Samples were preserved with a 10% sucrose/formalin solution ( $40 \text{ g sucrose L}^{-1}$ , in  $100 \text{ ml } 37\% \text{ formaldehyde L}^{-1}$ ).

The dominant *Daphnia* species are cyclical parthenogens that reproduce during summer months by parthenogenesis, yet overwinter as sexually produced diapausing eggs. Under high density and resource shortage, females will lay clutches of hormonally induced males and become sexual (Banta 1939), modifying the carapace into a saddle-like case, the ephippium, which houses one to two diapausing eggs. The ephippium usually has a characteristic shape and external sculpturing which aids identification but is not always distinctive under light microscopy.

Individuals from plankton samples and laboratory clones were imaged under a SPOT digital camera mounted atop an Olympus SZX12 stereomicroscope. Feed from the digital camera was manipulated by Image Pro Plus (Media Cybernetics) software on an HP Vectra (Hewlett-Packard) with recordable/rewritable CD-ROM capability. Helmet and spine lengths were measured following Jacobs (1961). For detailed morphological measurements, individuals were transferred to a 50:50 glycerin:water droplet on a glass slide, covered with a glass coverslip, and measured at  $\times 125$ –500 under a Zeiss Universal compound microscope.

*Genetic analysis*—Genetic analysis of *D. retrocurva* in Portage Lake allowed us to examine genetic changes in populations since colonization. Initial analysis (Kerfoot et al. 1999) encountered some stratigraphic problems. Over the past two decades, the world-wide systematics of *Daphnia* has undergone a revolution, with recognition of far more species than previously described (Hebert 1995; Colbourne and Hebert 1996). Hybridization is acknowledged as widespread in certain Australian, European, and New World species (Hebert 1985, 1995; Wolf and Mort 1986) and is a local problem with *D. mendotae* and *D. dentifera* (Taylor and Hebert 1992, 1993; Kerfoot et al. 1999, in press). However, hybridization has not been found in *D. retrocurva* within North America. The morphological characterization seems rather straightforward, and this species is rarely confused with other similar-sized species (Hebert 1995). Thus, we concentrated on this species for detailed genetic and cloning studies. Nevertheless, modern work on *Daphnia* almost always requires allozyme or mitochondrial (mt)DNA characterization, just to confirm which species or hybrids are present.

Sieved diapausing eggs from sediment cores were stored under low light and low temperature ( $4$ – $6^\circ\text{C}$ ) in walk-in Hobart constant-temperature chambers. Temperature- and light-controlled rearing experiments used Percival environmental chambers. Hatching success was tested directly by placing viable individual diapausing eggs in 100-mm petri dishes at  $10$ – $20^\circ\text{C}$  under long day length. Dishes were checked each day during the first week and at 2–4-d intervals until 24 d.

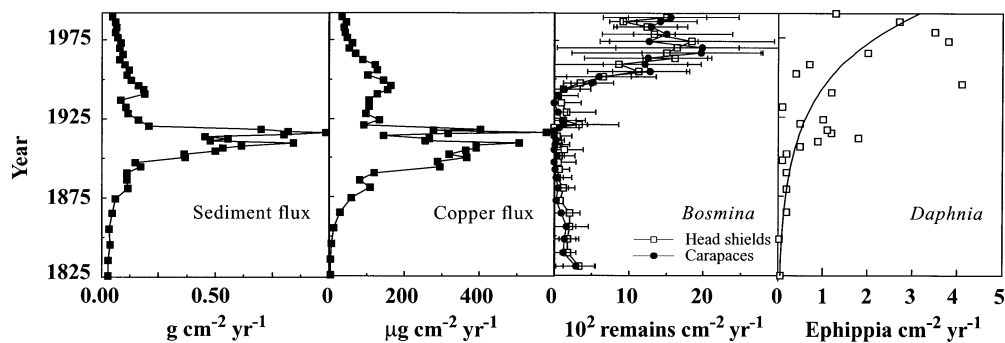


Fig. 3. Historic fluxes of sediment, copper, *Bosmina* head shields and carapaces, and *Daphnia* ephippia in Portage Lake sediments. The high sediment and copper fluxes correspond to the copper mining era, whereas the increased *Bosmina* remains and *Daphnia* ephippia after the mining era mark eutrophication during the recovery phase. Note that the fluxes of cladoceran remains exceed presettlement values from the 1950s to the present, indicating greater production than in presettlement strata.

Initial investigations at the Max Planck Institute used 2,031 eggs from three 1997 replicate Portage Lake cores. Seven later hatching experiments (4 Mar 99, 9 Mar 99, 10 Mar 99, 17 Mar 99, 18 Mar 99, 31 May 00, and 8 Jun 00) at Michigan Technological University used ephippia sieved from Ekman Dredge and 1998–1999 core samples. The latter experiments used 2,030 eggs.

*Daphnia* hatched from the original three Portage cores (1997, KB core) were raised as clonal lines and identified to species by cellulose acetate allozyme electrophoresis (Hebert and Beaton 1993; phosphoglucose isomerase, *Pgi*, EC 5.3.1.9; phosphoglucosmutase, *Pgm*, EC 5.4.2.1; aldehyde oxidase, *Ao*, EC 1.2.3.1; glutamate–oxaloacetate transaminase, *Got*, EC 2.6.1.1; lactate dehydrogenase, *Ldh*, EC 1.1.1.27) and mitochondrial mtDNA 12S/16S data (Colbourne and Hebert 1996). Viable *Daphnia* ephippia removed from later cores (sieved strata, KB core liner cores, 1998–1999 box corer) were run only for *Pgi* (EC 5.3.1.9). During the latter procedure, ephippia from slices were removed for common garden experiments.

Agreement with Hardy–Weinberg equilibria was tested on genotype frequencies by SYSTAT (chi-square with sequential Bonferroni correction; Wilkinson 1989) and BIOSYS (Swofford and Selander 1989).

**Common garden experiments**—As individuals hatched from diapausing eggs, we established clones not only for genetic characterization, but also for morphological comparisons. Highest hatching success was for the most recent strata, with hatching percentage decreasing with depth and age in sediment cores. Despite these difficulties, 671 clones were established over a great range of sediment depths and raised under common garden conditions (i.e., uniform temperature [21°C], light [9:15 light:dark], and food [*Chlamydomonas*] conditions). Clones were reared in 40-ml vials filled with 30 ml of Millipore-filtered Portage Lake water and interspersed in a test tube rack to ensure uniform rearing conditions. Only clones positively identified as *D. retrocurva* were used to investigate morphological differences. Species identification was verified as *D. retrocurva* by unique morphological characters (e.g., small comb on postabdomen, lack of ocellus)

and allozyme characterization. Males induced from crowded clones served as an additional species check. Regression analysis of morphological variables among clones from various depths was by SYSTAT (version 9.0, MGLH, regression and analysis of covariance, with Bonferroni correction; Sokal and Rohlf 1987; Wilkinson 1989).

## Results

**Portage Lake ecosystem perturbations**—Several ecosystem perturbations have occurred on the Keweenaw Peninsula over the last 150 yr (Fig. 3). As one of the first great North American metal mining rushes, native copper mining on the Keweenaw Peninsula began in the late 1840s, reached peaks between 1890 and 1925, and ended in 1968. The history of mining, enhanced copper flux, and environmental impacts are discussed elsewhere in detail (Kerfoot et al. 1994, 1999, 2002). Increased sediment and copper fluxes mark the copper mining era in sediment cores. At the turn of the last century, the slime clay discharges depressed certain species of phytoplankton and cladocerans but did not eliminate *Daphnia* from the Waterway (Kerfoot et al. 1999).

Following the mining era, a second, more recent perturbation was cultural eutrophication. Locally around the Keweenaw Peninsula, towns expanded hand-in-hand with copper production. In Keweenaw and Houghton Counties, the population increased to almost 90,000 by 1920, declined to around 37,000 by 1960, and remained at that level until the present (Kerfoot et al. 1999). However, construction of a municipal sewage treatment system and regional treatment plants did not begin until the late 1940s. The city systems were expanded from the 1950s to the 1970s, following a pattern common to many locations in North America. In 1995, a new, higher volume plant was constructed near Portage Lake, potentially accelerating nutrient input. Although the Waterway environment is oligotrophic to barely mesotrophic (June–July chlorophyll *a*, 3.2–4.2  $\mu\text{g L}^{-1}$ ), within Portage Lake, there are clear signs of progressive nutrient enrichment, with reduced Secchi disk transparency, occasional cyanobacterial blooms (*Microcystis*) in late August,

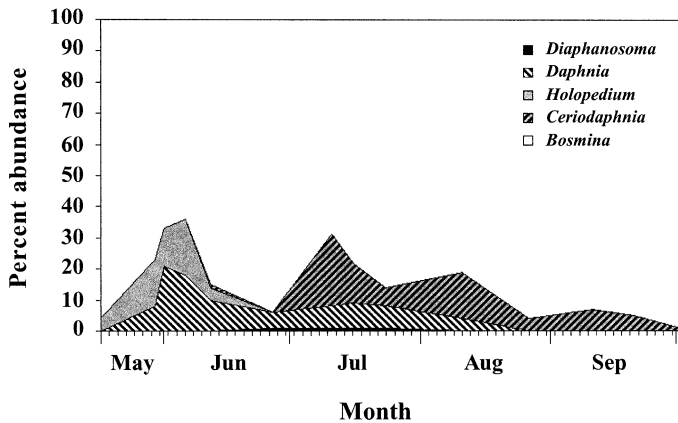


Fig. 4. Seasonal abundance (% individuals) of various herbivorous cladocerans in Portage Lake zooplankton samples (1998). *Bosmina* dominate cladoceran assemblages throughout the year, whereas *Holopedium* and *Ceriodaphnia* show seasonal succession. *D. retrocurva* are moderately abundant from May until late August ( $n = 117\text{--}583$ , mean = 403, SD = 135).

and oxygen depletion of hypolimnetic waters (low of 3–4 mg O<sub>2</sub> L<sup>-1</sup>).

At present in the Portage Lake portion of the Keweenaw Waterway, *Bosmina* dominates the herbivorous cladoceran assemblage (Fig. 4). The invertebrate predator, *L. kindtii*, is briefly present in May–June, but only as a few individuals (1–4 per vertical net haul). *Chaoborus* is present in hypolimnetic waters, but in low abundance. *Holopedium* is abundant in spring and is replaced by *Ceriodaphnia* in summer. Not surprisingly, microfossil assemblages are dominated by *Bosmina* carapaces and head shields (Fig. 3), which are resistant to decomposition, as well as numerous diapausing eggs (Fig. 5).

**Egg bank assemblages**—Sediment core records give us a historical perspective on modern-day zooplankton distributions. Viable diapausing eggs of crustaceans are common in Portage Lake sediments, forming a clear egg bank (Fig. 5). Copepod eggs (mainly *Skistodiptomus minutus*, *Leptodiptomus oregonensis*, some *Epischura*) are most numerous in core slices, with slice counts ranging from hundreds to thousands. Because the interior diameter of the core is 4.47 cm; a 1-cm core slice has a volume of 20.3 cm<sup>3</sup>, so dividing the egg count by 20.3 gives concentrations per cubic centimeter. Copepod egg concentrations have ranged between 4 and 72 eggs cm<sup>-3</sup> since the early 1950s. Cladoceran (pelagic species: *Holopedium*, *Daphnia*, *Bosmina*, *Diaphanosoma*, *Ceriodaphnia*; benthic species: *Simocephalus*, *Alona*) diapausing eggs also are abundant, ranging between 4 and 14 eggs cm<sup>-3</sup> since the early 1950s. Although the ephyppial egg assemblages reflect the presence of modern-day species in pelagic assemblages, *Holopedium* and *Daphnia* are overrepresented relative to percent pelagic abundance, whereas *Bosmina* and *Ceriodaphnia* are underrepresented (Figs. 4, 5). All these taxa extend back decades into the sedimentary record. *Bosmina* and *Holopedium* eggs are more abundant recently, and *Daphnia* showed a peak in the 1960s. Viable eggs of *Daphnia* extend down 15–20 cm, ~70–80 yr, and

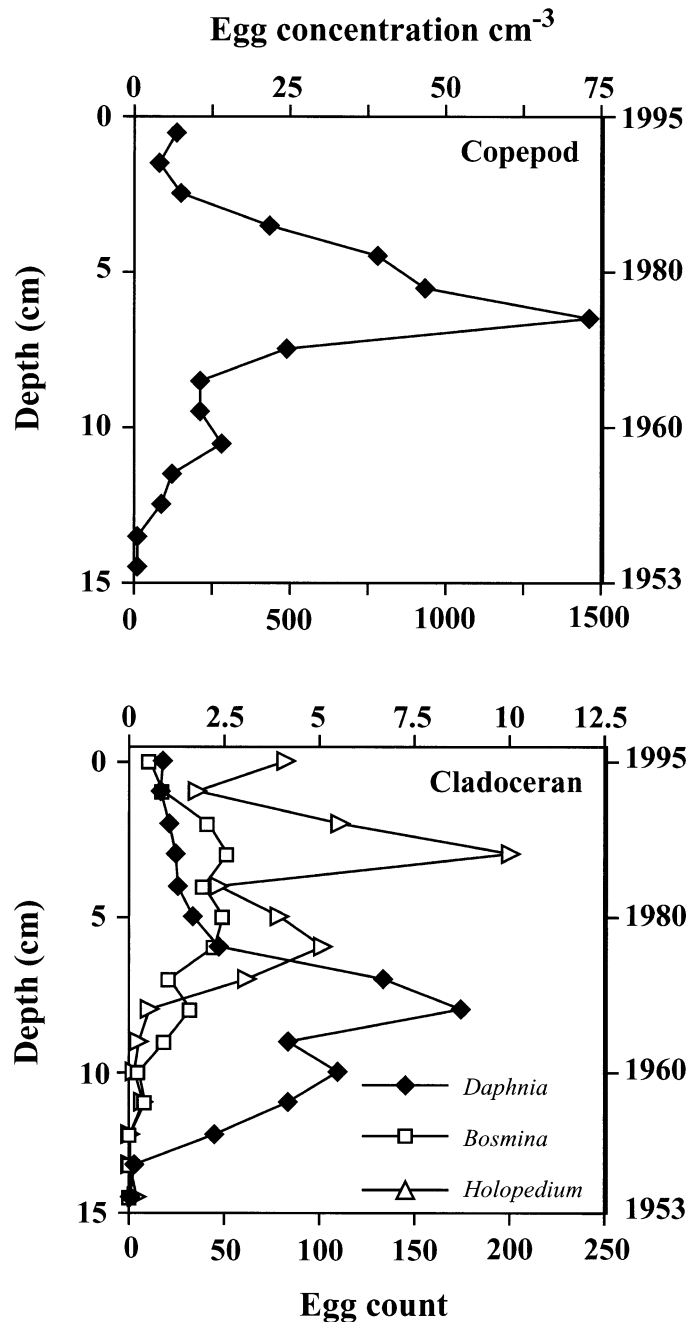


Fig. 5. The microcrustacean egg bank of Portage Lake illustrating egg abundance of various taxa with depth in the sedimentary record. Counts of eggs are for 1-cm slices of the core down to a depth of 15 cm. To convert counts into densities, divide by 20 cm<sup>3</sup> (the volume of a 1-cm slice). Note that the eggs of *Daphnia* are most abundant between 7 and 13 cm depth, whereas those of *Bosmina* and *Holopedium* are most abundant at 3–8 cm depth. Viable eggs of *Daphnia* continue back 60–80 yr.

are truncated by thick slime clay layers from mining discharges, shown to be toxic (Kerfoot et al. 1994, 1999). Empty ephyppial cases extend down further, for hundreds to thousands of years (Livingstone Piston core; W. C. Kerfoot pers. obs.).

In the top sediment layers, there were 100–500 *Daphnia*

Table 2. Predatory invertebrate remains and cladoceran ephippia from sieved 2-cm slices (replicate core No. 3, 5 Aug 99). *Leptodora* tallies are tail spines, *Chaoborus* are mandibles, and *Polypheumus* are diapausing egg cases. Ephippia are totals, without reference to intact diapausing eggs. To convert counts into densities, divide by the volume of the slice (40 cm<sup>3</sup>).

Year	Depth (cm)	Invertebrate predator remains			Cladoceran ephippia				
		<i>Leptodora</i> spines	<i>Chaoborus</i>	<i>Polypheumus</i>	<i>Daphnia retrocurva</i>	<i>Daphnia dentifera</i>	<i>Ceriodaphnia</i>	<i>Simocephalus</i>	<i>Alona</i>
1994	0–2	7	1	—	98	—	8	4	2
1987	2–4	21	—	—	245	—	12	1	6
1980	4–6	50	—	—	378	4	20	3	13
1974	6–8	39	—	—	439	4	38	3	17
1967	8–10	131	—	—	524	2	55	3	37
1961	10–12	136	—	1	643	6	21	4	22
1956	12–14	240	—	—	209	64	1	4	1
1953	14–16	104	—	4	42	41	1	0	0
1946	16–18	103	—	—	19	83	0	0	0
1941	18–20	139	—	8	85	180	2	0	0
1934	20–22	113	—	—	74	169	0	0	0
1921	24–26	6	—	1	13	136	1	0	1
1880	40–42	10	1	2	0	69	0	0	2
	50–52	13	3	36	3	92	0	0	0
	52–54	21	6	8	7	102	0	0	2

ephippia per 2-cm core slice, but only 18–47 viable diapausing eggs. Neglecting the top stratum (0–2 cm) enriched in recently produced ephippia, we can calculate representation in the egg bank relative to annual production. If the central core site is representative of deep-water sediments, over the last 30 yr the ratio of viable diapausing eggs to total ephippia suggests that only 5–7% of the original egg production now remains in the *Daphnia* egg bank (mean = 5.7%, SD = 0.4%,  $n = 3$  strata between 2 and 8 cm). The estimate assumes that ephippia were deposited with the full compliment of two eggs, which might not be the exact circumstance (see Kerfoot et al. 2004).

There is clear evidence of changing zooplankton assemblages through time. Presettlement strata (before 1850) contain filter combs, postabdomens, and mandibles of a large-bodied *Daphnia*. Fluxes of *Bosmina* and *Daphnia* microfossils are low throughout the mining era (Fig. 3). However, during recovery, fluxes exceed presettlement values. Fluxes of *Bosmina* remains increased sevenfold above presettlement values, with the increase beginning in the 1950s (Fig. 3). Over time, the flux of *Daphnia* ephippia also increased severalfold (10 $\times$ ), paralleling the increase in fluxes of *Bosmina* carapaces and head shields ( $r = 0.750$ ). In general, *Bosmina*, *Ceriodaphnia*, *Daphnia*, and *Simocephalus* total ephippial counts correlate well (Table 2; range of  $r = 0.606$ – $0.905$ ). Note, however, the difference in scale for *Daphnia* ephippia in Fig. 3 compared with *Bosmina* carapaces and head shields, emphasizing the relative scarcity of *Daphnia* ephippia as microfossils in sediments. In the 1997 cores, fluxes of *Daphnia* ephippia suggest low production in premining strata ( $0.03$ – $0.06$  ephippia cm<sup>-2</sup> yr<sup>-1</sup>) and gradual increases through the mining era, with post-1950s fluxes reaching 4 ephippia cm<sup>-2</sup> yr<sup>-1</sup>. Additional analysis from the 1998–1999 cores gave a mean flux for total *Daphnia* ephippia from 1950 to the present of  $3.1 \pm 1.3$  (mean  $\pm$  95% CL,  $n = 7$  time strata) compared with  $0.3$  ephippia cm<sup>-2</sup>

yr<sup>-1</sup> for 1850–1870, a 10-fold increase above background, suggesting general eutrophication of the Waterway during recovery.

Despite the overwhelming evidence for postmining recovery and increased productivity of pelagic and benthic species, indicating eutrophication, there is also sedimentary evidence for a modified balance of invertebrate and vertebrate (fish) predation over time. In 75- $\mu$ m sieved samples, *Leptodora* spines and mandibles and *Chaoborus* teeth were much more abundant in lower core strata (Table 2). The length of *Leptodora* spines was also decreased through time. At 20–22 cm depth, *Leptodora* spines were large ( $n = 44$ ; mean 95% CL =  $990 \pm 78$   $\mu$ m length), whereas in top strata they are scarce and small (6–8 cm,  $622 \pm 59$   $\mu$ m,  $n = 34$ ; 2–4 cm,  $710 \pm 98$   $\mu$ m,  $n = 19$ ). The difference in *Leptodora* spine lengths is interpreted primarily as a change in the instar distribution: a shift from a more balanced adult : juvenile age distribution in lower strata to a dominance of juveniles in recent strata. Moreover, the ratio of *Leptodora* tail spines to *Bosmina* carapaces has changed dramatically from deep (15 cm) to surface strata (Table 3). Sixty years ago, the ratio was  $\sim 12.6$ – $17.2$  *Leptodora* spines per  $10^3$  *Bosmina* carapaces. As *Bosmina* increased in abundance, the ratio decreased progressively to only 0.4–0.6 *Leptodora* spines per  $10^3$  *Bosmina* carapaces in surface strata. A similar trend is found for *Leptodora* mandibles (i.e., an order of magnitude decrease in ratio to *Bosmina* carapaces) because they are highly correlated with tail spine counts ( $r = 0.757$ ,  $p < 0.001$ ,  $n = 14$  strata).

*Appearance of D. retrocurva in Portage Lake*—Several *Daphnia* species are characteristic of the present-day Waterway and southern Lake Superior coastal regime (Kerfoot et al. in press). *D. dentifera* and scarce round-headed *D. mendotae* are present in the highly stained waters of the Sturgeon Sloughs, which discharge into the southern portion of the

Table 3. Frequency of *Leptodora* tail spines relative to *Bosmina* carapaces in Portage Lake sediments. Data pooled into 3-cm depth classes for chi-square test of homogeneity, five groups,  $\chi^2 = 198.3$ ,  $df = 4$ ,  $p < 0.005$ .

Depth (cm)	Year	<i>Bosmina</i> carapaces	<i>Leptodora</i> tail spines	Spines (per 10 <sup>3</sup> carapaces)
0–1	1995	2,164	1	0.5
1–2	1990	1,687	1	0.6
2–3	1988	2,472	2	0.4
3–4	1985	4,015	2	0.5
4–5	1982	3,893	3	0.8
5–6	1978	4,822	8	1.7
6–7	1976	3,063	3	1.0
7–8	1973	2,819	7	2.5
8–9	1968	3,062	12	3.9
9–10	1964	3,139	9	2.9
10–11	1962	2,402	19	7.9
11–12	1959	3,116	17	5.5
12–13	1957	3,174	14	4.4
13–14	1956	2,152	27	12.6
14–15	1953	1,630	28	17.2

Keweenaw Waterway. Both species occur sporadically in the Pike Bay region of Portage Lake, and occasionally in the deeper, hypolimnetic waters of Portage Lake. Highly transparent, large-bodied, helmeted *D. mendotae* are characteristic of summer coastal waters, surging in and out of the Keweenaw Waterway at both ends (North and South Entries) during the year as seiche and internal wave oscillations create cool sills that penetrate the warmer, productive waters of the Keweenaw Waterway (Churchill et al. in press; Kerfoot et al. in press). *D. retrocurva* is present at lower concentrations and persists longer in nearshore Lake Superior waters, reaching peak densities in August–September. The species is present in Keweenaw Bay (Kerfoot et al. in press), but at lower densities (8–57 individuals m<sup>-3</sup>) than *D. mendotae* and is most abundant in the southern, shallow region called L'Anse Bay.

At present, the smaller bodied, helmeted *D. retrocurva* dominates summer *Daphnia* assemblages within the Keweenaw Waterway, especially in Portage and Torch Lakes. May–June densities average 150–698 individuals m<sup>-3</sup>, whereas July densities can reach 1,330–2,390 individuals m<sup>-3</sup>. Diapausing egg production usually is initiated in July at a time when densities decline. August, September, and October densities are relatively low (0–106 individuals m<sup>-3</sup>).

The ephippia of *D. dentifera* are more heavily pigmented than those of *D. mendotae* and *D. retrocurva*, with honeycomb ornamentation (Kerfoot et al. in press). Moreover, the ephippia of *D. dentifera* (mean length  $\pm$  95% CL = 992  $\pm$  24  $\mu$ m,  $n = 120$ ) and *D. mendotae* (898  $\pm$  23  $\mu$ m,  $n = 86$ ) are larger and more triangular than the smaller, hemispherical ephippia of *D. retrocurva* (708  $\pm$  36  $\mu$ m,  $n = 30$ ), allowing us to easily distinguish the latter species from the former two in sediments. *Daphnia* ephippia are abundant in the Waterway and sheltered bay surficial sediments and generally reflect the nature of pelagic *Daphnia* assemblages (Kerfoot et al. in press).

Although *Daphnia* have been present in Portage Lake for hundreds of years, one recent revelation is that *D. retrocurva* and *D. mendotae* ephippia were abundant only during the past 150 yr in nearshore waters of Keweenaw Bay (i.e., since European arrival and forest clearance (Kerfoot et al. in press). In the L'Anse Bay core, Kerfoot et al. (in press) found that *Daphnia* appeared at 33 cm (Fig. 6) then became progressively more abundant in strata above 27 cm depth. *D. retrocurva* increased from the 1850s until the 1910s then declined relative to *D. mendotae*, which increased in the 1920s until the 1940s and became proportionately more abundant up to the present.

Within Portage Lake, *Daphnia* species composition showed substantial change within the ephippial assemblage. The Portage Lake assemblage was sensitive to the creation of the Waterway, which permitted increased mixture of inland and Lake Superior plankton. On the basis of the morphological characters of ephippia, filter combs, and postabdomens, the relatively large-bodied *Daphnia* in deep strata was *D. dentifera*. After the creation of the Waterway, *D. dentifera* gave way to a mixture of *D. retrocurva* and hybrids (*D. mendotae*  $\times$  *D. dentifera*  $\times$  *D. galeata* complex; Kerfoot et al. 1999 in press). *D. retrocurva* might have penetrated the Waterway during its peak abundance in Keweenaw Bay. The species then became common as smaller cladocerans (*Bosmina* and *Ceriodaphnia*) began a dramatic increase during eutrophication. The smaller, roundish, and more transparent *D. retrocurva* ephippia have dominated the egg bank for the last 40 yr.

*Genetics of D. retrocurva.*—Two sets of cores from the central, deep hole of Portage Lake were examined for genetic changes in *D. retrocurva* diapausing eggs (see *Materials and methods*). In the initial 1997 hatching experiments, 225 *Daphnia* eggs from Portage Lake cores hatched to establish isolates from a total of 2,031 eggs, giving a hatching percentage of 11.1% (Fig. 7). Seven additional hatching experiments were carried out during the summers of 1998–2000 with sieved ephippia from more recent core material (see *Materials and Methods*). After transfer from a 4°C incubator to Portage Lake water in petri dishes at room temperature (20°C), eggs started hatching within 2 d. Peak hatching occurred on days 4–8 and continued for a month thereafter. From a total of 2,030 eggs, 233 hatched (11.5%), which was very close to the original estimate (from 1997 experiments) of hatching success.

In the 1997 core experiments, hatchlings from diapausing eggs were used to establish 206 clonal lines, which were then analyzed for mtDNA restriction fragment length polymorphisms (RFLPs) of the small (12S) and large (16S) subunits (genes) coding for rDNA. In the mtDNA 12S/16S characterization, we uncovered only one polymorphic restriction enzyme: *Dra*I. The population appeared monomorphic at *Dde*I, *Dpn*II, *Hinf*I, *Bfa*I, *Sau*96I, as well as at *Rsa*I and *Taq*I. No restriction enzyme (cut) sites were found for *Hae*III, *Hpa*II, *Scr*FI, and *Bst*uI. At *Dra*I, only two haplotypes were detected that varied at a single restriction site. When we pooled data from 0 to 8 cm and 8 to 18 cm, there were no significant shifts in *Dra*I (haplotype) frequency (Table 4).

Furthermore, we analyzed electrophoretic variation at four

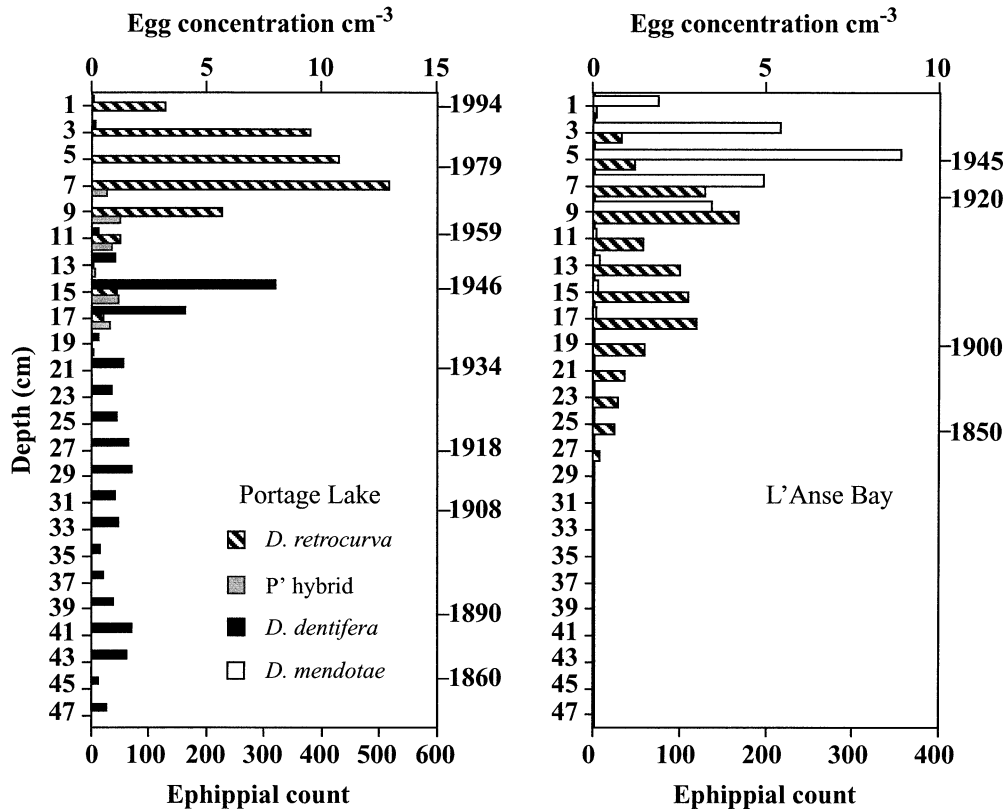


Fig. 6. *Daphnia* ephippia are assigned to species categories for Portage Lake and L'Anse Bay core strata. Counts are concentrations per 2-cm slice (slice volume = 40 cm<sup>3</sup>). Species determinations are made on the basis of morphology and hatching experiments. In Portage Lake, note the transition between dominance by *D. dentifera* in deeper strata and *D. retrocurva* in shallower strata. Where possible, from a combination of radiotracer studies (<sup>137</sup>Cs and <sup>210</sup>Pb, Kerfoot and Robbins 1999) and varves, dates have been indicated in the right-hand margin of figures. The nature of hybrid taxa (*D. dentifera* × *D. mendotae* × *D. galeata*) is discussed in Kerfoot et al. (in press). Figure redrawn from Kerfoot et al. (in press).

loci (*Pgm*, *Pgi*, *Ao*, *Got*). The set of *D. retrocurva* clones were fixed or nearly fixed at the *Ao* and *Got* loci. However, *Pgi* contained suggested stratigraphic differences, but the sample sizes were too small to adequately compare temporal trends (Table 4). The diapausing eggs from the Keweenaw Waterway documented two distinctly different populations: one was characterized by close agreement with Hardy–Weinberg equilibria (*D. retrocurva*, the P series), whereas another entity had a separately segregating series of alleles with a high frequency of heterozygotes (the P' series). Ephippia with alleles in the P' series occurred lower in the core strata and never occurred in combination with the regular P series of segregating alleles. The highly heterozygous assemblage (P') first appeared, as *D. dentifera* was dropping out, filling the transition between the larger bodied, pigmented *D. dentifera* and the smaller bodied, helmeted, and highly transparent *D. retrocurva*. This taxon (*D. mendotae* × *D. dentifera* hybrid complex) is treated elsewhere (Kerfoot et al. in press). We present the stratigraphic data here just to emphasize how morphologically similar ephippia can create problems that are sorted out with larger sample sizes and more detailed genetic studies.

To increase sample sizes, remove hatching bias, and help

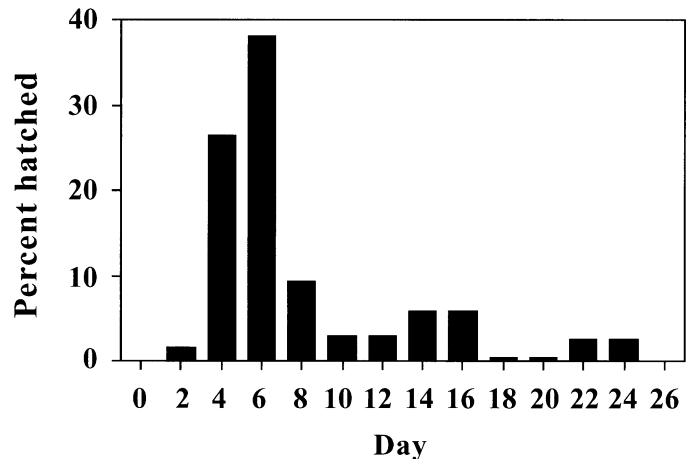


Fig. 7. Time schedule of hatching from *D. retrocurva* ephippia. Example comes from 1998 samples, 11.1% hatching success. Peak hatching occurred between days 4 and 8 and continued for a month.

Table 4. Mean number of viable diapausing eggs retrieved from sieved, 2-cm-thick slices of Portage Lake sediment cores (21 Sep 97;  $n = 3$  cores). The number of isolated *Daphnia* hatchlings (isolates) used to establish clonal cultures from the three sliced cores are tabulated and matched with the stratum date. Depth is midslice (cm). The number of clones run for *Pgi* and the frequency of the *F*-allele are also indicated in the right column. Data from 12S/16S mtDNA, *DraI* analysis is given below. Merging *DraI* data into two sets (1997–1973, 1966–1941) suggested no evidence for differences within the mtDNA haplotype series (see 95% CL below).

Year	Depth (cm)	Ephippia	Isolates	Clones ( <i>Pgi</i> )	<i>F</i> allele
<i>Pgi</i> allozyme					
1993	1	32	37	32	0.311
1986	3	43	43	29	0.241
1979	5	53	37	37	0.216
1973	7	60	12	12	0.208
1966	9	93	27	27	0.204
1959	11	78	14	14	0.321
1955	13	98	41	41	0.22
1952	15	159	13	13	0.231
1946	17	54	1	1	0.5
Totals		670	225	206	weighted 0.245
Haplotype					
	Depth, cm	Clones			
			A	B	
12S/16S mtDNA, <i>DraI</i>					
	1997–1973	0–8	32	0.484±0.366	0.516±0.366
	1966–1941	8–18	39	0.452±0.177	0.548±0.177

follow single-species populations through time, we collected a second set of cores in 1998–1999 from the Portage Lake deep hole (see *Materials and Methods*), sieved *Daphnia* ephippia from 2-cm slices, and directly ran the diapausing eggs for *Pgi* (Fig. 8). Here, sample sizes ranged between 65 and 214 for individual slices, allowing much more detailed comparisons within single species. *Pgi* allele frequencies for *D. retrocurva* are in Hardy–Weinberg equilibrium within summer plankton samples ( $\chi^2_3 = 0.2$ ,  $0.98 < p < 0.99$ ); that is, the genotype frequencies correspond to combinations expected from random segregation in the living population. The modern planktonic populations (Live, Fig. 8) also have genotype frequencies very similar to those found in diapausing eggs from surficial sediments (Live vs. 0–4 cm;  $\chi^2_3 = 1.6$ ,  $0.75 > p > 0.50$ ), suggesting little bias in surficial layers of the egg bank, at least at the *Pgi* locus. Despite the observation that only 5–6% of the produced diapausing eggs ended up in the egg bank (see Egg Bank Assemblages), the genotype frequencies at the *Pgi* locus are similar to living populations. That the correspondence to living populations extends down into surficial sediments is not surprising because top sediments are poorly consolidated and usually constitute a region of active mixing and exchange with overlying waters.

*Pgi* allozyme electromorph patterns again distinguished two separate breeding populations through time: one with four alleles (regular P series, *D. retrocurva*) and the other with three alleles (P' series). At the base of the viable egg bank (14–16 cm depth), *D. retrocurva* and the P' species were equally abundant. The P' species became scarce in Portage Lake main basin waters about 40 yr ago, whereas *D. retrocurva* persisted up to the present (Fig. 8, regular P series). Removing the influence of the P' species allows direct comparison of allele frequencies for *D. retrocurva* through

out the 80-yr core interval (Table 5). The fast allele ( $P_2 = F$  in Table 4) shows significant change with depth, decreasing in frequency from 0.34 at 14–16 cm depth to 0.24 in the top strata (Fig. 8;  $\chi^2_{12} = 103.0$ ,  $p < 0.0001$ , chi-square test of homogeneity across seven strata; sequential Bonferroni correction  $p = 0.05/7 = 0.007$ ).

*Morphology of D. retrocurva clones from Portage Lake: Ancestral phenotypes in common garden experiment*—Retrieving eggs and rearing offspring under controlled light, temperature, medium, and food conditions (common garden experiments) allowed us to examine microevolutionary differences between time horizons. *D. retrocurva* eggs were hatched from different strata of the Portage Lake cores to establish 671 clonal “isolates.” Growth of helmets within cultures was compared with regression analysis. All helmet regressions had significant to highly significant slopes, merely showing that helmets grew with body length. However, proportionate lengths decreased (so-called negative allometry) in cultures, a pattern expected when individuals are raised in the absence of chemical stimuli from fish or invertebrate predators (Jacobs 1961; Tollrian and Harvell 1999). Within this general pattern, regression slopes showed significantly greater growth rates of helmets in deeper, older sediments (Fig. 9; Table 6). A nested analysis of covariance on regression equations revealed significant differences between strata ( $F_6 = 5.7$ ,  $p = 0.003$ ), maintained with the Bonferroni correction ( $p < 0.007$ ).

Differences in another trait, tail spine lengths, were striking for clones from different strata and also showed strong trends through time. For deeper strata, tail spines were large and grew with a positive slope (Fig. 9; Table 6). Intermediate strata showed a decrease in spine lengths and a marked shift toward lower growth coefficients, whereas clones from shal-

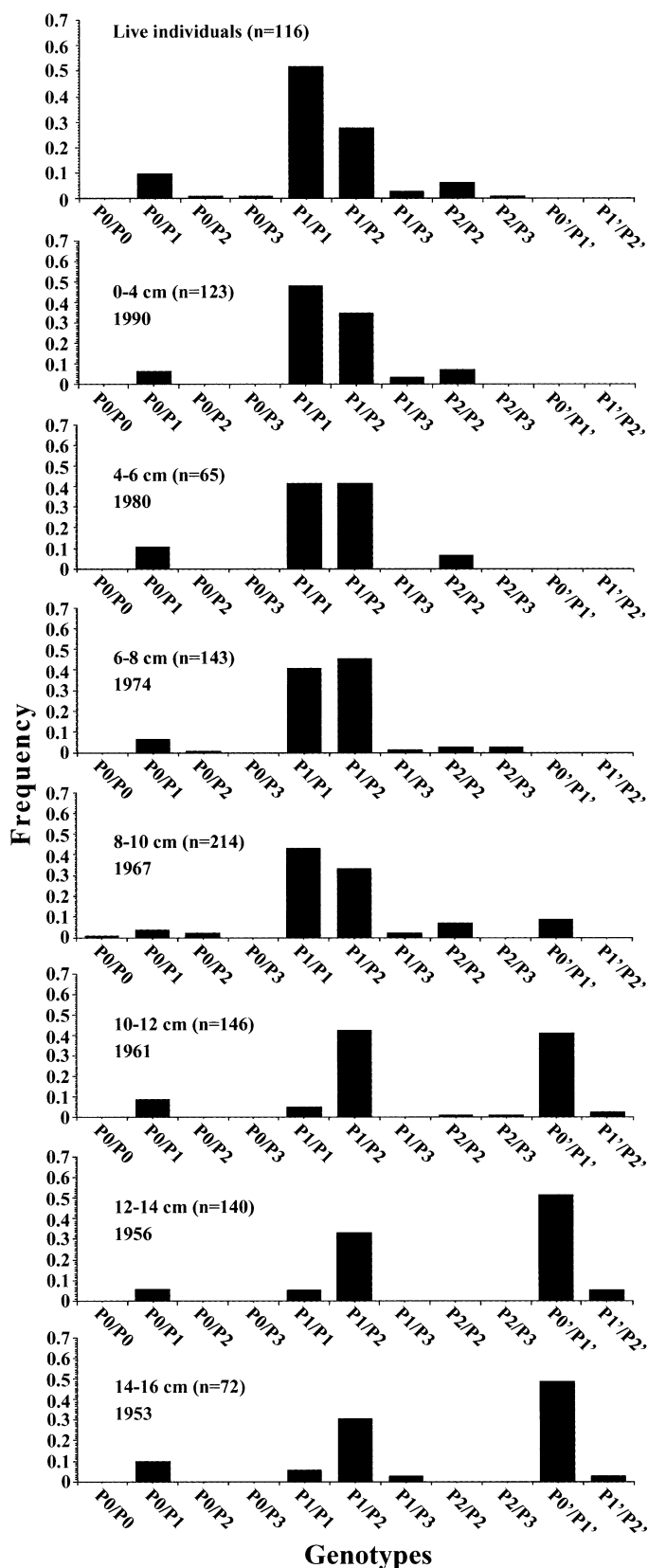


Fig. 8. *Pgi* allozyme frequencies for *D. retrocurva* (P series) through time. Samples compare living populations (Live) with diapausing eggs from surficial (0–4 cm) and deeper strata. Assignment

Table 5. *Pgi* allele frequencies in *D. retrocurva* diapausing eggs from Portage Lake (core 21 No. 2 replicate, 5 Aug 99). Cores were sliced at 2-cm intervals, then sediment was sieved through 350- and 75- $\mu$ m Nitex netting ( $n$  = sample size for eggs).

Depth (cm)	Year	$n$	Gene frequency			
			$P_0$	$P_1$	$P_2$	$P_3$
Live	—	138	0.030	0.685	0.240	0.048
0–4	1988	156	0.039	0.716	0.235	0.013
4–6	1973	65	0.054	0.677	0.270	—
6–8	1961	135	0.034	0.679	0.278	0.011
8–10	1950	194	0.026	0.700	0.227	0.048
10–12	1946	80	0.057	0.545	0.395	0.007
12–14	1940	61	0.066	0.558	0.377	—
14–16	1929	29	0.086	0.495	0.340	—

low strata exhibited negative regression slopes. A nested analysis of covariance on regressions in Table 6 demonstrated highly significant differences between regressions, with Bonferroni correction ( $F_6 = 68.1, p \ll 0.001$ ). Multiple samples from strata guarded against pseudoreplication (Table 6); thus, clear microevolutionary differences occurred over time in relative helmet and spine lengths. Moreover, the helmet and spine length differences were consistent with strata patterns for *Leptodora* in Portage Lake (Tables 2, 3) and simultaneously increasing populations of planktivorous fishes (perch, smelt). That is, the correspondence suggests that large helmets and long spines were selected in the presence of higher *Leptodora* densities, whereas the reverse was true in the presence of more dense piscivorous fish that reduced *Leptodora* densities. These trends have been observed in direct, shorter term lake experiments with *Leptodora* (Browman et al. 1989; McNaught 1993).

## Discussion

Ecologists are primarily concerned with the nature of environmental and biotic interactions—how biological interactions (predation, competition) influence the abundance and distribution of organisms. Secondly, we are interested in evolution of the phenotype, especially on traits that influence how populations interact. The evidence for historic environmental changes in Portage Lake was evident from sediment, copper, and crustacean microfossil fluxes. The copper mining perturbation of the late 1880s to early 1900s was followed by changes in species assemblages and by evidence for eutrophication. Simultaneous colonization of species (*D. retrocurva*, *Holopedium*, *Simocephalus*) and subsequent increased microfossil flux of almost all taxa argued for general increas-

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of dates under stratum depth is based on a combination of radio-tracer studies and cross-correlated varves (Fig. 2; Kerfoot et al. 1994, 1999; Kerfoot and Robbins 1999). The small, hemispherical ephippia in cores also include a hybrid complex (P' series; *D. dentifera*  $\times$  *D. mendotae*  $\times$  *D. galeata* complex), discussed in Kerfoot et al. (in press), which caused initial difficulties in species identifications.

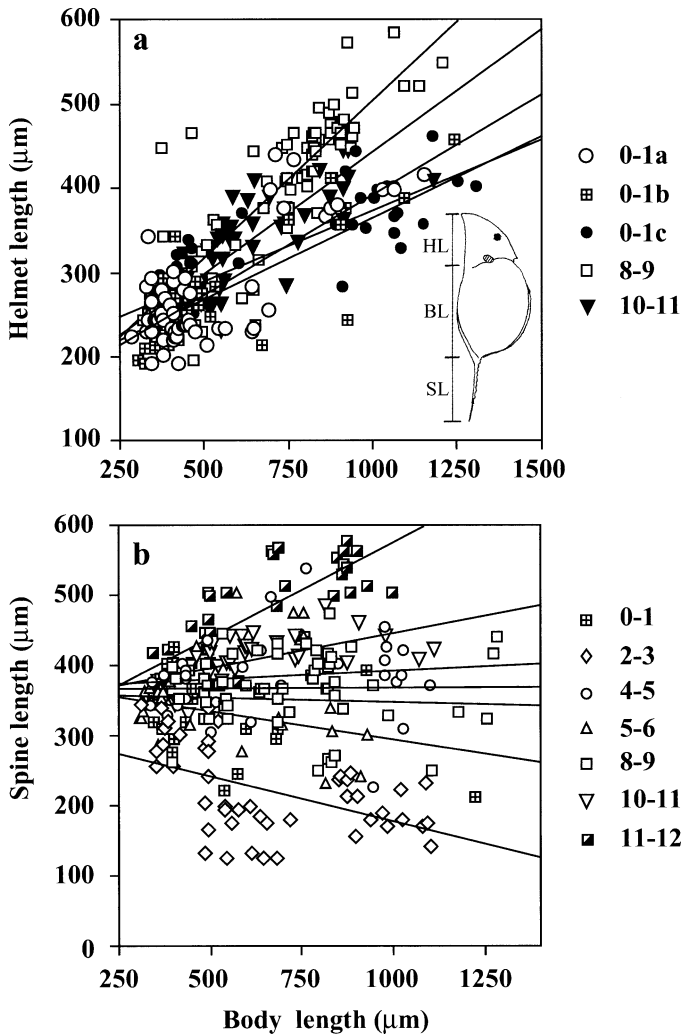


Fig. 9. Helmet and spine lengths of Portage Lake *D. retrocurva* clones established in common garden experiments. (a) Helmet growth relationships for various depths (cm); (b) relative tail spine lengths, ranging from positive growth in older samples to reduction in recent strata. Insert clarifies position of morphological measurements. Regression equations for various depths are given in Table 6.

es in productivity during recovery from the 1950s to the present. Initially low densities of *D. retrocurva* ephippia in presettlement strata from Portage Lake and the nearshore assemblage of Lake Superior might suggest that this species was one of the first recent invaders of Lake Superior, carried in from the warmer, southern Great Lakes (Michigan, Erie, Ontario) by ship traffic through the St. Marys River locks (Kerfoot et al. in press). The first published record of *D. retrocurva* in Lake Superior is from 1889 (Balcer et al. 1987), confirming its presence in the late 1800s. Alternatively, the increase may signal water quality changes associated with deforestation.

The microfossil record of Portage Lake sediments helps document the timing of species arrival (local colonization events) and the nature of changing assemblages associated with environmental conditions. Ephippial assemblages revealed that *D. dentifera* formerly occupied the stained waters

of the original Torch Lake–Portage Lake system. Initial geographic surveys suggested lower concentrations of planktivorous fishes. Originally, the Portage River drained the interior system into Keweenaw Bay, creating a flow against zooplankton transfer from Lake Superior. However, after construction of the Waterway, cool-water sills frequently penetrated the Waterway, as they do today, sometimes as far as Portage Lake (Churchill et al. in press). Coinciding with its peak abundance in Keweenaw Bay, *D. retrocurva* from the coastal zone possibly moved into the Keweenaw Waterway and colonized Portage Lake, although regional populations might also have been present in low abundance. More extensive geographic surveys and core genetic studies are needed to confirm these conjectures. However, after cessation of mining activities and increased cultural development, mixed waters became both clearer (less humic-stained) in summer and more productive, with introduced fishes (especially perch) dominating modern assemblages. As planktivorous perch and smelt became more abundant in the Keweenaw Waterway, we can hypothesize that the increased planktivory restricted *D. dentifera* to the stained, marshy waters of the Sturgeon Sloughs (Kerfoot et al. in press) and aided replacement by the smaller bodied and more transparent *D. retrocurva*. Some invertebrate predators (*Chaoborus*, *Polyphemus*) were more common early throughout the Waterway, whereas the highly transparent *Leptodora* became abundant during the initial phases of eutrophication in the 1950s and 1960s, then declined from the 1970s to the present under increasing planktivory (Table 2).

A decrease in conspicuous herbivorous and large-bodied carnivorous cladocerans (*Leptodora*, *Chaoborus*) is a common consequence of increasing planktivorous fish densities (Lunte and Luecke 1990; McNaught 1993). Mining discharges could have facilitated the rapid initial 1920s to 1950s transition in species because the slime clays in surficial sediments were toxic to living and ephippial *D. dentifera*, eliminating a viable egg bank (Kerfoot et al. 1999). *Bosmina* and *Ceriodaphnia*, two taxa usually suppressed under high *Leptodora* densities (McNaught 1993; McNaught et al. 2004), became abundant in Portage Lake with eutrophication, lower *Leptodora* densities, and higher planktivorous fish abundance.

Considerable work has been done elsewhere on local factors that influence *Daphnia* species abundance. Studies across North America suggest that *D. dentifera* is a strong exploitative competitor in lakes where visual vertebrate predation (planktivory) is negligible or of low intensity (Clark and Carter 1974; DeMott and Kerfoot 1982; DeMott 1983; Neill 1985). Conversely, *D. mendotae* and *D. retrocurva* are often associated with moderate vertebrate predation (e.g., yellow perch, cisco); thus, the sequence of cladoceran replacements in Portage Lake is consistent with observed increases in planktivorous fish associated with eutrophication and the decrease of resident invertebrate predators (*Leptodora*).

Under these changing environmental conditions, did *D. retrocurva* have to evolve just to maintain its position in the food web? Evaluating the prey response part of the Red Queen hypothesis required large sample sizes of ephippia and multiple approaches to genetic characterization. Initial

Table 6. Examples of helmet and spine length regressions from *Daphnia retrocurva* clones, Portage Lake. Eggs from ephippia were reared from 1-cm sieved core slices, used to establish clones, and raised under uniform (common garden) conditions (intercept in microns).

Depth (cm)	<i>n</i>	Regression equation	<i>r</i> <sup>2</sup>	SE slope	SE intercept
Helmet : body length					
0–1	56	$y=0.169x+206$	0.728	0.014	10
0–1	65	$y=0.195x+171$	0.566	0.012	11
0–1	60	$y=0.239x+154$	0.571	0.027	15
2–3	72	$y=0.250x+168$	0.668	0.021	14
2–3	46	$y=0.273x+94$	0.742	0.024	11
4–5	32	$y=0.234x+115$	0.791	0.022	8
5–6	32	$y=0.251x+99$	0.574	0.040	14
8–9	64	$y=0.314x+129$	0.819	0.019	13
8–9	106	$y=0.375x+129$	0.830	0.017	11
10–11	35	$y=0.287x+112$	0.845	0.021	8
10–11	66	$y=0.290x+153$	0.686	0.025	16
11–12	37	$y=0.311x+105$	0.650	0.039	16
Spine : body length					
0–1	60	$y=-0.069x+363$	0.041	0.044	23
0–1	65	$y=-0.295x+444$	0.507**	0.037	19
0–1	56	$y=-0.313x+462$	0.751**	0.025	17
2–3	72	$y=-0.064x+272$	0.088*	0.025	16
2–3	46	$y=-0.129x+190$	0.246**	0.034	15
4–5	32	$y=0.024x+229$	0.012	0.041	18
5–6	32	$y=-0.013x+225$	0.002	0.054	19
8–9	64	$y=0.001x+347$	0.000	0.028	20
8–9	106	$y=0.080x+328$	0.125**	0.021	14
10–11	66	$y=0.070x+351$	0.208**	0.017	11
10–11	35	$y=0.100x+216$	0.334**	0.025	10
11–12	37	$y=0.266x+191$	0.636**	0.034	14

\*  $P < 0.05$ ; \*\*  $P < 0.01$ .

screening with 206 clones produced mixed results. At one level, mtDNA RFLPs of the 12S/16S (genes) coding for rDNA exhibited no evidence for change, partly because only *Dra*I had sufficient variation to record change and because the 12S and 16S rDNA sequences are conservative (Colbourne and Hebert 1996). The initial allozyme checks did aid species identification and sorting, especially when similar-sized ephippia were present. The discovery of hybrids within the *D. mendotae* × *D. dentifera* × *D. galeata* complex complicated matters (Kerfoot et al. 1999 in press), but the characterization and rearing steps allowed separation of these taxa. Subsequent application of *Pgi* directly on *D. retrocurva* diapausing eggs from sediment cores clarified stratigraphic relationships and provided strong evidence for allozyme gene frequency changes. However, one could argue that the differences in early populations were founder effects carried over from colonization. Founder effects have been postulated for colonizing populations of exotic *Bythotrephes* sp. (Berg et al. 2002), *Daphnia* (Boileau et al. 1992), and *Eubosmina* (DeMelo and Hebert 1994).

Common garden experiments documented microevolutionary changes in *D. retrocurva* as both relative helmet size and tail spine length changed over the 60-yr interval. Similar changes of tail and anterior head (antennule) spine lengths have been described from the well-preserved carapaces and head shields of *Bosmina* in Portage Lake over the same interval (Kerfoot et al. 1999). The differences in *D. retrocurva* spine lengths are compatible with the observed changing bal-

ance between invertebrate predators (e.g., *Leptodora*) and cladocerans. The microfossil evidence (i.e., the changing ratio of *Leptodora* spine and mandible remains relative to *Bosmina* and *Daphnia* remains in sediment cores) emphasizes the importance of Red Queen responses. That is, the microfossil and experimental evidence suggests that *Leptodora* and cladocerans (*Daphnia*, *Bosmina*) could be responding to density- and frequency-dependent differences in the abundance of invertebrate predators. The common garden experiments verified progressive microgenetic changes in populations of *D. retrocurva* against the background of continually changing *Leptodora* density and frequency. With these experiments, we did not attempt to evaluate coevolutionary changes in the predator, yet this examination is possible. Although less abundant, the diapausing eggs of invertebrate predators such as *Leptodora* can be retrieved from sediments to test whether reciprocal behavioral or morphological responses took place in the predator-prey pair during the interval of conflict. Likewise, predator-prey experiments with resurrected predator and prey populations could test whether evolutionary adjustments stabilized population fluctuations through time. Unfortunately, the same cannot be done for fish interactions because eggs from local vertebrate species do not survive in sediments.

Progressive morphological changes in fossils (mollusk families) originally prompted Van Valen (1973) to postulate the Red Queen hypothesis. When Van Valen determined extinction rates for families, he obtained a straight line on a

log scale, suggesting a constant rate of extinction. He interpreted the morphological changes as indicative of progressive evolutionary change—that species must continually evolve just to stay in place. The responses were viewed as coevolutionary; that is, in order to maintain constant fitness, he postulated a reciprocal response between interacting pairs of species (predator and prey, competitors) through time. The environment of each species was interpreted as deteriorating at a constant rate as the species with which it interacted evolved, changing morphological traits. Maynard Smith (1976) criticized the concept, believing it unlikely that the fitness improvement of a single species was precisely balanced by a decrement in the fitness of another interacting species. Comparing evolutionary trait responses in both prey and predators through time will effectively test the Red Queen hypothesis.

Diapausing egg banks are considered analogous to seed banks in that individuals are stored in a noninteractive state for scores to hundreds of years (Hairston et al. 1995; Hairston 1996). These long-lived dormant stages contribute to a storage effect, with implications for long-term competition and maintenance of genetic diversity (Chesson 1983; Hairston et al. 1996). Three previously postulated effects are prolongation of competitive conflicts (Chesson 1986; Cáceres 1997), retardation of evolution (Hairston and De Stasio 1988), and maintenance of genetic variation (Ellner and Hairston 1994). The 80-yr-old egg banks in Portage Lake document relatively rapid evolution of prey populations. Where does the genetic variation come from that fuels the observed rapid selective response? One primary candidate is the storage effect produced by the egg bank. Another interesting alternative is that *D. retrocurva* was present in low abundance naturally but that immigrating populations from Lake Michigan imported novel genetic material that aided the observed selective response. The evidence for rapid evolution in *Pgi* also is reminiscent of changes documented for *D. galeata* in Lake Constance (Weider et al. 1997; Hairston et al. 1999a, 2001). In Lake Constance, eutrophication, changes in fish stock, and toxic blue-green blooms occurred over a span of 40 yr. Cousyn et al. (2001) also documented rapid evolution of *D. magna* in a small pond under the influence of fish predation. That is, despite the presence of egg banks, species are replacing each other frequently through time with relatively rapid evolution within species during the time of residency. In Portage Lake, the highly toxic Cu-rich slime clays could have facilitated community transition by truncating recruitment of previously resident species from the egg bank (Kerfoot et al. 1999). Globally, we believe that in many lakes, environmental changes are occurring so rapidly that they overwhelm the potential buffering influence of egg banks, emphasizing the importance of checking for evolutionary responses and offering a test of the coevolutionary Red Queen hypothesis.

Other evolutionary hypotheses are also amenable to the resurrection ecology approach. Over the species' geographic range, one could test the Kluge–Kerfoot hypothesis (Kluge and Kerfoot 1973): Are the most variable morphological traits the most likely to evolve? The latter hypothesis might simply reflect Fisher's (1930) fundamental theorem of natural selection that genetic variance is related to rates of evo-

lutionary divergence. The simultaneous preservation of morphology in genera like *Bosmina* and *Eubosmina* coupled with retrieval of ancestral ephippia for genetic characterization, common garden competition, and predation tests would lend itself to detailed examination of evolutionary responses at local and regional scales both in time and space.

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