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Reports

Tainted resurrection: metal pollution is linked with reduced hatching and high juvenile mortality in *Daphnia* egg banks

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Abstract. Many taxa, from plants to zooplankton, produce long-lasting dormant propagules capable of temporal dispersal. In some cases, propagules can persist for decades or even centuries before emerging from seed and egg banks. Despite impressive longevity, relatively little is known about how the chemical environment experienced before or during dormancy affects the fate and performance of individuals. This study examines the hatching rate and developmental success of *Daphnia* hatched from diapausing eggs isolated from sediments from four lakes that experienced varying levels of metal contamination. Two hundred seventy-three animals were hatched from lake sediments deposited over the past century. Hatching rate was negatively influenced by metal contamination and sediment age. There was a robust positive relationship between sediment metal concentrations and juvenile mortality in *Daphnia* hatching from those sediments. The negative effect of metals on *Daphnia* hatching and juvenile survival may stem from metal bioaccumulation, genetic effects, or reduced maternal investment in diapausing embryos. Regardless of the specific mechanism driving this trend, exposure to metals may impose strong selection on *Daphnia* diapausing egg banks.

Key words: Daphnia; *development; diapause; dormancy; egg banks; hatching rate; juvenile mortality; paleolimnology; resting eggs; seed banks; trace metals.*

Introduction

Many organisms rely on dormant life-history stages to transit harsh conditions. Some individuals do not emerge from dormancy the following season after deposition, but nevertheless remain viable. Over time, a dormant propagule bank accumulates, resulting in the storage of species and preservation of genetic diversity that may contribute to future generations through temporal dispersal (Warner and Chesson 1985). Not all propagules will successfully develop due to selection pressures experienced during dormancy (Fenner and Thompson 2005). Such selection, in turn, will shape the future species composition and genetic structure of populations that emerge from dormancy (Hairston et al. 1996); however, how selection acts on propagule banks remains an important yet unresolved question in evolutionary ecology.

In this study, dormancy is defined as a state of reduced metabolic activity, inclusive of diapause, a dormant state in some animals induced by environmental cues (Hairston and Fox 2013). Zooplankton egg banks in lake sediments provide an excellent system for reconstructing conditions experienced before and during dormancy with great temporal resolution. When sediments accumulate in layered deposits in undisturbed lake basins, they retain both diapausing eggs and traces of the chemical and biological conditions present in the water column at a given time. These records enable the reconstruction of historic environmental changes and corresponding ecological and evolutionary responses of populations (Hairston 1996).

During dormancy, propagules may experience a variety of stressors that can cull the seed or egg bank dramatically. Most research on selection in propagule banks focuses on biotic agents such as predation, disease, and aging (De Stasio 1989, Fenner and Thompson 2005). Our knowledge of the effects of the chemical environment on zooplankton egg bank dynamics is limited to the influence on hatching success. Metal contamination and lake acidification are suspected to have caused a reduction in hatching capacity of zooplankton diapausing eggs in the field (Hairston et al. 1999, Kerfoot et al. 1999), while experimental exposure to heavy metals led to decreased hatching success in marine copepod eggs (Jiang et al. 2007). We know very little about the effects of historic chemical exposure on post-hatching developmental success of dormant propagules. Chemical pollution may affect dormant propagules through two different but not mutually exclusive pathways: contaminants may have direct toxic effects during storage, and conditions experienced by past generations may affect the fitness of offspring.

Over the past century, human land use has intensified, resulting in increased release and accumulation of harmful chemicals, including metals. Numerous studies

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have measured increasing concentrations of mercury, cadmium, lead, and zinc in lake sediments over time, even in areas remote from industrial activity (e.g., Fitzgerald et al. 1998, Siver and Wozniak 2001, Audry et al. 2004). Relatively little is known about the consequences of metal exposure for resident lake species, particularly via contaminated sediments, over the course of multiple generations in a natural context (but see Klerks and Levinton 1989, Kerfoot et al. 1999, Perceval et al. 2006). However, tens of thousands of laboratory studies conducted for regulatory purposes have provided a body of information on the effects of toxicants in a laboratory setting, particularly using zooplankton taxa (e.g., US EPA's Ecotox database).

One of the most commonly used taxa in toxicity assays is *Daphnia*, a genus of filter-feeding zooplankton that plays a key role in lake food webs (Lampert 2011). Laboratory and field studies reveal that *Daphnia* species and populations can vary dramatically in response to the presence of metals (Barata et al. 2002, Muyssen et al. 2005). Most *Daphnia* produce diapausing eggs that can spend anywhere from years to a century or longer buried in lake sediments before hatching (Hairston and Cáceres 1996, Frisch et al. 2014). The impact of exposure to metals during this phase of the life cycle is unknown.

Using a paleolimnological approach, I investigated long-term effects of exposure of *Daphnia* diapausing eggs to metal contamination. I measured metal concentrations in sediments collected from four lakes to determine historic contamination histories and hatched *Daphnia* from diapausing eggs deposited in these sediments over the past 40–100 years. I tested whether sediment metal concentrations were associated with hatching success and juvenile mortality of *Daphnia* hatched from these egg banks.

Materials and Methods

Core collection and processing

Sampling was conducted in four study lakes in Connecticut, USA, to compare historic trends in metal contamination and *Daphnia* egg bank dynamics within and among these sites. A large-diameter (12.5 cm) sediment core was collected at the deepest basin of each lake by SCUBA divers who took care not to disturb the sediment–water interface. Cores ranged from 20 to 49 cm deep and were sectioned at 1.5-cm intervals. The design of the coring device and methods for sediment extrusion followed Benoit and Rozan (2001). Cores were processed within 24 h of collection. Homogenized subsamples for metal analysis were dried at 60°C for 24 h and ground to a fine powder in a mortar and pestle. Remaining wet sediments were stored in plastic specimen cups in the dark at 4°C.

Sediment geochronology

Approximately 100 mL of wet sediment subsample was packed into air-tight aluminum sample containers. To date the layers of sediment, radionucleotides were counted with a low-background planar Ge detector (GL2020R model; Canberra, Meriden, Connecticut, USA). Dry mass of sediment in each sample was estimated from bulk density measurements of subsamples at the corresponding depths. ^{210}Pb and ^{214}Pb (an estimate of ^{226}Ra) were counted in 10–14 intervals per core; total and unsupported ^{210}Pb were measured following Cutshall et al. (1983) after a $\geq$21 d equilibration period. The constant rate of supply (CRS) method was used to determine sediment age (Appleby and Oldfield 1983). ^{137}Cs was measured to verify the ^{210}Pb geochronology. ^{7}Be (half life = 53 d) was measured in the first and third slices of the cores immediately upon core collection, to establish integrity of the core surfaces and the depth of bioturbation in the lakes.

Metal analysis

Metals were measured in 11–20 sediment samples per core to create historic contamination profiles going back at least 100 yr into the past. Cadmium, chromium, copper, lead, and zinc (Cd, Cr, Cu, Pb, and Zn) were extracted from sediment samples using hot block acid digestion, following the US EPA 200.8 protocol (Creed et al. 1994). These metals were measured by inductively coupled plasma mass spectrometry (Thermo Finnigan Element 2 high resolution ICP-MS; Thermo Fisher, Waltham, Massachusetts, USA). Quality control included standards, calibration blanks, and duplicates, as well as reference and sample spikes. Recovery percentages calculated from NIST 1944 sediment reference material (New York–New Jersey waterway sediment; National Institute of Standards and Technology, Gaithersburg, Maryland, USA) differed across metals due to variation in volatility: Cr, 70% (4%; SD); Cu, 85% (4%); Zn, 86% (3%); Cd, 94% (4%); Pb, 93% (3%). Sediment mercury (Hg) concentrations were measured by atomic absorption spectrometry (Direct Mercury Analyzer DMA80; Milestone, Shelton, Connecticut). Duplicates, MESS-3 standard reference material (Beaufort Sea marine sediment; National Research Council, Ottawa, Ontario, Canada), and blanks were included for every 10 samples. The recovery rate for Hg in MESS-3 was 97.5% (5.7%; SD).

Daphnia *hatching, culturing, and identification*

Observations of *Daphnia* hatching and post-hatching performance were made over the course of two years (2012–2013) of hatching and culturing animals. Animals were hatched from three to six time periods in each lake, representing a range of metal contamination. *Daphnia* diapausing eggs (encased in ephippia; see Plate 1A) were isolated by filtering a known volume of sediment through a 50-μm sieve, after first ensuring that no ephippia were lost at this pore size. Filtered sediments were examined under a dissecting microscope at 10× power. Ephippia with one or two eggs inside were moved into 2-mL wells of COMBO medium (Kilham et al. 1998) and incubated in spring hatching conditions

(15°C, 14 h light : 10 h dark). Well plates were checked for hatchlings every other day for at least three weeks, after which new hatchlings became uncommon. Hatchlings were cultured in COMBO at 20°C (14 h light : 10 h dark) and fed a diet of *Scenedesmus obliquus*, ad libitum, until they reached maturity and could be identified to species.

Several parameters related to *Daphnia* hatching and early development were recorded. These data include hatching rate (proportion of diapausing embryos that hatched), time to hatching (number of days spent incubating before an individual hatched), and time to maturation (number of days post-hatching for *Daphnia* to produce offspring). In addition, some *Daphnia* hatchlings died before reproducing (see Plate 1B). Juvenile mortality is defined as the death of *Daphnia* individuals that hatched but failed to reach maturity. Hatchlings that reached maturity were identified using taxonomic keys (Pennak 1989, Hebert 1995). Hatchlings that failed to hatch or died before reaching maturity were identified to species based on morphological traits of the ephippium from which they hatched (size, shape, number and position of egg chambers, spinescence on the dorsal ridge).

Statistical analyses

The influence of historic heavy metal contamination on traits associated with *Daphnia* diapausing embryo development and post-hatching success was examined using multiple regression and multiple logistic regression. Four of the six metals (Cd, Hg, Pb, and Zn) were highly correlated with one another. To avoid overfitting the regression models with multiple collinear predictors, these metals were reduced to a single variable using principal components analysis (PCA). Variance explained and individual loadings for the metals PCA are provided in Appendix B: Table B1. The first principal component score was included as an independent variable in the regression models.

The relationship between *Daphnia* juvenile mortality and metal contamination of the sediments from which these animals were hatched was tested using logistic regression. The binomial dependent variable in the model was the event of *Daphnia* juvenile death before reaching maturity. The effect of metals (Cr, Cu, and PC1 of Cd, Hg, Pb, and Zn) was tested first. After testing the relationship between juvenile mortality and metals, additional independent variables (sediment age, lake, and species) were added sequentially to test for significance (all $P < 0.05$). Those variables that were significant and reduced the model Akaike information criterion (AIC) were kept in the final model.

The relationship between hatching success (probability of hatching) and sediment metal concentration was also examined using logistic regression. Here, the binomial dependent variable was the event of a diapausing embryo hatching. The effect of metals on time to hatching (number of days spent incubating before hatching) and time to maturation (number of days post hatching before clonally reproducing) was measured using multiple regression. The same set of independent variables was tested in these logistic regression and multiple regression models (metals, sediment age, lake, and species), with the same model selection criteria. All statistical analyses were conducted in R v. 3.0.2.

Results

Sediment geochronology

Sediments from three lakes (Black Pond, Alexander Lake, and Cedar Pond) showed log-linear decay of excess ^{210}Pb to supported levels. In Roseland Lake, sedimentation rate was markedly higher for sediments 0–20 yr old compared with sediments 20–100 yr old, but the monotonic decline of ^{210}Pb activity with core depth indicated that the core was undisturbed and use of the CRS method was appropriate (Appendix A: Fig. A1). ^{137}Cs peaks in 1963 confirmed the validity of ^{210}Pb determined dates. ^{7}Be was found only in the uppermost slice in all cores, indicating that sediments were not mixed after core collection and in-lake bioturbation was limited to 3 cm depth.

Metal contamination

Over the past century, metal contamination increased in all four lakes, though the degree and type of contamination, along with timing, differed among lakes (Fig. 1). Typically, contamination started in the early 1900s and peaked in the 1950–2000s; however, in Cedar Pond, metal concentrations peaked earlier in the 1900s and declined in recent decades. This difference in the timing of metal pollution may result from the fact that Cedar Pond is situated approximately 12 km upwind (NE) of New Haven, Connecticut, a region that experienced industrial activity in the earlier 1900s. The other three lakes are located in more rural northeastern Connecticut. Cd, Cu, and Hg, nonessential metals known to be particularly toxic to *Daphnia* (Bossuyt and Janssen 2005, Tsui and Wang 2006, Qu et al. 2013), showed some of the largest fluctuations. The most dramatic change across lakes was an 11-fold increase in Cu in Roseland Lake ca. 1960–1990, owing largely to the consistent application of several hundred kilograms of $CuSO_4$ to control algal blooms, a practice that continues today (Putnam County, Connecticut Water Department, *personal communication*).

Daphnia hatchling mortality, hatching rate, time to hatching, and maturation

Hatchling mortality was examined based on observations of a total of 273 *Daphnia* hatchlings from three species (*D. ambigua*, *D. parvula*, and *D. pulicaria*), four lakes, and 3–6 time periods per lake spanning ca. 1912–2011. Of these hatchlings, 59 juveniles died before reaching maturity. Often animals died within a few days

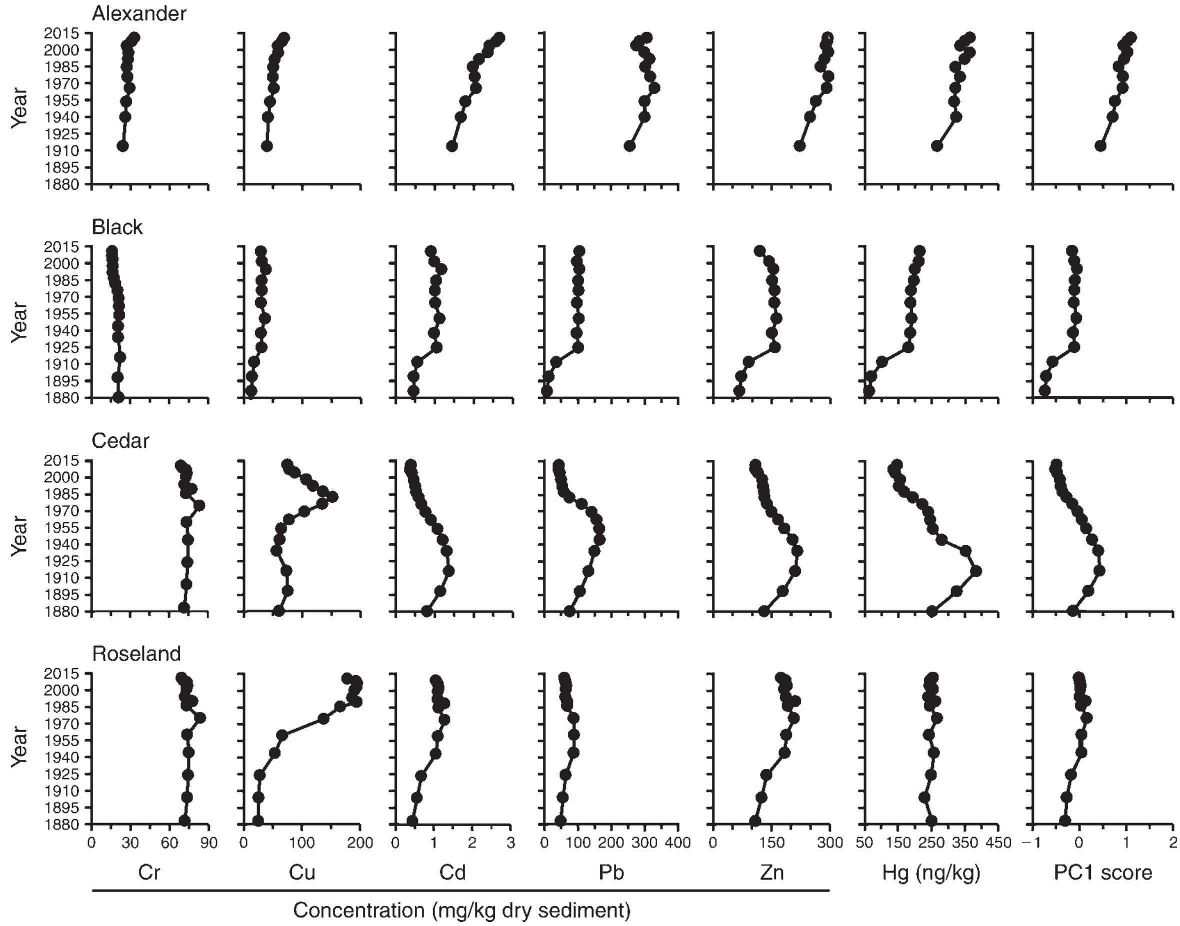

FIG. 1. Sediment profiles for chromium, copper, cadmium, lead, zinc, and mercury (Cd, Cu, Cd, Pb, Zn, Hg) for each lake (Alexander, Black, Cedar, and Roseland; all lakes are in Connecticut, USA). Note differences in scale among metals and difference in units for Hg. Each row represents data from one of the four lakes. First principal component (PC1) values show PC1 scores from analysis of Cd, Hg, Pb, and Zn.

of hatching. Mortality rates ranged from 0% to 45% for a given lake and time period (Appendix C: Table C1).

Hatching rate was determined for a subset of these individuals. *Daphnia* ephippia can have either one or two embryos present, so data on hatching rate was only included for those cases where the number of embryos present in the ephippium before incubation was noted. A total of 158 *Daphnia* hatched from a total of 417 diapausing embryos from the same lakes and time periods. Hatching data was available for four taxa (*D. ambigua*, *D. parvula*, *D. pulicaria*, and *D. mendotae*). Hatching rates ranged from 6% to 100% depending on the lake and time period (Appendix C: Table C1). Time to hatching was also observed for these individuals and ranged from 4 to 26 d. Time to maturation was observed for a total of 64 *Daphnia* from two time periods in two lakes and ranged from 6 to 16 d.

Principal component analysis

The first principal component explained 90.6% of the variation in Cd, Hg, Pb, and Zn, and the four metals had loadings ranging from 1.798 to 1.933 (Appendix B: Table B1). Thus, an increase in PC1 represents a parallel increase in Cd, Hg, Pb, and Zn.

Regression analysis

There was a strong, positive relationship between *Daphnia* juvenile mortality and contamination by Cd, Hg, Pb, and Zn (PC1; Table 1). Cr and Cu contamination, within the concentration range present in the sediment cores, had no significant effect on juvenile mortality ($P = 0.999$ and 0.631, respectively). When tested in combination with the PC1 of Cd, Hg, Pb, and Zn, lake of origin explained some of the variation in *Daphnia* juvenile mortality and was included in the final model (Table 1; log-likelihood $= -133.777$; Goodman-Kruskal gamma measure of association $= 0.38$; Pearson goodness-of-fit test: $P = 0.411$). The positive relationship between juvenile mortality and metal contamination remained qualitatively consistent but differed slightly among lakes (Fig. 2). Variation among lakes in absolute metal concentrations likely reflects some combination of

Table 1. Logistic regression results for the final models selected examining *Daphnia* juvenile mortality and *Daphnia* hatching rate.

Predictor	Coefficient	SE coefficient	Z	P	Odds ratio
Juvenile mortality					
Constant	−5.346	1.940	−2.756	0.006	
PC1 (Cd, Hg, Pb, Zn)	4.516	1.926	2.345	0.019	91.45
Lake					
Black	2.840	2.445	1.161	0.246	17.11
Cedar	5.450	2.683	2.031	0.042	232.67
Roseland	3.580	1.805	1.983	0.047	35.87
Hatching rate					
Constant	4.476	1.095	4.089	<0.001	
PC1 (Cd, Hg, Pb, Zn)	−2.280	1.105	−2.063	0.039	0.102
Cu	−0.023	0.007	−3.519	<0.001	0.977
Sediment age	−0.029	0.006	−4.715	<0.001	0.971
Lake					
Black	−3.595	1.271	−2.829	0.005	0.027
Cedar	−1.636	1.575	−1.039	0.299	0.195
Roseland	0.923	1.428	0.646	0.518	2.516
Species					
D. mendotae	−3.485	0.764	−4.564	<0.001	0.031
D. parvula	−1.429	0.361	−3.959	<0.001	0.240
D. pulicaria	−2.266	0.440	−5.148	<0.001	0.104

Notes: PC1 refers to first principal component. *Daphnia* juvenile mortality was modeled as P(mortality) = 1/(1+exp − ($\beta_0 + \beta_1$PC1+ β_2 lake) and hatching rate was modeled as P(hatching) = 1/(1+exp − ($\beta_0 + \beta_1$PC1+ β_2 Cu + β_3 age $_+$ β_4 lake + β_5 species). Regressions were significant at $P <$ 0.05.

differences in sedimentation rate, sediment organic content, lake basin morphometry, and metal pollution source. Sediment age was not a significant predictor in combination with metals PC1 or alone ($P = 0.362$ and 0.121 respectively); species identity was marginally significant when combined with metals PC1 and lake (*P* values for species = 0.096 and 0.090).

Daphnia hatching rate (probability of hatching) was negatively associated with Cu and the PC1 of Cd, Hg, Pb, and Zn, but only when sediment age was included in the model. The final model selected for predicting hatching rate included Cu, PC1 of Cd, Hg, Pb, and Zn, sediment age, lake, and species (Table 1; log-likelihood = −217.527; Goodman-Kruskal gamma measure of association = 0.63; Pearson goodness-of-fit test: $P = 0.326$). *Daphnia* were more likely to hatch from more recently deposited sediments and when metal concentrations were lower. *Daphnia ambigua* were more likely to hatch than *D. pulicaria*, *D. parvula*, and *D. mendotae*. Hatching was less likely from Black Pond.

Time to hatching was very weakly positively associated with sediment age ($R^2 = 7.7\%$, $P < 0.001$), and was unrelated to metal contamination, lake of origin, or species. Time to maturation was not significantly associated with any of the predictor variables tested.

Discussion

Despite the fact that many organisms employ long-lasting dormant stages, we know little about the selective pressures that these propagules face and even less about these effects on development. This study uncovers a strong effect of the chemical environment on the post-hatching success of diapausing invertebrates. *Daphnia* were less likely to hatch from metal-contaminated lake sediments, and those animals that did hatch suffered significantly increased juvenile mortality.

Differences among lakes in the timing of metal contamination as well as the degree of contamination over time support the statistical evidence that metal contamination, and not age, is contributing to the trend in juvenile mortality. *Daphnia* hatched from Roseland and Alexander Lakes from more recent sediments where metal contamination was at its peak (Fig. 1) suffered higher juvenile mortality (Fig. 2). The timing of metal

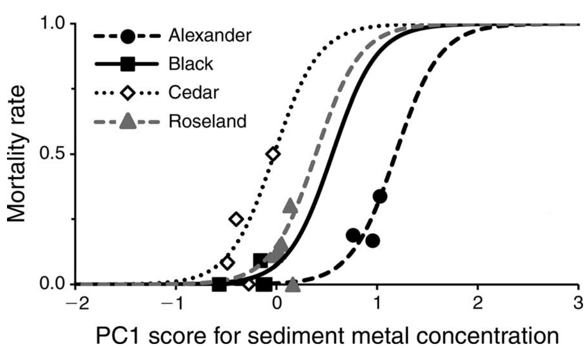

Fig. 2. Relationship between heavy metal concentrations (PC1 of Hg, Pb, Cd, and Zn concentrations in dry sediment) and juvenile mortality (as a proportion) of *Daphnia* hatched from those sediments ($n = 273$ animals) from the four lakes. Metal concentrations and PC1 values are positively correlated, and loadings for each metal range from 1.798 to 1.933. Point data of mortality rates calculated for each lake/time period are overlaid by the logistic regression model, which is fitted to the binary mortality data and takes into account the respective lake.

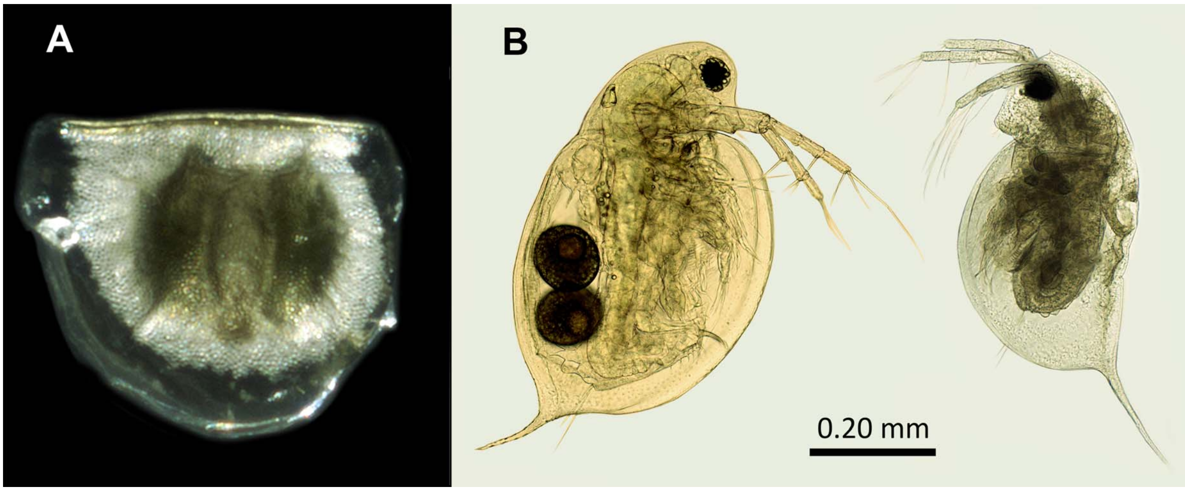

PLATE 1. (A) *Daphnia ambigua* ephippium, (B) healthy maturing 4 day old *D. ambigua* clone (left), and dying 2 day old juvenile *D. ambigua* clone (right). Both *D. ambigua* specimens were hatched from ephippia from Roseland Lake from sediment dated ca. 2007. The dying *D. ambigua* clone was preserved in 70% ethanol to avoid decomposition prior to photographing, while the maturing *D. ambigua* clone was fixed with 10% formaldehyde immediately before photographing. Photo credit: Eric Lazo-Wasem, Yale Peabody Museum of Natural History.

contamination in Cedar Pond was reversed, with peak concentrations in the mid-1900s; yet juvenile mortality still tracked metal contamination, not sediment age. In Black Pond, where metal contamination changed very little over the past century, juvenile mortality was extremely rare (a single individual from ca. 2011 died after hatching).

In contrast, both sediment age and metal contamination negatively affected hatching rate. While researchers have observed reduced *Daphnia* egg bank viability in lakes contaminated with heavy metals (Hairston et al. 1999, Kerfoot et al. 1999), this is the first study measuring the effect of historic metal contamination on *Daphnia* diapausing egg hatching rate.

The observational nature of this study makes it difficult to resolve the mechanisms behind these trends. Yet, by drawing upon the large body of research that has explored *Daphnia* responses to metals, we can make predictions about how metal contamination may be affecting the *Daphnia* egg bank. Concentrations of the four metals largely associated with these trends, Cd, Hg, Pb, and Zn, are highly correlated. While it is not possible to say which metal or combination of metals is at work, all of these metals are toxic to *Daphnia* and may have fitness consequences. Sublethal exposure to Cd, Pb, Hg, and Zn can lead to smaller body size in progeny (Guan and Wang 2006), reduced reproductive output (De Schamphelaere et al. 2004), and developmental abnormalities (Khangarot and Das 2009).

Multigenerational exposure to metals may have affected the *Daphnia* through a variety of mechanisms. Zooplankton are known to bioaccumulate Cd, Hg, and Zn in the field (Chen et al. 2000, Ward et al. 2012) and laboratory (Memmert 1987, Tsui and Wang 2004, Guan and Wang 2006) and may transfer this metal burden to their progeny (Tsui and Wang 2004). An elevated metal body burden is associated with fitness costs (De Schamphelaere et al. 2004), including increased incidence of developmental abnormalities (Khangarot and Das 2009). Maternal effects may also play a role in the observed response. *Daphnia* are known to provide differential investment in progeny depending on environmental conditions (e.g., Tessier and Consolatti 1991). Historically elevated metal exposure may have affected maternal investment in the diapausing eggs, leading to a reduction in *Daphnia* developmental performance.

Reduced hatching rate and increased juvenile mortality in *Daphnia* from metal-contaminated time periods may also be the result of genetic effects. *Daphnia* populations can vary genetically in sensitivity to metals (Barata et al. 2002), and selection has led to adaptation to metals in the field (Ribeiro et al. 2012) and lab (Ward and Robinson 2005). Metal resistance is hypothesized to come at an energetic cost and may affect metabolism of essential metals (Van Straalen and Hoffmann 2000); such a cost has been observed in *Daphnia* (Agra et al. 2011) and other invertebrates (Postma et al. 1995, Shirley and Sibly 1999, Mireji et al. 2010). *Daphnia* in this study from metal-contaminated time periods may have evolved to tolerate metal exposure, but this adaptation could come at a cost in uncontaminated conditions. Additionally, some metals can have genotoxic effects; exposure to Cd, Hg, and Pb has led to genetic damage in plants (Steinkellner et al. 1998), mussels (Bolognesi et al. 1999), and humans (Hengstler et al. 2003).

Regardless of the mechanisms driving this trend, the ecological and evolutionary implications of the abiotic environment exerting strong selective pressure on diapausing egg banks deserve further attention. *Daphnia* are key members of lake food webs (Lampert 2011), and egg banks can play an important role in long-term *Daphnia* population dynamics (Hairston and Kearns 2002). Metal contamination may be having unknown, complex effects on the way these egg banks function. If a legacy of metal contamination selects against temporal dispersal from polluted time periods, this could potentially affect the ability of populations to adapt to changing environmental conditions. Alternatively, if the observed pattern represents selection for genotypes resistant to metal pollution, then egg banks could provide variation needed to deal with current environmental conditions.

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Supplemental Material

Ecological Archives

Appendices A–C are available online: http://dx.doi.org/10.1890/14-1663.1.sm

Reports