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Population biology of a failed invasion: Paleolimnology of *Daphnia exilis* in upstate New York

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Abstract

Viable diapausing eggs of *Daphnia exilis,* a species within the *Daphnia similis* species complex, occur in sediments of Onondaga Lake, New York. The discovery of this species, which otherwise occurs exclusively in temporary saline ponds in southwestern North America, represents a range extension of 1,000 km. ^{210}Pb-dating of the sediments containing diapausing eggs indicates that *D. exilis* was present in Onondaga Lake between the mid-1920s and the early 1980s. The species' introduction, successful colonization, and subsequent disappearance from the water column correspond temporally with distinct events in the history of industrial activity along the shores of the lake and with the paleoecological record of this activity deposited in Onondaga Lake sediments. Only the most recently deposited diapausing eggs (late 1970s to early 1980s) hatch during laboratory incubation; older eggs may not be viable because of toxic concentrations of mercury pollution in older sediments. The *D. exilis* eggs that have hatched have had strikingly low genetic (allozyme) variation in comparison with the variation documented for populations in the southwestern United States by Hebert and Finston (1993). Exploration of various invasion scenarios through simulated introduction of genotypes from the southwestern United States suggests that a single genotype established the *D. exilis* population in Onondaga Lake. These observations document the ecological and microevolutionary patterns associated with an invasion by an exotic crustacean that currently persists only in the sediment egg bank.

Range expansions by aquatic taxa have become increasingly common in inland bodies of water in the United States and elsewhere (Mills et al. 1993, 1994). Although many colonizations by exotic species have resulted in the species' successful establishment in new geographic areas, it seems likely that a large number of introductions have been unsuccessful or successful only for limited periods. The frequency of failed introductions is, however, hard to assess (Lodge 1993), either because such failures occur rarely or because they are difficult to detect. The failure of an invading species to persist after it has become successfully established (i.e., after it has been abundant for more than 1 yr) is even less commonly documented: in his review of invasions, Lodge (1993) does not even consider this possibility.

One source of information about the appearance and disappearance of planktonic species lies in lake sediments, which store histories of population and community dynamics for many organisms with tissues resistant to decomposition (Deevey 1942; Frey 1958; Kerfoot 1974). Furthermore, many species of zooplankton make diapausing eggs that can remain viable in lake sediments for periods ranging from many decades to several centuries (Hairston et al. 1995; Hairston 1996). Even nonviable eggs may contain genetic material from past populations. Thus, lake sediments can be useful in elucidating temporal changes that occur within a zooplankton gene pool (Weider et al. 1997). Here we report one such example.

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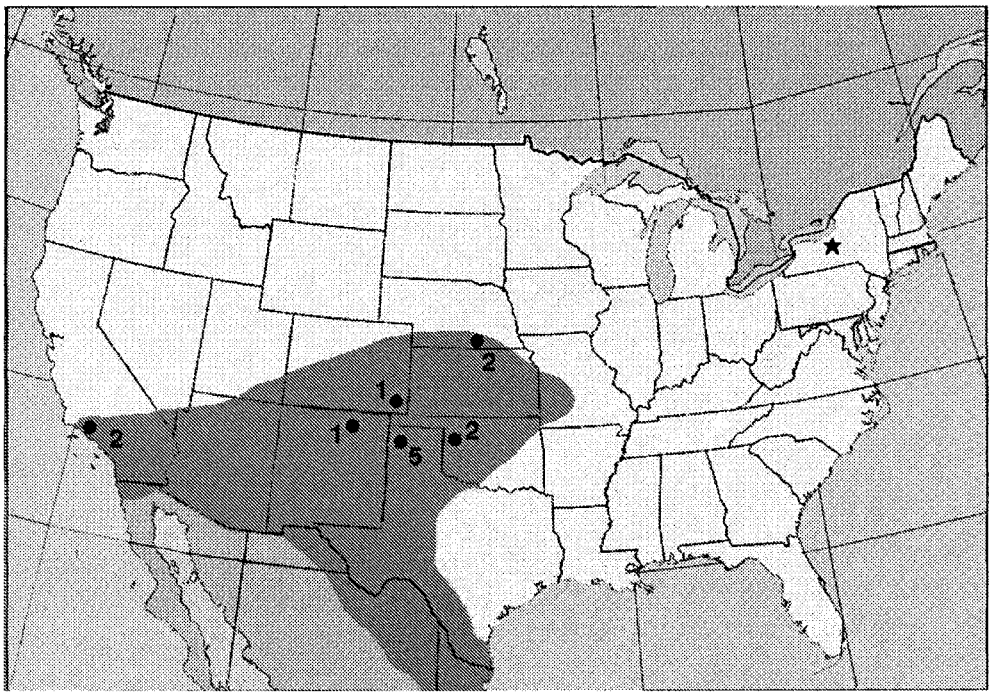

Fig. 1. The geographic distribution of *Daphnia exilis.* The species range depicted by Hebert (1995) is shown as the darkly shaded area. The sample sites used by Hebert and Finston (1993) are shown as filled circles with numbers giving the number of populations (i.e., separate ponds) sampled at each site. The location of Onondaga Lake (this study) is indicated by a star.

During an investigation of the distribution of zooplankton diapausing eggs in the sediments of Onondaga Lake, New York, we discovered large numbers of ephippia belonging to *Daphnia exilis.* Until now, this species has been reported exclusively from shallow, saline, fishless, temporary ponds of the southwestern United States and northeastern Mexico (Hebert and Finston 1993; Hebert 1995; Colbourne et al. 1997). Because the easternmost edge of its range has been considered to be in the state of Missouri, the presence of *D. exilis* in Onondaga Lake represents a single-population range extension of more than 1,000 km. Although the results we report here show that the species was clearly present in large numbers in Onondaga Lake for several decades during the 20th century, and although some of the embryos within its ephippia remain viable at present, the species apparently has not occurred in the water column of the lake for the past 15 yr. This history of presence and absence corresponds with the history of chemical industry on the shore of Onondaga Lake, which, for six decades during the 20th century, released salt (NaCl) wastes into the basin (Effler et al. 1996*a*), substantially increasing water-column salinity.

Our observations of colonization and invasion followed by extinction (except for the egg bank) for *D. exilis* contrast markedly with recent reports of successful invasions of North American lacustrine habitats by other exotic planktonic cladocerans. These invasions have occurred both in the recent past (e.g., *Bythotrephes cederstroemi,* Lehman 1987; and *Daphnia lumholtzi,* Havel et al. 1995) and over a longer time scale, similar to that for *D. exilis* (e.g., *Bosmina coregoni,* Lieder 1991; De Melo and Hebert 1994*a*). Data from the diapausing-egg bank for the Onondaga Lake *D. exilis* population provide insights into a six-decade-long history of the dynamics of an exotic species introduction during both the colonization and extinction phases.

Because diapausing eggs contain genetic as well as morphological information, zooplankton sediment egg banks also provide opportunities for studying the population genetic consequences of natural colonization events. Egg banks can thus add a temporal dimension to De Melo and Hebert's (1994*b*) observation that range expansions by exotic species provide opportunities for studying genetic processes during founder events. For *D. exilis* in Onondaga Lake, we are able to compare allozyme data from individuals hatched from lake sediments with data from the populations in the southwestern United States studied by Hebert and Finston (1993). The results reveal remarkably low genetic variation in Onondaga Lake, suggesting very low population size in the relatively recent past.

Materials and methods

Study organism—D. exilis is a member of the subgenus *Ctenodaphnia.* The biogeography of the *Ctenodaphnia* in North America was initially described by Brooks (1957), who suggested that only two species, *Daphnia similis* and *Daphnia magna,* were present on this continent. More recently, Hebert and Finston (1993) used allozyme data to conclude that *D. similis* is in fact a species complex composed of three sister taxa, with *D. similis* sen. str. and *Daphnia*

salina living in the northwestern United States and southwestern Canada and *D. exilis* living in the southwestern United States. Hebert (1995) depicts the range of *D. exilis* extending into northeastern Mexico (Fig. 1). Hebert and Finston (1993) and Hebert (1995) reported finding *D. exilis* in temporary ponds of wide-ranging conductivity (10^2 to 10^4 $\mu S\ cm^{-1}$). Ovigerous females are relatively large in body size (1.8–4.5 mm body length, excluding the shell spine), and their distribution in shallow, saline, temporary ponds may well be related to the absence of fish from these habitats (Hebert and Finston 1993; Hebert 1995; Colbourne et al. 1997).

Study site—Onondaga Lake is located at the northern city limit of Syracuse, New York (43°06'54''N, 76°14'34''W). The lake has relatively steep sides with two basins: a northern basin, 18 m deep, and a southern basin, 19 m deep; the two basins are separated by a slightly shallower ''saddle'' 17 m in depth. Its surface area is 12 km^2. The lake is situated on the northern edge of the Appalachian Uplands, which are formed by outcrops of the Salina Group containing evaporites (salt deposits) from the Upper Silurian period. The brines that drain from this formation make Onondaga Lake naturally saline (Perkins and Romanowicz 1996) and have attracted chemical industries to the region beginning as early as 1804 (Effler and Harnett 1996).

Coring and core processing—Three sediment cores were collected from the saddle location at 17 m in Onondaga Lake in three different years. On 20 June 1995, a 4.7-cm diameter gravity core (39 cm long) was taken remotely from a boat, and on 30 May 1996 and 13 May 1997, 7-cm diameter piston cores (81 cm and 92 cm long, respectively) were taken by a scuba diver. All cores were wrapped immediately in aluminum foil, returned to the laboratory, stored refrigerated (4°C), and sliced within 2 d of collection. The gravity core was sliced at 1-cm intervals between 0 and 25 cm, and at 2-cm intervals between 25 and 39 cm. The piston cores were sliced at 1-cm intervals between 0 and 20 cm, at 2-cm intervals between 20 and 40 cm, and at 4-cm intervals between 40 and 92 cm. For each core slice, the outer sediment layer that had dragged along the wall of the core tube was separated from the inner sediments using a cookie cutter (a cutter of 4.4-cm diameter for the gravity core and a cutter of 5.9-cm diameter for the piston cores) and discarded.

Sediment dating—Subsamples from 22 stratigraphic intervals were analyzed for ^{210}Pb activity to establish a chronology for the 1997 piston core. ^{210}Pb activity was measured by ^{210}Po distillation and by alpha spectrometry methods (Eakins and Morrison 1978), and dates and sedimentation rates were calculated according to the constant rate of supply model (Appleby and Oldfield 1983). Dry density (dry mass per volume of fresh sediment), water content, organic content, and carbonate content of all core slices were determined by standard loss-on-ignition techniques (Dean 1974).

Analysis of egg densities—Sediments were suspended in 1.6-μm–filtered lake water and washed through a 150-μm mesh. A stereo-dissecting microscope was used to search the retained material for *Daphnia* ephippia. All sediment collected from all slices of the gravity core was searched. For the piston cores, either an entire slice or two to three subsamples were searched, depending upon egg density. At minimum, 12% of each core slice was analyzed. For the piston cores, each ephippium observed was opened, and the number of eggs present was recorded. For the gravity core, only the number of ephippial cases was recorded. Isolated ephippia with diapausing eggs were placed in 7-ml wells of 12-well plastic tissue culture plates in filtered lake water and maintained at 10°C, with a 14 : 10 light : dark (LD) photoperiod. The diapausing eggs were monitored weekly for hatching for a minimum of 10 months. Most hatching occurred within 6 weeks after extraction from the sediments, and no hatching took place after 11 weeks. The neonates from the eggs that hatched from the piston core taken in 1996 were cultured in filtered lake water and fed laboratory-cultured *Scenedesmus* sp.; the neonates were maintained at 15°C, with a 13.5 : 10.5 LD photoperiod.

Genetic analysis—One *D. exilis* from each of 44 isoclonal cultures established from individuals hatched from Onondaga Lake was analyzed for allozyme variation using a cellulose acetate medium. These *Daphnia* included 1 animal hatched from an egg found at 13–14 cm in the 1996 core, 22 hatched from eggs found at 20–22 cm in the 1996 core, and 21 hatched from eggs found at 22–24 cm in the 1996 core. No *D. exilis* hatched from eggs found at other sediment depths in this core (*see Results*). Fifteen putative loci were scored for each individual, using staining recipes adapted from Hebert and Beaton (1993). These putative loci included the 11 loci screened by Hebert and Finston (1993), the only existing electrophoretic analysis of *D. exilis*: *Pgm* (two loci), *Gpi, Ldh, Mpi, Aat* (two loci), *Fum, Mdh, Me,* and *Ao.* In addition, we screened four loci not included by Hebert and Finston (1993) in the principal part of their survey: *Idh, Mdh* (a second locus), *G3pdh* and *Ark.* All 44 clones were screened for all loci, with the exception of *G3pdh* ($n = 42$) and *Aat*-1 ($n = 22$). Sample sizes are comparable to those obtained by Hebert and Finston (1993), who analyzed between 22 and 44 individuals per population.

Results

Key characters for distinguishing adult *D. exilis* from closely related species are molecular and morphological in nature (Hebert and Finston 1993). In addition, distinctive morphologies of ephippia permit confidence in identification. We first noted the presence of a *Ctenodaphnia* in Onondaga Lake based on the fact that the ventral margin of the carapace was attached to many of the ephippia we obtained from the sediment cores. This fact alone is remarkable, because the only two species in this subgenus previously known to occur east of the Mississippi River are *Daphnia ephemerella,* which occurs in temporary vernal ponds, and *D. lumholtzi,* which is a recent African invader of southeastern reservoirs (Hebert 1995). Characters that identify the ephippia from Onondaga Lake as belonging to *D. exilis* are an anterior extension and an attached carapace ventral margin that are substantially shorter than the body of the ephippium. Indi-

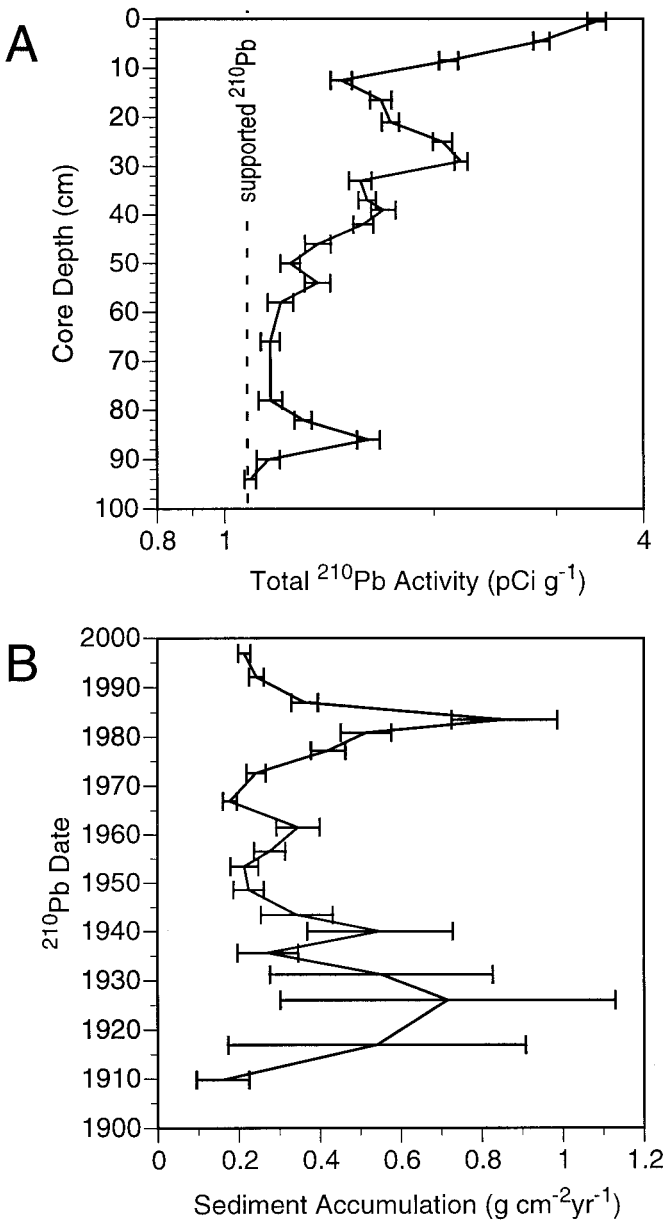

Fig. 2. Total ^{210}Pb activity vs. sediment depth (A) and sediment accumulation rates vs. ^{210}Pb dates (B) in the 1997 Onondaga saddle core. Error bars (± 1 SD) were determined by first-order error propagation of counting uncertainty (Binford 1990).

viduals that hatched from these ephippia possessed morphological traits characteristic of *D. exilis*: rostrum terminating in a broad triangle, prominent antennular mounds, and pigmented ocellus. Finally, both the *Ldh* and *Me* loci have mobilities very similar to those for *D. magna,* again characters consistent with *D. exilis* (*see* Hebert and Finston 1993). Voucher specimens have been deposited with the New York State Museum.

Sediment ages and accumulation rates—Total ^{210}Pb activities in the 1997 piston core decrease irregularly with depth to a minimum value of 1.1 pCi g^{-1} at 96 cm (Fig. 2A). The profile is distinctly nonmonotonic, and ^{210}Pb values below 50 cm are barely above supported (background) ^{210}Pb, which is estimated here by the lowermost level in the core. Such conditions—low ^{210}Pb and a poorly constrained background—yield an uncertain chronology, especially for older dates in the lower part of the core. It is likely, for instance, that supported ^{210}Pb is not constant but instead varies stratigraphically with shifts in sediment lithology. This inconsistency becomes a particular problem when ^{210}Pb values are very near background, as they are in the Onondaga Lake profile. Nonetheless, the oldest date in the resulting constant rate of supply chronology (1904 ± 14 yr at 84 cm) is consistent with a stratigraphic increase in carbonate content at this depth, which Effler et al. (1996*b*) attribute to the onset of Ca^{+} discharge from soda ash production in 1884. More recent dates, especially those marking the appearance of *D. exilis*, should be more reliable than those at the base of the core.

Sediment dating for the 1997 piston core using ^{210}Pb activity reveals highly variable rates of sedimentation during the past century (Fig. 2B) that correspond with changing industrial activity in the watershed of Onondaga Lake. Standard deviations of estimated sediment age are very large near the base of the core due to high sediment accumulation rates and consequent dilution of ^{210}Pb activities, bringing values close to supported (background) levels. High sediment accumulation during the 1920s, 1930s, and 1940s was almost certainly caused by inputs from chemical industry waste beds that were active along the shore of the lake adjacent to the saddle site during the period 1926 to1944 (Effler and Harnett 1996). Indeed, it seems quite possible that the slightly shallower saddle between the two basins exists solely because of the deposition from these waste beds. After the shoreline waste beds were shut down in 1944, sedimentation rates declined and remained relatively low until the mid-1970s (Fig. 2). The reason for the brief spike in sedimentation rate during the period 1975 to 1985 is uncertain, but the spike may be related to an unusually high sediment load from Onondaga Creek, the principal tributary to the lake (Perkins and Romanowicz 1996).

Diapausing egg density and viability—All three cores show the same general pattern of *D. exilis* densities as a function of depth, although the depths of the major features differ somewhat among cores (Fig. 3). Ephippia, and therefore eggs, are virtually absent from the recent surface layers of sediment. Underneath the recent layers is a distinct layer of high ephippial abundance between about 15 and 40–50 cm (^{210}Pb-dated as 1980 and 1952–1940). Below this layer, the 1996 and 1997 piston cores show a period in which *D. exilis* eggs were absent, again followed by a brief period at about 60 cm (1930) when eggs were present. Finally, below this relatively small, deep pulse of abundance, no *D. exilis* eggs occur—at least to the bottom of the 1996 core at 85 cm and the 1997 core at 92 cm. Two other subtle patterns also appear to be consistent among the three cores. There is a very low abundance of ephippia and eggs about halfway between the major pulse and the sediment surface (at 10–11 cm, 13–14 cm, or 8–9 cm for the 1995, 1996, and 1997 cores, respectively; this depth was ^{210}Pb-dated in the 1997

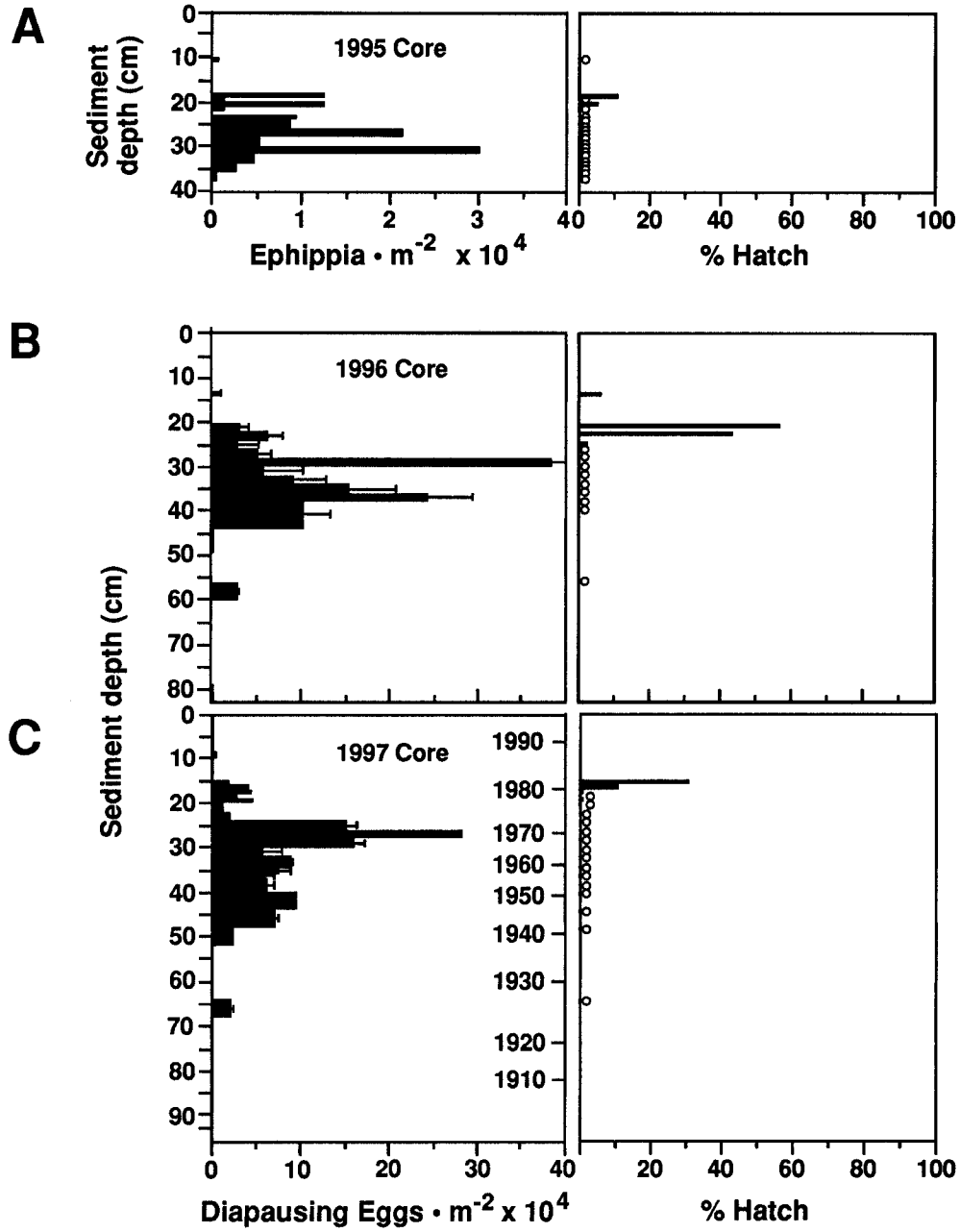

Fig. 3. Distribution of *Daphnia exilis* ephippia or eggs (± 1 SE) in Onondaga Lake sediments as a function of sediment depth and the percentage of incubated eggs that hatched at each depth. Zeros denote depths where none of the incubated eggs hatched. (A) 1995 39-cm gravity core, showing ephippial densities. For this core only ephippia, and not the eggs within, were counted. The calculation of percentage hatched assumes two eggs per ephippium at the time of collection. (B) 1996 85-cm piston core, showing egg densities. (C) 1997 96-cm piston core, showing egg densities. The dates for the 1997 core are calculated from ^{210}Pb dating (*see Results*).

core to be 1986), and there is a distinct abundance maximum within the major pulse (at 31–32 cm, 29–31 cm, and 26–28 cm for the 1995, 1996, and 1997 cores, respectively; this depth was ^{210}Pb-dated to be about 1967). The differences among the cores in the exact depths of the various features may be attributable either to variable sedimentation rates among coring locations or possibly to differences in coring methods (gravity vs. piston coring). More important for our purposes here, however, is the broad consistency of the major patterns. Estimated sediment ages (Fig. 3C) show that *D. exilis* diapausing eggs first appear in Onondaga Lake during the mid-1920s. Diapausing eggs are absent for the core depths from between about 1927 and 1940, after which time the major peak in abundance appears in the sediment. The

Table 1. Genetic variation of *Daphnia exilis* hatched from diapausing eggs from Onondaga Lake sediments dated to be about 20 yr old (i.e., about 1976). These results are compared with the genetic variation reported by Hebert and Finston (1993) for 13 populations from the southwestern United States. P = percentage of loci that were polymorphic, P_{95} = percentage of loci that were polymorphic (<95% criterion), H_o = observed percentage of individuals that were heterozygous, H_E = percentage of individuals expected to be heterozygous.

Population	P (%)	P_{95} (%)	H_o (%)	H_E (%)
Southwestern United States (Hebert and Finston [1993])				
11 loci	44.1	37.8	14.1	13.5
Onondaga Lake				
11 loci (in common with Hebert and Finston [1993])	0.0	0.0	0.0	0.0
15 loci	7.1	7.1	3.4	3.3

eggs reach their greatest density in core depths from about 1970 and then decline to low numbers, finally disappearing from the core depths from the early 1980s.

There is also a distinct, repeated pattern in the apparent viability of diapausing *D. exilis* eggs from the three cores. Only the eggs collected from the very top of the major pulse (i.e., the youngest eggs) hatched. In the 1995 gravity core, six individuals hatched from 40 ephippia incubated from the 18–21 cm sediment depths. None of the 237 ephippia incubated from below this depth hatched. This result is better quantified in the 1996 and 1997 piston cores, where the diapausing eggs within ephippia were counted prior to incubation. From the 1996 and 1997 cores, a maximum of 57% and 31%, respectively, of eggs from this depth hatched (Fig. 3B,C), with rapid declines in egg viability below this peak. Between 20 and 26 cm in the 1996 core, 55 eggs hatched of the 159 incubated. Below 26 cm, none of 454 incubated eggs were apparently viable, although many of them looked very similar under light microscopy to eggs from higher in the core that had hatched. Between 15 and 20 cm (1981–1976) in the 1997 core, 34 of 485 incubated eggs hatched. Below 20 cm, none of 1,394 incubated eggs hatched.

Genetic variation—Of the 15 allozyme loci screened for the *D. exilis* that hatched from the 1996 core, only *Mdh-2* was polymorphic, with two alleles present at frequencies of 0.45 and 0.55. Expected heterozygosity (H_E) (calculated by pooling all 44 individuals) agreed closely with observed heterozygosity (H_O) at this locus ($H_O = 0.45$, $H_E = 0.50$). There were no differences in allele frequencies among *D. exilis* hatched from the 13–14 cm, 20–22 cm, or 22–24 cm portions of the core ($X^2 = 2.49$, 2 df, $P = 0.29$).

In each of a number of comparisons, these observed values of heterozygosity are consistently lower than those reported by Hebert and Finston (1993) for populations of *D. exilis* collected from the species' normal western range. Individual heterozygosity, averaged across loci, was 13.5% for the 13 *D. exilis* populations studied by Hebert and Finston, and only 3.3% for those hatched from Onondaga Lake sediments (Table 1). Furthermore, individual heterozygosity for the *D. exilis* hatched from Onondaga Lake was lower than that of any single population in the southwestern United States. However, because Hebert and Finston (1993) presumably chose loci based on their usefulness in distinguishing population and phylogenetic structure, the polymorphism they reported may be biased toward high values. Thus, a comparison with the Onondaga Lake *D. exilis* population using only the 11 loci used in their study may be more relevant. Within this subset of loci, the Onondaga Lake *D. exilis* shows no genetic variation (i.e., heterozygosity is zero; Table 1). Because individual (observed) heterozygosity was not reported on a population- or locus-specific basis by Hebert and Finston (1993), we calculated expected heterozygosity (averaged over loci) for each population in their study; these 13 heterozygosity values were approximately normally distributed. Assuming normality, the zero heterozygosity for the Onondaga Lake population falls well outside of the 99.999% confidence interval for the western populations.

Discussion

The history of the invasion of Onondaga Lake by the exotic daphniid *D. exilis* can be reconstructed if we assume that its presence and absence in the water column can be inferred from the distribution of diapausing eggs in the sediments. The species was absent from the lake at least as far back in time as the late 1800s (Fig. 2C) and probably had never been previously present. The species colonized Onondaga Lake during the 1920s, and after an absence of about a decade, it reappeared in the 1930s and successfully established a thriving population that persisted until the late 1970s. By the early 1980s, *D. exilis* was again absent from Onondaga Lake except for a residual pool of viable diapausing eggs in the sediments. Based on comparisons of these data with the paleolimnological record seen in cores taken by others (*see* Effler et al. 1996*b*), it appears highly likely that the colonization, establishment, and eventual failure of *D. exilis* in this lake were all the result of human influence.

Given the known biogeography of *D. exilis* (Fig. 1) and the types of habitats in which it typically occurs (i.e., shallow, temporary, saline ponds west of the Mississippi River), it is surprising to discover this species in a relatively large, deep, and permanent lake in the northeastern United States. Indeed, the discovery is so unexpected that a previous report of *D. similis* (i.e., *D. exilis*) in the water column of Onondaga Lake in 1969 (Waterman 1971) was questioned as a likely misidentification (Meyer and Effler 1980). Waterman's discovery of this species in the lake in 1969, however, is fully consistent with the maximum egg density at a sediment depth dated by ^{210}Pb as 1970.

Unfortunately, 1969 is the earliest year in which any known zooplankton study was undertaken at Onondaga Lake (Auer et al. 1996), and no archived Onondaga Lake zooplankton samples exist from the time period over which *D. exilis* diapausing eggs are present in the sediments. Other studies were carried out in 1978 (Meyer and Effler 1980), 1979–1981 and 1986–1989 (Siegfried et al. 1996), 1994 (Makarewicz et al. 1995), and 1996–1997 (Hairston unpubl. data). In none of these studies was *D. exilis* observed.

The appearance of *D. exilis* in Onondaga Lake in the 1920s can almost certainly be attributed to dispersal from outside the lake. The absence of ephippia for the preceding 50 yr makes it highly unlikely that there was an introduction from a preexisting egg bank. Furthermore, the complete lack of evidence of *D. exilis* populations living in other lakes in northeastern North America suggests that the invasion must have come directly or indirectly from habitats in the southwestern United States, at least 1,000 km distant. We cannot rule out the possibility that this *Daphnia* species was present in water bodies in the immediate region of Onondaga Lake during the 1920s, especially since the species occasionally occurs in the southwestern United States in pools with relatively low salinity (*see below*). Nevertheless, the absence of known shallow, saline, temporary habitats where the species more typically occurs argues strongly against nearby populations as a dispersal source. One possible dispersal vector from the southwestern United States may have been mud clinging to equipment belonging to the chemical company established at the shore of Onondaga Lake in 1881; this company also owned a lead mine in Missouri (Cominolli 1990), the easternmost edge of the natural range of *D. exilis* (Hebert 1995).

Speculation about dispersal mechanisms raises the question of how *D. exilis* survived in Onondaga Lake, a water body atypical of the species' natural habitat. We believe that the answer, again, most likely lies in the history of industrial development on the lake shore. Geological deposits of NaCl and limestone in the local area led to the establishment of a soda ash (Na_2CO_3) industry at the south end of the lake in 1881 (Cominolli 1990; Effler and Harnett 1996). Beginning in 1926, large waste beds containing high concentrations of $CaCO_3$ and NaCl were established along the central western shore of the lake. The resulting rise in water-column salinity is apparent in the paleolimnological sediment record for Onondaga Lake (Effler et al. 1996*a*). A marked increase in the concentration of $CaCO_3$ is apparent at the same sediment depth as the occurrence of a steep rise in the proportion of halophilic taxa in the diatom community. In our 1997 saddle core, this rise in $CaCO_3$, presumably accompanied by increased salinity, begins at 84 cm and peaks at 72–76 cm or about 1920, close to the time that *D. exilis* eggs first appear in the sediment (Fig. 2C). Between 1968 and 1990, the volume-weighted salinity of Onondaga Lake was measured directly (Effler et al. 1996*a*). Values varied between 2.5 and 3.5 g liter^{-1} (i.e., approximately 5.1 to 7.1 mS cm^{-1}) through 1985. After the industrial production of soda ash was terminated in 1986, water-column salinity declined rapidly until, by 1990, it had reached its current value of about 1 g liter^{-1} (2.0 mS cm^{-1}).

Lodge (1993) pointed out that habitat disturbance is commonly cited as a factor favoring successful invasion by exotic species. Our observations for *D. exilis* in Onondaga Lake are consistent with this interpretation: the salinity of the water was elevated to levels unnaturally high for lakes in northeastern North America (including Onondaga Lake itself). Unlike many freshwater zooplankton (Remane and Schlieper 1971), *D. exilis* is able to thrive in salinities up to at least 7.5 mS cm^{-1} (Hebert and Finston 1993). It is not, however, an obligate resident of saline habitats, and Hebert and Finston (1993) collected *D. exilis* from ponds in the southwestern United States with salinities as low as 0.065 mS cm^{-1}. We cultured the *D. exilis* from Onondaga Lake sediments in current (1990s) lake water (2.2 mS cm^{-1}). More critical to the species' success than salinity may be the absence of effective zooplanktivory by fish. Hebert and Finston collected *D. exilis* exclusively from temporary ponds lacking fish. Although historical data on fish populations in Onondaga Lake are meager, it seems likely that the relatively high salinity during the period 1920 to 1986 may have driven many fish from the system, since most freshwater fishes are stenohaline (Evans 1993; Moyle and Cech 1996). Collections made in 1927, 1946, and 1960 provide no data on fish abundance, but all of these collections had substantially lower diversity of species, including zooplanktivores, than in collections made more recently, in 1989–1991 (Auer et al. 1996), after the salinity of the lake had declined to levels presumably closer to the natural level. It seems likely that the remediation of salt pollution in 1986 led to the current absence of *D. exilis* from the water column of Onondaga Lake. In this case, reversal of habitat disturbance has also reversed invasion by an exotic species.

Only the most recently produced *D. exilis* diapausing eggs hatched. No hatching occurred in eggs collected from lower than 21 cm in the 1995 core, 26 cm in the 1996 core, or 20 cm in the 1997 core, even though large numbers of eggs from lower depths were incubated. The likely explanation for this pattern lies, once again, in the history of industrial pollution of the lake. Beginning in 1946, mercury waste was created in the production of chlorine gas using the mercury-cell chloralkali process and released into Onondaga Lake (Effler and Harnett 1996). By the late 1960s, inputs had reached 10 kg Hg day^{-1}. After the U.S. Department of Justice took legal action in 1970, the load was reduced substantially, to 0.5 kg Hg day^{-1}, and then eliminated when the industry shut down in the late 1980s. A mercury signal is distinct in Onondaga Lake sediments, with concentrations averaging about 45 μg Hg per gram dry weight for sediment depths corresponding to the period 1946–1970 (Effler et al. 1996*b*). In the 1997 core that we dated, this interval covers sediment depths between about 44 cm (roughly 1946) and 24 cm (roughly 1970). It is just above this sediment horizon that we begin to observe egg hatching. Alternative explanations, however, are that eggs older than about 25 yr (i.e., early 1970s) have simply become nonviable because of aging, or that the correct hatching cue for these older eggs has not yet been discovered. The few studies that have explored the viability of *Daphnia* diapausing eggs under natural conditions found substantial declines in hatching success in eggs older than about 30 yr (Carvalho and Wolf 1989; Weider et al. 1997), though Cáceres (1998) achieved hatching from ephippia of *Daphnia pulicaria* and *Daphnia galeata mendotae* collected from 125-yr-old sediment. Nevertheless, the coincidence of the sediment depths at which hatching success drops to zero with the depths at which mercury concentrations increase to very high levels suggests a causal relationship between the hatchability of *D. exilis* eggs and heavy-metal pollution. Whether mercury in the water column of the lake caused the eggs to become nonviable at the time that they were produced or mercury in the sediments

acted to make the eggs nonviable over the period of burial is uncertain. It seems very likely, however, that industrial pollution has already had a substantial impact on the potential role of the *D. exilis* egg bank in ecological and evolutionary processes in the future.

In comparison with previously studied populations of *D. exilis* (Hebert and Finston 1993), levels of allozyme variation found in individuals hatched from the 13–24 cm sediment depths in Onondaga Lake are extremely low. The number of individuals we analyzed ($n = 44$ for 13 loci, $n = 42$ for 1 locus, $n = 22$ for 1 locus) is only moderate, but our results are bolstered by the fact that similar results were obtained for animals hatched from three sediment depths (i.e., three different time frames). Our observations are consistent with a sudden colonization of Onondaga Lake by very low numbers of *D. exilis* in the early part of this century. Population genetics theory gives clear predictions concerning the effect of population bottlenecks on genetic variation (Nei et al. 1975; Chakraborty and Nei 1977; Hartl and Clark 1989). Although there has been considerable debate surrounding the founding of new populations from a small number of individuals, clearly some genetic changes during invasion-associated bottlenecks may be expected from theoretical considerations (Mayr 1965; Nei et al. 1975; Barton and Charlesworth 1984; Carson and Templeton 1984), from studies of threatened species (O'Brien et al. 1987), and from the results of laboratory experiments (Bryant and Meffert 1991; Leberg 1992; but *see* Rice and Hostert 1993). Numerous studies have sought to document these changes early in the colonization process for a variety of species, including several freshwater vertebrates and invertebrates (Black et al. 1988; Hebert et al. 1989; Weider 1991; Vriejenhoek and Graven 1992; Berg and Garton 1994; Marsden et al. 1995; Dougherty et al. 1996). Typically, it is expected that brief drops in population size should result only in the loss of rare alleles, not overall heterozygosity (Balanya et al. 1994; Marsden et al. 1995). However, loss of variation can occur if the number of founders is very small or if the bottleneck lasts for many generations (Baker and Moeed 1987; Ward and Andrew 1995). For populations of freshwater invertebrates from northern Canada, Boileau et al. (1992) hypothesized that unexpectedly high allozyme differentiation among populations could be attributed to extreme founder effects as individual populations became established. Because *Daphnia* are parthenogenetic for much of the year, successful colonizations by one or a few individuals could easily take place. The extremely low level of heterozygosity in the Onondaga Lake population of *D. exilis* provides empirical support for this hypothesis.

How many *D. exilis* founded the Onondaga Lake population? To answer this question, we simulated invasions based on the assumption that gene frequencies at the 11 loci studied by Hebert and Finston (1993) for 13 populations in the southwestern United States are an unbiased representation of the source population that founded the Onondaga Lake population. Scenarios involving 1, 2, 3, 5, and 20 founders were explored. For each scenario, we performed 1,000 bootstraps in which we first chose one of the 13 populations at random and then randomly sampled it for 2n genes (*D. exilis* is diploid) at each locus. Only the 95% confidence interval (CI) for the single-colonist scenario overlaps with the zero heterozygosity observed in Onondaga Lake for these 11 loci (Fig. 4), although an invasion of two individuals becomes possible if a more conservative 99% CI is used. Furthermore, only one of the additional 4 loci we studied, *Mdh*-2, showed any genetic variation. The two *Mdh-2* alleles in the Onondaga Lake *D. exilis* that we hatched are present in a ratio of 45 : 55, which is not significantly different from 50 : 50. It seems that the single colonizer was heterozygous at this locus.

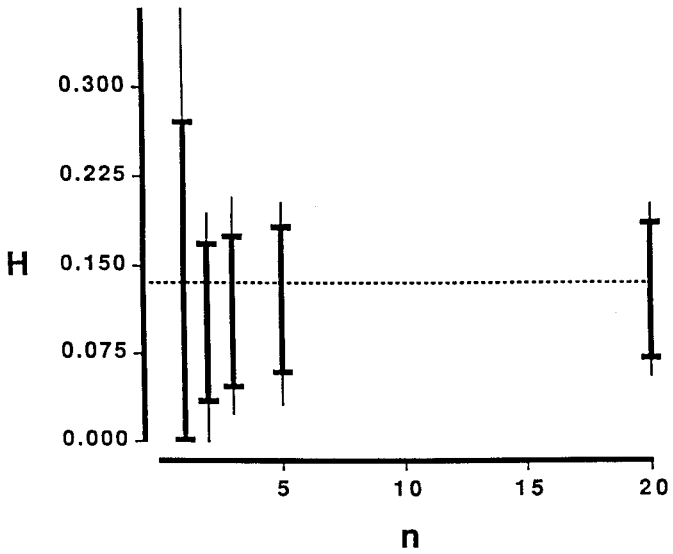

Fig. 4. Allozyme heterozygosity (H) expected in groups of *Daphnia exilis* drawn at random from each of 13 populations studied by Hebert and Finston (1993). We created scenarios involving 1, 2, 3, 5, and 20 founding colonists sampled at random (2 alleles per locus) from a single source population, and then we performed 1,000 simulations for each. Predictions of genetic variation are based on the 11 loci studied by Hebert and Finston (1993). Bars indicate 95% (thick lines) and 99% (thin lines) confidence limits, and the dashed line indicates expected heterozygosity averaged across all 13 southwestern U.S. populations. Only establishment by a single individual (95% CI) or by two individuals (99% CI) can explain the very low genetic variation (H = 0) in the *D. exilis* population in Onondaga Lake.

We only have data for *Daphnia* hatching from the most recently deposited diapausing eggs, so it is not possible to distinguish whether low population size occurred at the time of initial invasion in the mid-1920s or subsequently, in the mid-1930s, following the decade-long period of absence between about 1927 and about 1939 (Fig. 3). This reinvasion may represent either a second external dispersal event or reintroduction from the egg bank. The latter scenario is possible because we have definitive evidence (presented here) that diapausing eggs of *D. exilis* can remain viable in Onondaga Lake sediment for much longer than this 12-yr period of absence. With our data, it is not possible to distinguish between the two possibilities. However, because of the large population size of *D. exilis*, most or all genetic variability was likely to have been lost during either the initial invasion or the reinvasion, rather than during the extended period that the species was present in the lake. Clonal re-

production in similar daphniids can take place in as short a time as a week, and a great many generations may occur over the course of a summer growing season. Thus, the population could easily have grown to millions of individuals within the first year. In fact, if we assume that each *Daphnia* in the water column only produced a single ephippium, then the annual population size in the whole lake, in order to produce the 2.5×10^4 diapausing eggs per square meter (1×10^4 eggs m^{-2} y^{-1}) seen in both the 64–68 cm and 48–52 cm core slices (Fig. 3C) must have been on the order of 4×10^9 (given the known sedimentation rates and the volume of the water column of the lake and assuming conservatively that these sediment egg densities cover only the central tenth of the lake bottom). It seems reasonable, therefore, to use animals hatched from ephippia of the established population in Onondaga Lake to make inferences regarding an invasion that occurred some 40 yr earlier. See Boileau et al. (1992) for similar observations on founder effects in other freshwater invertebrates. A more detailed analysis of population genetic structure in the egg-bank population of *D. exilis* in Onondaga Lake is currently in progress, using microsatellite deoxyribonucleic acid that can be extracted from nonviable eggs taken from the full range of the sediment depths where they occur.

Our observation of extremely low genetic variation in *D. exilis* in Onondaga Lake stands in marked contrast to observations of high allelic diversity in populations of other invading cladocerans. De Melo and Hebert (1994*b*) interpret the high levels of allozyme heterozygosity in a number of animals (particularly *Bosmina coregoni* from the Laurentian Great Lakes) as evidence for repeated successful invasions by large numbers of individuals. Introduction and establishment of an exotic species requires the existence of both a suitable dispersal mechanism and an invasible system. For *D. exilis* in Onondaga Lake, dispersal appears to have been an isolated event (perhaps related to transport by industrial equipment), and invasibility was almost certainly mediated by an alteration of lake salinity by industry. The latter feature of the environment has proved to be reversible, and it appears that the elimination of *D. exilis* from the water column can be attributed to the cessation of the industrial activity. For both of these reasons, the genetic variation we observed in this failed invasion is substantially lower than that seen in the systems reviewed by De Melo and Hebert (1994*b*). Despite the apparent failure of *D. exilis* in Onondaga lake, viable eggs do remain in the egg bank should environmental conditions again become favorable for this species. Any boat anchor can easily penetrate the 20–25 cm necessary to mix the buried diapausing eggs back to the sediment surface.

References

APPLEBY, P. G., AND F. OLDFIELD. 1983. The assessment of ^{210}Pb data from sites with varying sediment accumulation rates. Hydrobiologia **103:** 29–35.

AUER, M. T., AND OTHERS. 1996. Biology, p. 384–534. *In* S. W. Effler [ed.], Limnological and engineering analysis of a polluted urban lake: Prelude to environmental management of Onondaga Lake, New York. Springer.

BAKER, A. J., AND A. MOEED. 1987. Rapid genetic differentiation and founder effect in colonizing populations of common mynas (*Acridotheres tristis*). Evolution **41:** 525–538.

BALANYA, J., C. SEGARRA, A. PREVOSTI, AND L. SERRA. 1994. Colonization of America by *Drosophila subobscura*: The founder event and a rapid expansion. J. Hered. **85:** 427–432.

BARTON, N. H., AND B. CHARLESWORTH. 1984. Genetic revolutions, founder effects, and speciation. Annu. Rev. Ecol. Syst. **15:** 133–164.

BERG, D. J., AND D. W. GARTON. 1994. Genetic differentiation in North American and European populations of the cladoceran *Bythotrephes.* Limnol. Oceanogr. **39:** 1503–1516.

BLACK, W. C. I., J. A. FERRARI, K. S. RAI, AND D. SPRENGER. 1988. Breeding structure of a colonizing species *Aedes albopictus skuse* in the USA. Heredity **60:** 173–182.

BINFORD, M. W. 1990. Calculation and uncertainty analysis of ^{210}Pb dates for PIRLA project lake sediment cores. J. Paleolimnol. **3:** 253–267.

BOILEAU, M. G., P. D. N. HEBERT, AND S. S. SCHWARTZ. 1992. Non-equilibrium gene frequency divergence: Persistent founder effects in natural populations. J. Evol. Biol. **5:** 25–39.

BROOKS, J. L. 1957. The systematics of North American *Daphnia.* Mem. Conn. Acad. Arts Sci. 13.

BRYANT, E. H., AND L. M. MEFFERT. 1991. The effects of bottlenecks on genetic variation, fitness, and quantitative traits in the housefly, p. 591–601. *In* E. C. Dudley [ed.], The unity of evolutionary biology, v. 2, Dioscorides. Proc. 4th Int. Congr. Syst. Evol. Biol.

CÁCERES, C. E. 1998. Interspecific variation in the abundance, production and emergence of *Daphnia* diapausing eggs. Ecology. **79:** 1699–1718.

CARSON, H. L., AND A. R. TEMPLETON. 1984. Genetic revolutions in relation to speciation phenomena: The founding of new populations. Annu. Rev. Ecol. Syst. **15:** 97–132.

CARVALHO, G. R., AND H. G. WOLF. 1989. Resting eggs of lake-*Daphnia*. I. Distribution, abundance and hatching of eggs collected from various depths in lake sediments. Freshw. Biol. **22:** 459–470.

CHAKRABORTY, R., AND M. NEI. 1977. Bottleneck effects on average heterozygosity and genetic distance with the stepwise mutation model. Evolution **31:** 347–356.

COLBOURNE, J. K., P. D. N. HEBERT, AND D. J. TAYLOR. 1997. Evolutionary origins of phenotypic diversity in *Daphnia,* p. 163–188. *In* T. J. Givnish and K. J. Sytsma [eds.], Molecular evolution and adaptive radiation. Cambridge.

COMINOLLI, R. 1990. Smokestacks allegro: The story of Solvay, a remarkable industrial/immigrant village (1880–1920). Center for Migration Studies.

DEAN, W. E., JR. 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition: Comparison with other methods. J. Sediment. Petrol. **44:** 242–248.

DEEVEY, E. 1942. Studies on Connecticut lake sediments. III: The biostratonomy of Lindsley Pond. Am. J. Sci. **240:** 233–264.

DE MELO, R., AND P. D. N. HEBERT. 1994*a*. A taxonomic reevaluation of North American Bosminidae. Can. J. Zool. **72:** 1808–1825.

———, AND ———. 1994*b*. Founder effects and geographical variation in the invading cladoceran *Bosmina (Eubosmina) coregoni* Baird 1857 in North America. Heredity **73:** 490–499.

DOUGHERTY, J. D., W. S. MOORE, AND J. L. RAM. 1996. Mitochondrial DNA analysis of round goby (*Neogobius melanostomus*) and tubenose goby (*Proterorhinus marmoratus*) in the Great Lakes basin. Can. J. Fish. Aquat. Sci. **53:** 474–480.

EAKINS, J. D., AND R. T. MORRISON. 1978. A new procedure for the determination of lead-210 in lake and marine sediments. Int. J. Appl. Radiat. Isot. **29:** 531–536.

EFFLER, S. W., AND G. HARNETT. 1996. Background, p. 1–31. *In* S. W. Effler [ed.], Limnological and engineering analysis of a polluted urban lake: Prelude to environmental management of Onondaga Lake, New York. Springer.

———, AND OTHERS. 1996*a*. Chemistry, p. 263–383. *In* S. W. Effler [ed.], Limnological and engineering analysis of a polluted urban lake: Prelude to environmental management of Onondaga Lake, New York. Springer.

———, M. T. AUER, N. JOHNSON, M. PENN, AND H. C. ROWELL. 1996*b*. Sediments, p. 600–666. *In* S. W. Effler [ed.], Limnological and engineering analysis of a polluted urban lake: Prelude to environmental management of Onondaga Lake, New York. Springer.

EVANS, D. H. 1993. Osmotic and ionic regulation, p. 315–341. *In* D. H. Evans [ed.], The physiology of fishes. CRC Press.

FREY, D. G. 1958. The late glacial cladoceran fauna of a small lake. Arch. Hydrobiol. **54:** 209–275.

HAIRSTON, N. G., JR. 1996. Zooplankton egg banks as biotic reservoirs in changing environments. Limnol. Oceanogr. **41:** 1087–1092.

———, R. A. VAN BRUNT, C. M. KEARNS, AND D. R. ENGSTROM. 1995. Age and survivorship of diapausing eggs in a sediment egg bank. Ecology **76:** 1706–1711.

HARTL, D. L., AND A. G. CLARK. 1989. Principles of population genetics, 2nd ed. Sinauer.

HAVEL, J. E., W. R. MABEE, AND J. R. JONES. 1995. Invasion of the exotic cladoceran *Daphnia lumholtzi* into North American reservoirs. Can. J. Fish. Aquat. Sci. **52:** 151–160.

HEBERT, P. D. N. 1995. The *Daphnia* of North America: An illustrated fauna [computer program]. Version 1. CD-ROM AuthorWare.

———, AND M. J. BEATON. 1993. Methodologies for allozyme analysis using cellulose acetate electrophoresis: A practical handbook, revised ed. Helena Laboratories.

———, AND T. L. FINSTON. 1993. A taxonomic reevaluation of North American *Daphnia* (Crustacea: Cladocera). I. The *D. similis* complex. Can. J. Zool. **71:** 908–925.

———, B. W. MUNCASTER, AND G. L. MACKIE. 1989. Ecological and genetic studies on *Dreissena polymorpha* Pallas, a new mollusc in the Great Lakes, USA and Canada. Can. J. Fish. Aquat. Sci. **46:** 1587–1591.

KERFOOT, W. C. 1974. Net accumulation rates and the history of cladoceran communities. Ecology **55:** 51–61.

LEBERG, P. L. 1992. Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. Evolution **46:** 477–494.

LEHMAN, J. T. 1987. Palearctic predator invades North American Great Lakes. Oecologia (Berl.) **74:** 478–480.

LIEDER, U. 1991. The *Bosmina kessleri*-like morphotype of *Eubosmina* in Lake Muskoka, Ontario, Canada, as putative interspecific hybrids. Hydrobiologia **225:** 71–80.

LODGE, D. M. 1993. Species invasions and deletions: Community effects and responses to climate and habitat change, p. 367–387. *In* P. M. Karieva, J. G. Kingsolver, and R. B. Huey [eds.], Biotic interactions and global change. Sinauer.

MAKAREWICZ, J. C., B. CADY, T. LEWIS, J. BUTTNER, AND J. HAYNES. 1995. Phytoplankton, zooplankton, macrobenthos and ichthyoplankton abundance, biomass and species composition in Onondaga Lake, 1994. Onondaga Manag. Conf.

MARSDEN, J. E., A. SPIDLE, AND B. MAY. 1995. Genetic similarity among zebra mussel populations within North America and Europe. Can. J. Fish. Aquat. Sci. **52:** 836–847.

MAYR, E. 1965. Animal species and evolution. Belknap.

MEYER, M. A., AND S. W. EFFLER. 1980. Changes in the zooplankton of Onondaga Lake 1969–1978. Environ. Pollut. (Ser. A) **23:** 131–152.

MILLS, E. L., J. H. LEACH, J. T. CARLTON, AND C. L. SECOR. 1993. Exotic species in the Great Lakes: A history of biotic crises and anthropogenic introductions. J. Gt Lakes Res. **19:** 1–54.

———, ———, ———, AND ——— 1994. Exotic species and the integrity of the Great Lakes: Lessons from the past. BioScience **44:** 666–676.

MOYLE, P. B., AND J. J. CECH, JR. 1996. Fishes: An introduction to ichthyology, 3rd ed. Prentice Hall.

NEI, M., T. MARUYAMA, AND R. CHAKRABORTY. 1975. The bottleneck effect and genetic variability in populations. Evolution **29:** 1–10.

O'BRIEN, S. J., D. E. WILDT, M. BUSH, T. M. CARO, C. FITZGIBBON, I. AGGUNDEY, AND R. A. LEAKEY. 1987. East African cheetahs: Evidence for two population bottlenecks. Proc. Natl. Acad. Sci. USA **84:** 508–511.

PERKINS, M. G., AND E. A. ROMANOWICZ. 1996. Hydrological setting, p. 32–96. *In* S. W. Effler [ed.], Limnological and engineering analysis of a polluted urban lake: Prelude to environmental management of Onondaga Lake, New York. Springer.

REMANE, A., AND C. SCHLIEPER. 1971. Biology of brackish water. Die Binnengewässer, v. 25. E. Schweizerbart'sche.

RICE, W. R., AND E. E. HOSTERT. 1993. Laboratory experiments on speciation: What have we learned in 40 years? Evolution **47:** 1637–1653.

SIEGFRIED, C., N. A. AUER, AND S. W. EFFLER. 1996. Changes in the zooplankton of Onondaga Lake: Causes and implications. Lake Reservoir Manag. **12:** 59–71.

VRIEJENHOEK, R. C., AND M. A. GRAVEN. 1992. Population genetics of Egyptian *Biomphalaria alexandrina* (Gastropoda: Planorbidae). J. Hered. **83:** 255–261.

WARD, R. D., AND J. ANDREW. 1995. Population genetics of the northern Pacific seastar *Asterias amurensis* (Echinodermata: Asteriidae): Allozyme differentiation among Japanese, Russian, and recently introduced Tasmanian populations. Mar. Biol. **124:** 99–109.

WATERMAN, G. 1971. Onondaga Lake zooplankton. Washington: Water Quality Office, U.S. Environmental Protection Agency. Onondaga Lake Study Project No. 11060, FAE 4/71, p. 3610384.

WEIDER, L. J. 1991. Allozymic variation in *Bythotrephes cederstroemi,* a recent invader of the Great Lakes. J. Gt Lakes Res. **17:** 141–143.

WEIDER, L. J., W. LAMPERT, M. WESSELS, J. K. COLBOURNE, AND P. LIMBURG. 1997. Long-term genetic shifts in a microcrustacean egg bank associated with anthropogenic changes in the Lake Constance ecosystem. Proc. R. Soc. Lond. B **264:** 1613–1618.

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