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Viability of invertebrate diapausing eggs collected from residual ballast sediment

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Abstract

Natural or anthropogenic movement of sediments may be an important vector for the dispersal of invertebrate resting stages between water bodies. Here we record the presence of invertebrate diapausing eggs in residual sediments from transoceanic vessels and explore whether these may pose an invasion risk. Viability of diapausing eggs was explored under light and dark conditions using sediment collected from eleven tanks on nine vessels operating on the Great Lakes. Seventeen cladoceran, copepod, and rotifer taxa were identified. Four of the species hatched have not yet been reported as established in the Great Lakes. Egg viability for individual species varied from 0% to 92%. Exposure to saline water may impact egg viability of some freshwater species. Generally, the proportion of eggs hatched in light and dark treatments did not differ significantly, indicating that light was not required to terminate diapause. As a result, eggs could potentially hatch in dark ballast tanks when immersed in freshwater loaded as ballast during operation on the Great Lakes. Viability of diapausing eggs differed among ballast tanks on a single vessel, indicating that tanks with independent ballast histories have different invasion risks. While additional work is needed to quantify risk, results from this study indicate that vessels entering the Great Lakes with only residual ballast are a potential vector for the introduction of new nonindigenous species during multiport operations.

Freshwater invertebrates achieve dispersal via transport of their desiccation-resistant dormant stages in flowing waters, wind, or by ectozoochorous or endozoochorous animal vectors (*see* Bilton et al. 2001; Cáceres and Soluk 2002; Figuerola and Green 2002). Water currents are likely responsible for most short-distance dispersal events, particularly of stream-dwelling taxa (Bilton et al. 2001). Wind and animal vectors also disperse invertebrates (Figuerola and Green 2002), although recent work indicates that these vectors may be species-specific or relatively unimportant (Jenkins and Underwood 1998; Cáceres and Soluk 2002). Movement of sediment represents an alternative dispersal medium that could transport resting stages of many taxa. As an example, sediment associated with heavy machinery has been linked to dispersal of both rotifer and copepod species (Koste and Shiel 1989; Hairston et al. 1999).

Hebert and Cristescu (2002) estimated that the rate of human-mediated dispersal of crustacean zooplankton to the Laurentian Great Lakes exceeds the natural rate by up to 50,000 fold. Transoceanic ships have been the dominant transport vector of nonindigenous species (NIS) to the Great Lakes for most of the 20th century (Mills et al. 1993; Ricciardi and MacIsaac 2000; Ricciardi 2001). Regulations enacted in 1993 effectively require open-ocean ballast exchange for vessels inbound to the Great Lakes with freshwater or brackish water if the water is to be discharged into the lakes (United States Coast Guard 1993). Open-ocean exchange purges most freshwater organisms, while remaining individuals should be killed when tanks are refilled with saline water. The invasion risk posed by these vessels appears to be much lower than that posed by ships entering without ballast water (MacIsaac et al. 2002). The latter vessels, officially classified as no ballast on board (NOBOB), are exempt from current ballast water regulations (United States Coast Guard 1993).

NOBOB vessels, which dominate trade into the Great Lakes, cannot completely empty their ballast tanks due to structural and operational limitations and carry an average

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of 60 tonnes of unpumpable water and sediments (Colautti et al. in press; P. Jenkins unpubl. data). MacIsaac et al. (2002) estimated that residual water in NOBOB vessels could carry up to 10^5 zooplankton. Inbound NOBOB vessels could serve as a vector of NIS when they load and then discharge ballast water during multiport operations on the Great Lakes. Typically, multiport ships load ballast water when circumstances dictate (e.g., when off-loading cargo) to balance longitudinal stresses and to maintain directional stability. At the last port of call on the Great Lakes, the residual ballast—now mixed with Great Lakes ballast water—is discharged as outbound cargo is loaded. The addition and subsequent release of water in the Great Lakes may, therefore, pose an invasion risk because it provides a direct vector for discharge of live organisms or viable resting stages of NIS.

Most previous ballast research has focused on metazoans and microorganisms resident in ballast water (Williams et al. 1988; Ruiz et al. 2000; Drake et al. 2001). Far less attention has been given to ballast sediments and the resting stages therein. However, Hallegraeff and Bolch (1991) were able to successfully germinate 20 dinoflagellate species from dormant cysts carried in residual sediment, while Kelly (1993) found that ballast sediments contained viable spores and cysts of diatoms, euglenoid flagellates, ciliates, and dinoflagellates.

There exist at least two mechanisms by which invertebrate NIS may be introduced to the Great Lakes via diapausing eggs contained within residual sediments of NOBOB vessels. First, diapausing eggs (i.e., fertilized embryos enclosed in a protective case that lie in a dormant state) may be resuspended from sediments after the addition of freshwater to ballast tanks, such that they are available in the water column for direct discharge to the Great Lakes (Locke et al. 1993). Alternatively, if diapausing eggs were stimulated to hatch inside ballast tanks after loading Great Lakes ballast water, live organisms might be expelled during subsequent deballasting on the upper lakes. In this study, we assess the viability of diapausing eggs in laboratory experiments and test the hypothesis that light is required as a hatching stimulus for diapausing eggs collected from ballast sediments.

Materials and methods

Sample collection—Residual sediments were collected from transoceanic vessels entering the Great Lakes in December 2000 and between May and December 2001 at the ports of Hamilton and Thorold, Ontario, Canada, and Cleveland, Ohio, U.S.A. Vessels were selected opportunistically without regard to previous ports of call (i.e., probable sources of ballast water). Residual sediment was collected from at least one tank per ship, and additional tanks were sampled depending upon the time period available and the ease and safety with which the tanks could be accessed. Samples were collected from at least five areas within each tank using sterile scoops and spatulas. Sediments were generally collected along longitudinal shell frames that trapped sediment in areas away from drainage flows. Approximately 4 kg of sediment from a single tank, if available, was composited and later used for experimentation. Temperature and salinity of residual ballast water, if any was present, were recorded at the time of sampling using a Fisher Scientific Traceable® thermometer and a portable optical refractometer. Salinity of sediment pore water was subsequently determined in the laboratory.

Egg density counts—Four 40-g subsamples (wet weight) were taken from each sample (except ship 1) and preserved in 95% ethanol. Each subsample was washed through a 45-μm sieve to remove fine sediment. Eggs were subsequently separated from the remaining sediment using a Ludox® HS 40 protocol after Burgess (2001). Mean density of invertebrate diapausing eggs was calculated from the four subsamples after enumeration under a dissecting microscope. Egg densities for ship 1 were estimated by size fractionating a 130-g sediment subsample from each tank using 1-mm, 500-μm, and 250-μm sieves. Each size fraction was examined under a dissecting microscope, and total egg density was calculated per gram of whole sediment. Egg densities from ship 1 were extrapolated to 40 g to compare with other tanks.

Viability experiments—Unprocessed sediments were stored in plastic containers in the dark at 4°C for at least 4 weeks to allow a refractory period before hatching experiments commenced (*see* Grice and Marcus 1981; Schwartz and Hebert 1987). After this time, diapausing eggs were isolated from sediment using the sugar flotation method, modified after Hairston et al. (1995). Sediment was processed through a 45-μm sieve, and the retained material was washed into centrifuge tubes using a 1 : 1 mixture (w : v) of sucrose and water. This material was centrifuged at approximately 650 rpm ($\sim$7.7 m s^{-2}) for 5 min. The supernatant was then decanted and rinsed thoroughly with water through a 45-μm mesh before being transferred to a counting dish. Diapausing eggs were immediately recovered from the supernatant and sorted by size and gross morphology under a dissecting microscope, selecting only fully intact, apparently healthy eggs. Eggs were then separated into replicates, with 10 or 20 eggs of one type in each (depending on available quantity), and placed into vials containing 15 ml of sterile synthetic pond water (Hebert and Crease 1980). At least three replicates, depending on egg quantity, were placed into each of two treatments consisting of a light : dark cycle of 16 : 8 or of 0 : 24 h, at 20°C. Exposure to light was minimized during the separation procedure by quickly placing eggs of the dark treatment into vials wrapped in aluminum foil, although some exposure to light occurred during sieving and at the microscope. A sufficient number of eggs for replicated experiments was collected from samples obtained from seven ships (Table 1). Sediment from one double-bottom tank contained only a sparse population of *Bosmina* eggs; thus, the number of eggs per replicate in this experiment varied from 4 to 20, although the same number of eggs was used in each light and dark treatment. A total of 12 distinct egg types were distinguished from residual sediments (Fig. 1). Diapausing eggs could be reliably sorted at the genus level, resulting in a total of 17 taxa emerging from the 12 egg morphotypes. In addition, the hatching of unidentified *Diaphanosoma* sp., *Daphnia* sp., and calanoid copepods may

Table 1. List of species hatched from ballast sediments through quantitative and qualitative hatching studies. Species with N/A replicates were too sparse to obtain enough eggs for fully replicated experiments and were hatched only for identification purposes. Salinity was measured from residual ballast water in tanks and as pore water extracted from sediment. FP = forepeak tank, DB = double-bottom tank.

Ship	No. of replicates	Species	Salinity: Ballast water	Salinity: Pore water
1(FP)	5	*Daphnia longiremis*	46	37
	N/A	Chydoridae		
2(DB)	N/A	*Moina affinis*	24	34
3(FP)	3	*Bosmina liederi*	2	2
	N/A	*Daphnia* sp.		
	N/A	*Diaphanosoma* sp.		
3(DB)	4	*Bosmina liederi*	22	20
	N/A	*Daphnia ambigua*		
4(FP)	3	*Brachionus calyciflorus*	5	10
	N/A	Calanoid copepod		
	N/A	*Daphnia magna*		
	N/A	*Diaphanosoma brachyurum*		
	N/A	*Filinia* sp.		
	N/A	*Moina micrura*		
4(DB)	3	*Brachionus calyciflorus*	23	18
	N/A	*Brachionus budapestinensis*		
	N/A	*Daphnia magna*		
5(DB)	5	*Asplanchna girodi*	2	0
	5	*Brachionus budapestinensis*		
	N/A	*Brachionus angularis*		
	N/A	*Ceriodaphnia pulchella*		
	N/A	*Moina micrura*		
6(DB)	N/A	*Daphnia* sp.	N/A	35
7(DB)	5	*Bosmina liederi*	0	2
	N/A	*Brachionus calyciflorus*		
	N/A	*Brachionus diversicornis*		
	N/A	*Brachionus budapestinensis*		
	N/A	*Ploesoma* sp.		
8(DB)	5	*Brachionus calyciflorus*	20	19
	N/A	*Brachionus budapestinensis*		
	N/A	*Brachionus angularis*		
	3	*Bosmina* sp. (0 hatched)		
	4	Calanoid copepod		
9(DB)	5	*Brachionus calyciflorus*	37	28

represent additional species, but these organisms were inadequately developed for identification with greater resolution. Only seven of the twelve egg types were found in sufficient density for fully replicated quantitative experiments (*see Table 1*). However, when egg densities were insufficient, eggs were still incubated for identification purposes. Eggs of two species, *Brachionus calyciflorus* and *Bosmina liederi,* were fully replicated from numerous tanks and were used in four and two trials, respectively. Therefore a total of 12 replicated trials, using seven egg types, were executed. Six trials were run for rotifer species, five for cladoceran taxa, and one for a calanoid copepod (Table 1). Controls containing synthetic pond water were kept in each treatment group to monitor for introduction of organisms from the environment. Vials were checked for emergence every 24 h for 20 d. Light exposure for eggs in the dark treatment was kept to a minimum, being generally less than 30 s per day. Water was renewed every 5 d and eggs examined for fungal infection or degeneration. Eggs that were contaminated with fungus were discarded. Few cultures (<1%) had to be discarded for this reason. The number of hatched individuals was recorded daily. Live individuals were removed to separate vials and fed ~12,000 cells ml^{-1} *Cryptomonas* sp. regularly (University of Toronto Culture Collection 338). All hatched animals were raised to adulthood, when possible, to aid in identification. Taxa were identified using standard taxonomic keys. Because the geographical origin of hatched individuals was unknown, at least two keys were used. Immature *Daphnia magna* were identified using a cytochrome oxidase 1 assay (P. D. N. Hebert pers. comm.). Throughout the duration of the study, all waste, filtered sediments, and unhatched eggs were autoclaved prior to disposal to minimize the possibility of environmental contamination. Variation in the cumulative proportion of diapausing eggs hatched between light and dark treatments was analyzed using a one-way analysis of variance (ANOVA) with repeated measures using Systat 7.0 (SPSS Inc. 1997). The proportions of eggs hatched were normalized using an arcsine square root transformation

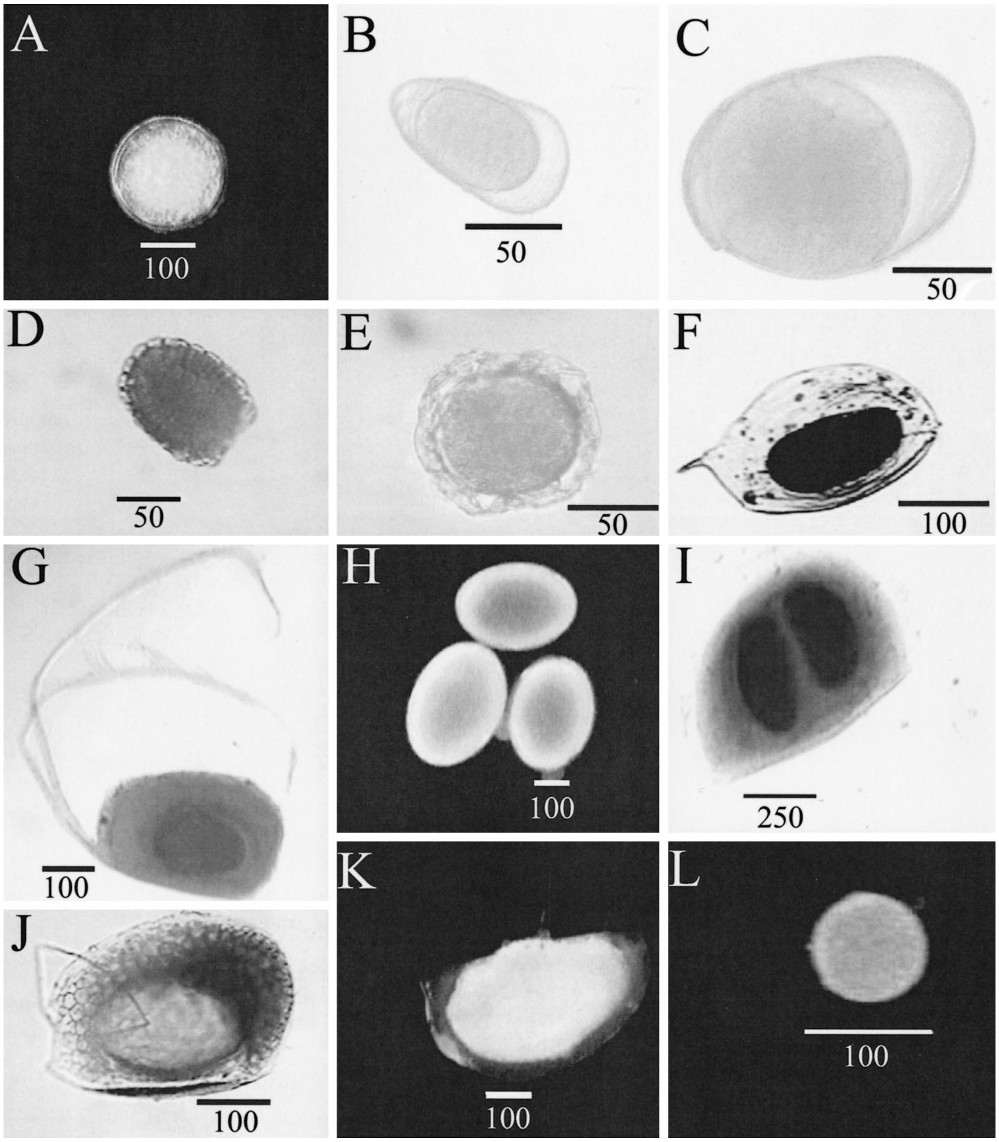

Fig. 1. Resting egg morphotypes successfully recovered from ballast sediments. Rotifera: (A) *Asplanchna girodi,* (B) *Brachionus budapestinensis,* (C) *B. calyciflorus,* (D) *Filinia* spp., (E) *Ploesoma* spp. Cladocera: (F) *Bosmina* spp., (G) Chydoridae, (H) various Cladocera, (I) *Daphnia* spp., (J) and (K) *Moina* spp. Copepoda: (L) calanoid copepod. Scale bars (μm) are included on each image.

prior to analysis. Analyses were conducted only using days when hatching occurred in at least one of the replicates.

Results

Viability experiments on invertebrate diapausing eggs were conducted on ballast sediment samples retrieved from nine vessels. Salinity of residual water in ballast tanks varied from 0‰ to 46‰, while that of ballast sediment pore water was only slightly less variable (i.e., 0‰ to 37‰) (Table 1). Forepeak and double-bottom tanks from the same ship had disparate ballasting histories. In general, forepeak tanks had not been flushed with saline water, as evidenced by the low salinity of their residual ballast water. For example, the respective salinities of residual water in forepeak and double-bottom tanks for ship 3 were 2‰ and 22‰, while those in ship 4 were 5‰ and 23‰ (Table 1). Species' resting egg densities were generally quite low (i.e., <10 eggs per 40 g sediment) (Table 2). There were, however, four instances in which egg density was quite high (>50 eggs per 40 g sediment) for *Daphnia* spp., *Bosmina* spp., *Brachionus budapestinensis*, and *B. calyciflorus.* Cladoceran ephippia were encountered most commonly in ballast sediment, but often at the lowest density. By contrast, diapausing eggs were recovered for fewer rotifer taxa, but they were typically found at much higher densities. Rotifers (*Asplanchna girodi*, *Brachionus budapestinensis*, and *B. calyciflorus*) generally began to hatch within 24 h of incubation, while calanoid copepods and cladocerans (*Bosmina liederi* and *Daphnia longiremis*) began to hatch at day 3. The average proportion of eggs hatched ranged from 0% to 92% and was slightly higher in light (40%) than in dark (33%) treatments (Fig. 2). Rotifer diapausing eggs exhibited the greatest hatch rate at 92% and averaged 49% over all trials. Typically less than 20% of cladoceran eggs hatched, although in one trial *Bosmina liederi* averaged a 72% hatch rate. Aside from the unusually high viability of *B. liederi* diapausing eggs obtained from it, this tank had no unusual attributes of which we are aware. The copepod trial achieved an 11% hatch rate. Both the number of species present and the viability of eggs varied among ships and between tanks on a single vessel (*see Table 1 and Fig. 2*). No strong relationship between hatch rate and pore-water salinity was evident (Fig. 3), although high (>65%) hatching success was achieved only for diapausing eggs derived from tanks with low salinity. A significant difference (ANOVA, $P < 0.05$) was observed between light and dark treatments in two trials (Table 3; Fig. 2D,I); in both instances, hatch rate in the light treatment exceeded that in the dark. However, this pattern was repeated neither for other taxa nor for all the trials combined (Table 3). Some diapausing eggs hatched in both light and dark treatments for all but two trials, both of which involved *Bosmina* species. One of these trials had no individuals hatch in either treatment. Two *Brachionus* trials exhibited significant divergence of hatch rates through time, with the light treatment having a higher, steeper curve in both cases (Table 3; Fig. 2B,D). Overall, there was a very high degree of correspondence between the percent of diapausing eggs that hatched in the light and dark treatments (Pearson correlation 0.93; Fig. 4).

Discussion

This is the first study to experimentally demonstrate that diapausing eggs of invertebrate species are present and remain viable after transportation in ballast tanks of transoceanic ships. Dormancy is a commonly encountered life history strategy in aquatic species that ensures long-term survival through adverse conditions (*see* Bilton et al. 2001). Resting stages, which can survive periods of anoxia, desiccation, and fluctuating temperature (Dodson and Frey 1991; Wallace and Snell 1991; Lutz et al. 1992), are also essential for dispersal. Colonization success is likely related to the number of dispersal events and the number of propagules in each event. Considering the density of diapausing eggs in residual sediment and the number of transoceanic vessels transiting the Great Lakes, the dispersal of diapausing eggs via NOBOB vessels may be an important invasion mechanism.

Jenkins and Buikema (1998) and Shurin (2000) reported that zooplankton community composition appears to be dispersal limited. Shipping activities could clearly effect dispersal of species or subspecies within genera that typically have restricted natural distributions. For example, although the genus *Brachionus* is considered cosmopolitan, *B. diversicornis*—a species encountered in one of our ballast sediment samples—has not been reported from either North or South America (Dumont 1983). Likewise, most *Daphnia* species occur in only two or three of the world's six zoogeographical zones (Hebert 1978) and could potentially experience range extensions mediated by shipping activities. In point of fact, an analysis of the cytochrome oxidase 1 gene revealed that the *Daphnia magna* individuals hatched in our study originated from a European, not North American, source (P. D. N. Hebert pers. comm.). If residual ballast sediments are important dispersal vectors for invertebrates, then the Great Lakes may remain vulnerable to invasion despite legal safeguards that prevent the discharge of untreated, freshwater ballast.

In order to estimate the risk of invasion posed by diapausing eggs in residual ballast sediments, it is necessary to consider both their density and viability. Maximum densities of diapausing eggs in natural populations typically range from 2.0×10^3 to 3.0×10^6 eggs m^{-2} (Hairston 1996). If we assume 1 g of sediment is equivalent to 1 cm^3, which seems reasonable considering the high water content of ballast sediment, our observed densities of diapausing eggs (range of 5.0×10^2 to 4.3×10^5 eggs m^{-2}) are typically an order of magnitude lower than densities observed in natural

Table 2. Average density of diapausing eggs in 40-g subsamples of residual ballast sediments. Eggs classified as rare were not encountered in all four subsamples and had an average density <2 eggs per 40 g. FP = forepeak tank, DB = double-bottom tank.

Ship	Species	No. eggs per 40 g
1(DB)	*Daphnia* spp.	202
	Chydoridae	rare
2(DB)	*Moina* spp.	3
3(FP)	*Bosmina* spp.	56
	Daphnia spp.	3
	Diaphanosoma spp.	rare
3(DB)	*Bosmina* spp.	4
	Daphnia spp.	rare
4(FP)	*Brachionus calyciflorus*	4
	*Brachionus budapestinensis**	4
	Calanoid copepod	rare
	Daphnia spp.	rare
4(DB)	*Brachionus calyciflorus*	7
	Brachionus spp.	37
	Daphnia spp.	rare
5(DB)	*Asplanchna* spp.	14
	Brachionus calyciflorus	51
	*Brachionus budapestinensis**	1703
	Ceriodaphnia spp.	rare
	Moina spp.	rare
6(DB)	*Daphnia* spp.	rare
7(DB)	*Bosmina* spp.	6
	Brachionus calyciflorus	2
	*Brachionus budapestinensis**	rare
	Ploesoma spp.	rare
8(DB)	*Brachionus calyciflorus*	6
	*Brachionus budapestinensis**	17
	Bosmina spp.	rare
	Calanoid copepod	3
9(DB)	*Brachionus calyciflorus*	23

* While 99% of the organisms hatched from these eggs were *Brachionus budapestinensis,* individuals of *B. angularis* and *B. diversicornis* also hatched from this egg morphotype.

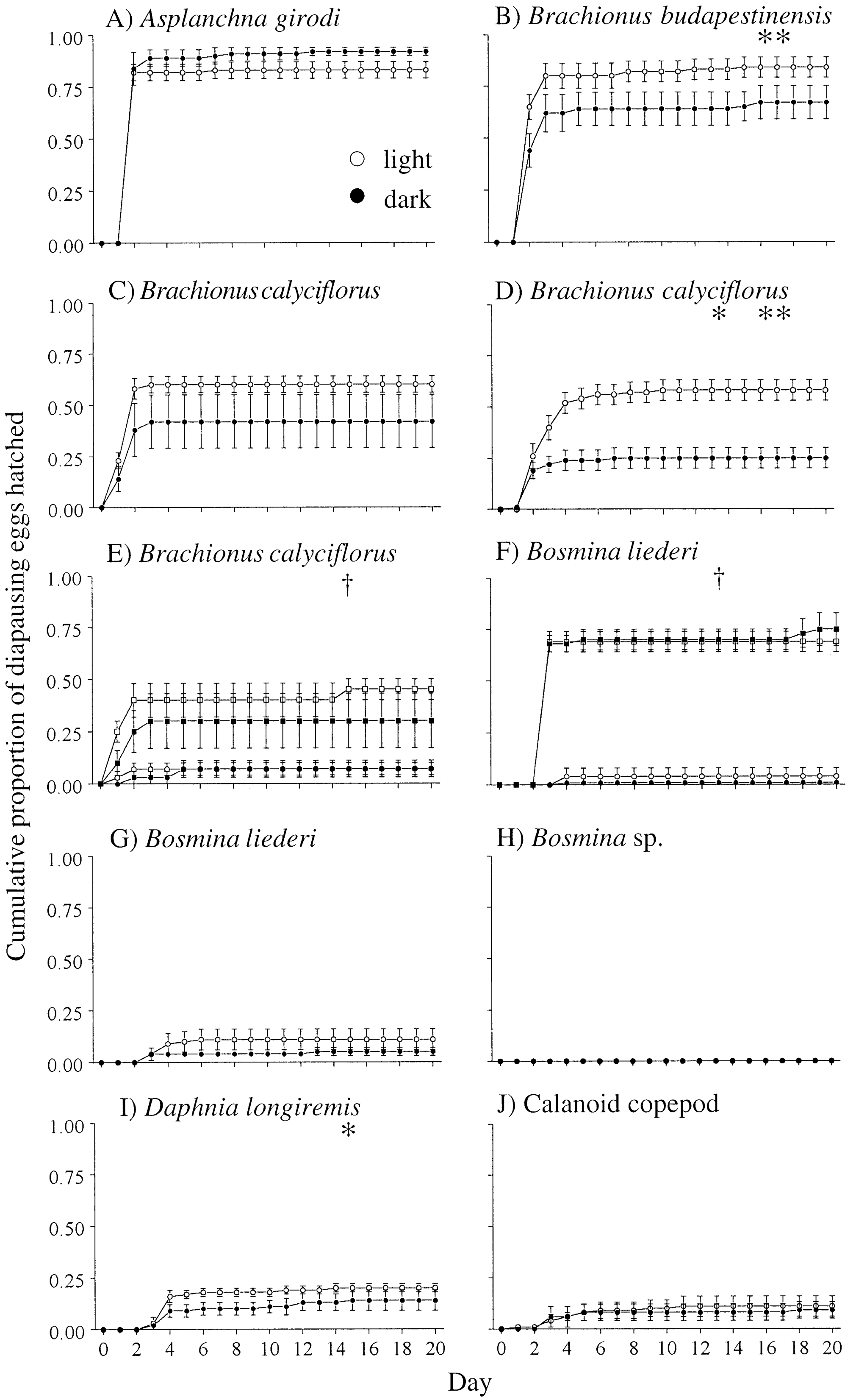

Fig. 2. Mean (±SE) cumulative proportion of diapausing eggs hatched under light and dark treatments, by taxon. (A) *Asplanchna girodi,* (B) *Brachionus budapestinensis,* (C) *Brachionus calyciflorus* (ship 8), (D) *B. calyciflorus* (ship 9), (E) *B. calyciflorus* (ship 4), (F) *Bosmina liederi* (ship 3), (G) *B. liederi* (ship 7), (H) *Bosmina* sp., (I) *Daphnia longiremis,* and (J) calanoid copepod.

environments. However, two of the nine vessels had egg densities (5.1×10^4 and 4.3×10^5 eggs m^{-2}) similar to those reported from ponds, lakes, and nearshore marine sediments. In our experience, sediment generally makes up less than 25% of unpumpable ballast. Based on our range of egg densities, a NOBOB vessel with 10 tonnes of residual sediment could carry 1.6×10^6 to 2.6×10^9 diapausing eggs. With an average hatch rate of 33% in the dark treatment, this translates to approximately 5.3×10^5 to 8.6×10^8 viable propagules per vessel.

The diversity of plankton potentially dispersed by diapausing eggs in ships' residual ballast sediment is quite high. As our study revealed, diapausing eggs of rotifers, cladocerans, and calanoid copepods all proved viable after recovery from ballast sediments. However, diapausing eggs of cladocerans and rotifers were much more abundant in ballast sediment than were those of calanoid copepods. In particular, diapausing eggs of the genera *Brachionus* and *Daphnia* were most commonly encountered in residual sediments, with each being recorded from six tanks, although *Brachionus* eggs were much more abundant in ballast sediments than the latter genus. We observed significant differences in hatching success between light and dark treatments in only two of the twelve trials. This indicates that invertebrate diapausing eggs are generally as likely to hatch in the dark confines of ballast tanks after the intake of freshwater ballast as they are if expelled into a lighted, lake environment. It is interesting and potentially ecologically important that diapausing eggs can be experimentally induced to hatch in the dark, since day length has commonly been identified as an important stimulus for hatching of invertebrate diapausing eggs (e.g., Dodson and Frey 1991; Wallace and Snell 1991). However, prolonged storage in constant dark conditions has been suggested to eliminate the requirement of light to induce hatching (Stross 1966). Diapause eggs may be entrained in ballast sediments, without light stimuli, for weeks, months, or years. We propose that the isolation of diapause eggs from ballast sediments and subsequent exposure to oxygenated media, combined with transfer from cold storage (4°C) to warm incubation (20°C), is sufficient to terminate diapause without exposure to light. Alternatively, it is possible that even brief exposure to lighted conditions, as occurred during our examination procedures, was sufficient to initiate emergence, although we believe this is highly unlikely.

The dense nature of residual ballast sediments is likely to cause the diapausing eggs to be entrapped when loaded with sediment, suggesting that there exists only a small chance that these eggs will be resuspended and expelled with ballast water. Rather, it is more plausible that planktonic individuals stimulated to hatch from diapausing eggs inside the tanks will be readily available for discharge. It is possible that only those eggs present in surficial sediments may be stimulated to hatch within ballast tanks, as some invertebrate diapausing eggs do not hatch while buried (Marcus 1996). Therefore, the large number of viable eggs above may be an overestimation of the risk presented unless they are released from sediments during turbulent ballast operations or are discharged into the Great Lakes directly. Ultimately, in situ studies are required to determine whether viability from laboratory experiments translates into real risk for vessels operating on the Great Lakes.

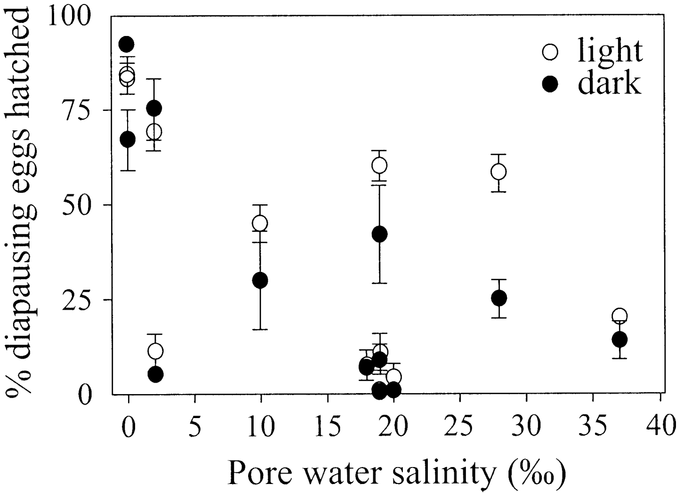

Fig. 3. Mean (±SE) viability of diapausing eggs as a function of pore-water salinity.

The majority of diapausing eggs were hatched during the first 10, and often 4, days of the experiments. This pattern is consistent with results from other studies. For example, Schwartz and Hebert (1987) found that *Daphnia* eggs begin to hatch within 3 to 5 d when exposed to optimal conditions, while May (1987) showed that the majority of rotifer resting eggs hatched by day ten, and Madhupratap et al. (1996) reported that eggs of four copepod species hatched within 2 d. Most transoceanic NOBOB vessels spend between 7 and 14 d during their inbound voyage on the Great Lakes, during which time they discharge cargo and load ballast water at a series of ports. This interval appears sufficient for diapausing eggs to hatch within tanks, potentially resulting in the production of zooplankton available for discharge with ballast water at the terminal port of call.

Of the 17 taxa that were hatched in our study, four have not been reported as established in the Great Lakes. Two of these species, *Moina affinis* and *Asplanchna girodi*, occur in the southern United States and/or Mexico (Edmondson 1966; Stemberger 1979; Hebert 1995). It is probable that the eggs of these species were produced by adults, or entered the tanks directly, in ballast loaded at a southern port. For example, *M. affinis* was hatched from a vessel that had recently ballasted at Brownsville, Texas, and Vera Cruz, Mexico. The ship containing diapausing eggs of the European strain of *Daphnia magna* had recently carried ballast from several

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Circle and square symbols represent double-bottom and forepeak tanks, respectively. Error bars less than 0.03 are hidden by graph symbol. * and ** denote trials for which differences in treatment and treatment × time were significant, respectively. † denotes a significant difference between tanks on a single vessel. All other growth curves differed insignificantly from each other (ANOVA, $P > 0.05$).

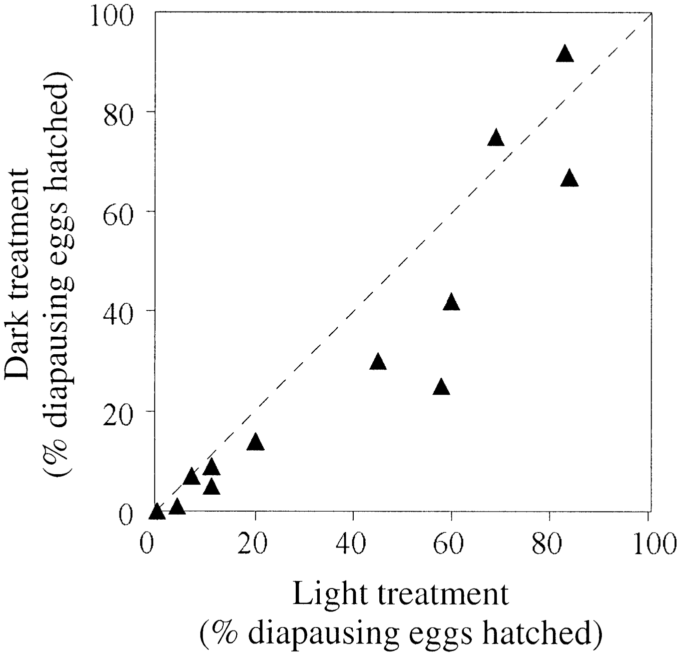

Fig. 4. Percent diapausing eggs that hatched in the dark relative to that in the light treatment. Each point represents one trial. The Pearson correlation is 0.93.

European countries (Portugal, Germany, Holland, and Finland). The fourth species, *Brachionus diversicornis,* has been reported from Australia, Europe, Asia, and Africa but not from either North or South America save for a misidentification of *B. havanaensis* in the Great Lakes (Stemberger 1979; Dumont 1983). The vessel carrying this species had recently ballasted in Poland (Baltic Sea) and Algeria (Mediterranean). We therefore postulate that Europe or Africa is the likely source for this NIS.

The diversity and viability of diapausing eggs collected from ballast sediments presented here should be considered a minimum estimate. Our study was limited by our ability to find and to recognize eggs, and our experiments were designed to assess viability of taxa capable of growing only in freshwater at 20°C. We detected no difference in appearance between eggs that did and did not hatch, although it is possible that unhatched eggs were viable but required either longer incubation periods or different refractory (i.e., pretreatment) or ''hatch-out'' conditions. Conditions required to induce hatching are complex and vary among taxa, although temperatures during the refractory and hatching incubation periods are both known to impact the rate of hatching (Schwartz and Hebert 1987). While 20°C will induce hatching of temperate taxa, which are currently of greatest concern to the Great Lakes, arctic or tropical species may perform poorly under these conditions. For example, in one case we observed a hatch rate of only 11% for calanoid copepod eggs at 20°C, while 67–96% of freshwater copepods from natural sediments have been reported to emerge at 8–10°C (De Stasio 1989; Parker et al. 1996). These differences may have resulted from the different evolutionary histories of these species and thus may not reflect true egg viability. Furthermore, if eggs have not passed through the necessary refractory period, then even viable diapausing eggs will not hatch under optimal conditions (Grice and Marcus 1981).

Viability of diapausing eggs was highly variable across ships and between the forepeak and double-bottom tanks within a single vessel (Fig. 2). These findings are consistent with previous reports of intertank variability for dinoflagellates and their cysts in ballast water and sediments, respectively (Hallegraeff 1998; Hamer et al. 2000). This variation may be a consequence of exposure to dissimilar water sources and salinity regimes in different ballast tanks. Forepeak tanks are typically managed differently than double-bottom tanks and are seldom flushed in midocean. In contrast, it is a common management procedure to flush double-bottom tanks midocean in an attempt to reduce sediment buildup. This procedure may also have the added benefit of reducing the number of viable dormant organisms associated with sediments. Differences in operational procedure and tank

Table 3. Analysis of variance with repeated measures demonstrating the effect of illumination treatment on the hatch rate of diapausing eggs. Data were arcsine square root transformed prior to analysis. Significance levels for *F* values: NS ($p > 0.10$), * ($p < 0.05$), ** ($p < 0.0001$). FP = forepeak tank, DB = double-bottom tank.

		ANOVA effects *F* value (df)		
Ship	Organism	Treatment	Time	Time × treatment
1(DB)	*Daphnia longiremis*	6.5* (1, 8)	16.8** (12, 96)	0.6$^{\mathrm{NS}}$ (12, 96)
3(FP)	*Bosmina liederi*	0.1$^{\mathrm{NS}}$ (1, 5)	4.0** (6, 30)	0.3$^{\mathrm{NS}}$ (6, 30)
3(DB)	*Bosmina liederi*	0.1$^{\mathrm{NS}}$ (1, 7)	1.8* (20, 140)	0.1$^{\mathrm{NS}}$ (20, 140)
4(FP)	*Brachionus calyciflorus*	0.3$^{\mathrm{NS}}$ (1, 7)	0.0** (20, 140)	0.9$^{\mathrm{NS}}$ (20, 140)
4(DB)	*Brachionus calyciflorus*	0.1$^{\mathrm{NS}}$ (1, 4)	0.1$^{\mathrm{NS}}$ (3, 12)	0.1$^{\mathrm{NS}}$ (3, 12)
5(DB)	*Asplanchna girodi*	1.7$^{\mathrm{NS}}$ (1, 8)	4.9** (6, 48)	1.7$^{\mathrm{NS}}$ (6, 48)
	Brachionus budapestinensis	3.3$^{\mathrm{NS}}$ (1, 8)	1.9** (8, 64)	2.5* (8, 64)
7(DB)	*Bosmina liederi*	0.2$^{\mathrm{NS}}$ (1, 8)	15.2** (6, 48)	0.9$^{\mathrm{NS}}$ (6, 48)
8(DB)	*Brachionus calyciflorus*	2.6$^{\mathrm{NS}}$ (1, 9)	56.0** (3, 27)	1.5$^{\mathrm{NS}}$ (3, 27)
	Bosmina sp.	0.7$^{\mathrm{NS}}$ (1, 5)	0.5$^{\mathrm{NS}}$ (20, 100)	0.7$^{\mathrm{NS}}$ (20, 100)
	Calanoid copepod	0.1$^{\mathrm{NS}}$ (1, 7)	9.8** (7, 49)	0.2$^{\mathrm{NS}}$ (7, 49)
9(DB)	*Brachionus calyciflorus*	15.6* (1, 8)	1.4** (9, 72)	11.4** (9, 72)
	All trials combined	0.4$^{\mathrm{NS}}$ (1, 97)	91.5** (20, 1,940)	0.3$^{\mathrm{NS}}$ (20, 1,940)

histories likely result in egg banks of different age, species composition, and physiological condition. Consequently, these tanks would present different risk profiles to recipient systems like the Great Lakes (Hallegraeff 1998; Hamer et al. 2000). Viitasalo and Katajisto (1994) reported that many factors affect the viability of resting stages, such as energy reserves of the eggs, genotype, environmental conditions during egg formation, parental condition, and sediment conditions such as anoxia due to burial. Future research examining the effect of variable salinity conditions on the viability of diapausing eggs with identical histories will be of particular management importance. The salinity tolerances of the taxa involved in this study are previously unexplored. Our limited data suggest that exposure to high-salinity water may reduce viability of diapausing eggs of freshwater species and potentially reduce the risk posed by NOBOB vessels to the Great Lakes, as the highest hatch rates were observed only for eggs recovered from tanks with relatively low residual ballast water salinity. Yet even at high salinities, a significant fraction of eggs often did hatch. If diapausing eggs of freshwater invertebrates are indeed capable of surviving drastic and abrupt changes in salinity, it could have vast implications for the long-term persistence of these taxa in environments experiencing temporary salinity increases, such as tidal estuaries and in water bodies subject to evaporation. Finally, the widespread presence of viable diapausing eggs in residual ballast sediments, produced by a diverse group of invertebrate taxa, indicates that commercial NOBOB vessels have the potential to vector NIS to the Great Lakes.

References

BILTON, D. T., J. R. FREELAND, AND B. OKAMURA. 2001. Dispersal in freshwater invertebrates. Annu. Rev. Ecol. Syst. **32:** 159–181.

BURGESS, R. 2001. An improved protocol for separating meiofauna from sediments using colloidal silica sols. Mar. Ecol. Prog. Ser. **214:** 161–165.

CÁCERES, C. E., AND D. A. SOLUK. 2002. Blowing in the wind: A field test of overland dispersal and colonization by aquatic invertebrates. Oecologia **131:** 402–408.

COLAUTTI, R. I., A. J. NIIMI, C. D. A. VAN OVERDIJK, E. L. MILLS, K. HOLECK, AND H. J. MACISAAC. In press. Spatial and temporal analysis of transoceanic shipping vectors to the Great Lakes. *In* G. M. Ruiz, J. T. Carlton, and R. N. Mack [eds.], Invasion pathways: Analysis of invasion patterns and pathway management. Island Press.

DE STASIO, B. T., JR. 1989. The seed bank of a freshwater crustacean: Copepodology for the plant ecologist. Ecology **70:** 1377–1389.

DODSON, S. I., AND D. G. FREY. 1991. Cladocera and other Branchiopoda, p. 723–786. *In* J. H. Thorp and A. P. Covich [eds.], Ecology and classification of North American freshwater invertebrates. Academic.

DRAKE, L. A., K. CHOI, G. M. RUIZ, AND F. C. DOBBS. 2001. Global redistribution of bacterioplankton and virioplankton communities. Biol. Invasions **3:** 193–199.

DUMONT, H. J. 1983. Biogeography of rotifers. Hydrobiologia **104:** 19–30.

EDMONDSON, W. T. 1966. Freshwater biology. Wiley.

FIGUEROLA, J., AND A. J. GREEN. 2002. Dispersal of aquatic organisms by waterbirds: A review of past research and priorities for future studies. Freshw. Biol. **47:** 483–494.

GRICE, G. D., AND N. H. MARCUS. 1981. Dormant eggs of marine copepods. Oceanogr. Mar. Biol. Annu. Rev. **19:** 125–140.

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

———, L. J. PERRY, A. J. BOHONAK, M. Q. FELLOWS, C. M. KEARNS, AND D. R. ENGSTROM. 1999. Population biology of a failed invasion: Paleolimnology of *Daphnia exilis* in upstate New York. Limnol. Oceanogr. **44:** 447–486.

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

HALLEGRAEFF, G. M. 1998. Transport of toxic dinoflagellate cysts via ships' ballast water: Bioeconomic risk assessment and efficacy of possible ballast water management strategies. Mar. Ecol. Prog. Ser. **168:** 297–309.

———, AND G. C. BOLCH. 1991. Transport of toxic dinoflagellate cysts via ships' ballast water. Mar. Pollut. Bull. **22:** 27–30.

HAMER, J. P., T. A. MCCOLLIN, AND I. A. N. LUCAS. 2000. Dinoflagellate cysts in ballast tank sediments: Between tank variability. Mar. Pollut. Bull. **40:** 731–733.

HEBERT, P. D. N. 1978. The population biology of *Daphnia* (Crustacea, Daphnidae). Biol. Rev. **53:** 387–426.

———. 1995. The *Daphnia* of North America: An illustrated fauna. Version 1. Department of Zoology, University of Guelph, Ontario, Canada.

———, AND T. J. CREASE. 1980. Clonal existence in *Daphnia pulex* (Leydig): Another planktonic paradox. Science **207:** 1363–1365.

———, AND M. E. A. CRISTESCU. 2002. Genetic perspectives on invasions: The case of the Cladocera. Can. J. Fish. Aquat. Sci. **59:** 1229–1234.

JENKINS, D. G., AND A. L. BUIKEMA, JR. 1998. Do similar communities develop in similar sites? A test with zooplankton structure and function. Ecol. Monogr. **68:** 421–443.

———, AND M. O. UNDERWOOD. 1998. Zooplankton may not disperse readily in wind, rain, or waterfowl. Hydrobiologia **387/388:** 15–21.

KELLY, J. M. 1993. Ballast water and sediments as mechanisms for unwanted species introductions into Washington state. J. Shellfish Res. **12:** 405–410.

KOSTE, W., AND R. J. SHIEL. 1989. Rotifera from Australian inland waters, 3. Euchlanidae, Mytilinidae, and Trichotriidae (Rotifera: Monogononta). Trans. R. Soc. S. Aust. **113:** 85–114.

LOCKE, A., D. M. REID, H. C. VAN LEEUWEN, W. G. SPRULES, AND J. T. CARLTON. 1993. Ballast water exchange as a means of controlling dispersal of freshwater organisms by ships. Can. J. Fish. Aquat. Sci. **50:** 2086–2093.

LUTZ, R. V., N. H. MARCUS, AND J. P. CHANTON. 1992. Effects of low oxygen concentrations on the hatching and viability of eggs of marine calanoid copepods. Mar. Biol. **114:** 241–247.

MACISAAC, H. J., T. C. ROBBINS, AND M. A. LEWIS. 2002. Modeling ships' ballast water as invasion threats to the Great Lakes. Can. J. Fish. Aquat. Sci. **59:** 1245–1256.

MADHUPRATAP, M., S. NEHRING, AND J. LENZ. 1996. Resting eggs of zooplankton (Copepoda and Cladocera) from the Kiel Bay and adjacent waters (southwestern Baltic). Mar. Biol. **125:** 77–87.

MARCUS, N. H. 1996. Ecological and evolutionary significance of resting eggs in marine copepods: Past, present, and future studies. Hydrobiologia **320:** 141–152.

MAY, L. 1987. Effect of incubation temperature on the hatching of rotifer resting eggs collected from sediments. Hydrobiologia **147:** 335–338.

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.

PARKER, B. R., F. M. WILHELM, AND D. W. SCHINDLER. 1996. Recovery of *Hesperodiaptomus articus* populations from diapausing eggs following elimination by stocked salmonids. Can. J. Zool. **74:** 1292–1297.

RICCIARDI, A. 2001. Facilitative interactions among aquatic invaders: Is an ''invasional meltdown'' occurring in the Great Lakes? Can. J. Fish. Aquat. Sci. **58:** 2513–2525.

———, AND H. J. MACISAAC. 2000. Recent mass invasion of the North American Great Lakes by Ponto-Caspian species. Trends Ecol. Evol. **15:** 62–65.

RUIZ, G. M., P. W. FOFONOFF, J. T. CARLTON, M. J. WONHAM, AND A. H. HINES. 2000. Invasion of coastal marine communities in North America: Apparent patterns, processes, and biases. Annu. Rev. Ecol. Syst. **31:** 481–531.

SCHWARTZ, S. S., AND P. D. HEBERT. 1987. Methods for the activation of the resting eggs of *Daphnia.* Freshw. Biol. **17:** 373–379.

SHURIN, J. B. 2000. Dispersal limitation, invasion resistance, and the structure of pond zooplankton communities. Ecology **81:** 3074–3086.

STEMBERGER, R. S. 1979. A guide to rotifers of the Laurentian Great Lakes. Report no. EPA-600/4-79-021. Environmental Monitoring and Support Laboratory, Office of Research and Development. U. S. Environmental Protection Agency.

STROSS, R. G. 1966. Light and temperature requirements for diapause development and release in *Daphnia.* Ecology **47:** 368–374.

UNITED STATES COAST GUARD. 1993. Ballast water management for vessels entering the Great Lakes. Code of Federal Regulations 33-CFR Part 151.1510.

VIITASALO, M., AND T. KATAJISTO. 1994. Mesozooplankton resting eggs in the Baltic Sea: Identification and vertical distribution in laminated and mixed sediments. Mar. Biol. **120:** 455–465.

WALLACE, R. L., AND T. W. SNELL. 1991. Rotifera, p. 187–248. *In* J. H. Thorp and A. P. Covich [eds.], Ecology and classification of North American freshwater invertebrates. Academic.

WILLIAMS, R. J., F. B. GRIFFITHS, E. J. VAN DER WAL, AND J. KELLY. 1988. Cargo vessel ballast water as a vector for the transport of nonindigenous marine species. Estuar. Coast. Shelf Sci. **26:** 409–420.

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