

## Viability of invertebrate diapausing eggs exposed to saltwater: implications for Great Lakes' ship ballast management

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### Abstract

International shipping has been the dominant vector of nonindigenous species introductions to the Laurentian Great Lakes over the past century. Apparent ballast-mediated invasions have been recorded in recent years, despite the implementation of voluntary ballast water exchange regulations in 1989. Since unregulated 'no-ballast-on-board' vessels currently dominate inbound traffic to the Great Lakes, it has been proposed that live or dormant organisms contained in residual ballast of these vessels may be partially responsible for recent invasions. Alternatively, euryhaline species may pose a significant invasion threat because they can potentially survive ballast exchange. In this study, we explored whether exposure to open-ocean water (32‰) reduced the viability of invertebrate diapausing eggs in ballast sediments. Sediments collected from three transoceanic ships and from three freshwater habitats were exposed to open-ocean seawater. Egg viability, assessed as the abundance of taxa hatched between exposed and unexposed sediments, was not affected by saltwater exposure in any experiment. Species richness of hatched diapausing eggs was reduced by saltwater exposure in only one of seven trials. Our results indicate that oligostenohaline zooplankton may pose an invasion risk because their diapausing eggs are largely resistant to exposure to open-ocean saltwater.

### Introduction

The dominant vector for introduction of nonindigenous species (NIS) to the Laurentian Great Lakes during the latter half of the 20th century has been discharge of ballast water from transoceanic ships (Mills et al. 1993; Ricciardi and MacIsaac 2000; Ricciardi 2001). Recognition of this problem resulted in the enactment of voluntary ballast water exchange (BWE) guidelines in 1989, and mandatory regulations in 1993, for vessels entering the system with fresh or brackish ballast water. This legislation effectively requires replacement of existing fresh or brackish ballast

water with open-ocean water if the ballast will be discharged into the Great Lakes (Locke et al. 1991, 1993; United States Coast Guard 1993). BWE reduces the threat of species introductions by either flushing potential invaders from ballast tanks or by killing them with exposure to highly saline seawater (Locke et al. 1991, 1993). BWE regulations only apply to ships that declare 'ballast-on-board' (BOB) status upon entry to the lakes (United States Coast Guard 1993). Ships that declare 'no-ballast-on-board' (NOBOB) status are considered to have empty ballast tanks, and are therefore exempt from BWE regulations. However, these vessels contain an average of

60 tonnes of un pumpable residual water and sediment, the latter of which may contain up to tens of millions of viable invertebrate diapausing eggs per tonne (Bailey et al. 2003). These vessels represent a potential vector for NIS introduction because they comprise more than 90% of inbound traffic to the Great Lakes (Colautti et al. 2003). Since approximately 55% of these vessels will load and discharge ballast water while operating on the Great Lakes (Colautti et al. 2003), invertebrates present as diapausing eggs may have the opportunity to hatch in ballast tanks and subsequently invade the Great Lakes when ballast water is discharged into the system. Alternatively, these resting stages may be released with ballast water if resuspension occurs during deballasting activities.

MacIsaac et al. (2002) suggested that NOBOB vessels collectively constitute a greater risk for NIS introduction to the Great Lakes than do vessels containing tanks filled with seawater. To reduce the importance of this vector, various treatment options require assessment. Diapausing eggs contained within ballast sediments may pose a particularly difficult life stage to eliminate owing to their inherent tolerance of a wide array of adverse environmental conditions, including desiccation, freezing and anoxia (Gilbert 1974; Marcus and Lutz 1998). However, effects of saltwater exposure on the viability of diapausing eggs produced by freshwater species are little known. Bailey et al. (2004) found that the viability of diapausing eggs of two cladocerans (*Bosmina liederi*, *Daphnia longiremis*) and one rotifer (*Brachionus calyciflorus*) was reduced following saltwater exposure; however, their results were not truly indicative of the effectiveness of BWE as the exposure regimes used only a limited number of species, and were conducted on eggs removed from sediments.

In this study we examine whether the viability and/or taxonomic richness of diapausing invertebrate eggs found in sediments from ballast tanks are affected by exposure to open-ocean water (32‰). If exposure to saline water markedly reduces egg viability or the resulting species richness of hatched organisms, then ballasting a small lens of saltwater into NOBOB ships (i.e. partial BWE) could reduce the risk of future introductions. We also consider viability responses of

diapausing eggs in sediments collected from natural freshwater habitats because these taxa presumably have a history of exposure to freshwater conditions only, and would, therefore, be comparatively naïve to saline conditions.

## Materials and methods

### Sample collection

Sediments from ballast tanks were obtained from transoceanic ships entering the Great Lakes between November 2001 and June 2002. Sediment was collected from at least five areas within each tank using sterile scoops and spatulas (see Bailey et al. 2003). Sediments from four ballast tanks (three vessels), containing a high density of diapausing invertebrate eggs, were selected for experimentation. A 200 g subsample was removed from each sediment upon return to the laboratory to measure pore water salinity; salinity of the supernatant was measured using an optical refractometer after centrifugation at  $3300 \times g$  for 15 min. Open-ocean water was collected from a loaded ballast tank of a vessel transiting the Great Lakes and stored in carboys at 4 °C for later use in the salinity exposure experiments. This water was vacuum filtered through a 2.5- $\mu\text{m}$  paper filter (Whatman #5) and adjusted to a salinity of 32‰ by addition of sterile synthetic pond water (Hebert and Crease 1980).

Due to the unknown origin of the ballast sediments, and the possibility of previous exposure to saline water, samples were also collected between June and December 2002 from natural freshwater habitats (Table 1). The use of these sediments provided a greater variety of organisms than that collected from ballast tanks, and ensured that a lack of any effects observed after saltwater exposure for eggs from ballast sediments was not the result of past salinity exposure (i.e. less tolerant diapausing eggs from ballast sediments may have already been extirpated due to past ballasting history). Sample sites from natural habitats were selected so as to obtain a high richness and abundance of diapausing invertebrate eggs. Freshwater sediments were collected using a 15 cm  $\times$  15 cm ponar grab and emptied into a bucket for transport and storage. Pore

Table 1. Sample site location and pore water salinity of sediments collected for exposure experiments.

Experiment	Sample site	Location	Pore salinity (‰)
1 NS	River Canard, Amherstburg, ON	42°10'07" N 83°05'52" W	0
2 NS	Malden Park Pond, Windsor, ON	42°16'36" N 83°03'41" W	0
3 NS	Western Lake Erie, Colchester, ON	41°58'57" N 82°55'56" W	0
4 BT	Ballast tank 1	Hamilton Harbour, ON, CA	4
5 BT	Ballast tank 2	Toronto Harbour, ON, CA	10
6 BT	Ballast tank 3	Hamilton Harbour, ON, CA	20
7 BT	Ballast tank 4	Cleveland Harbour, OH, USA	2

NS – natural sediment; BT – ballast tank.

water salinities from freshwater habitats were measured from small (10 g) sediment samples, using the same methods as for the ballast sediments.

#### *Ballast water exchange experiments*

Sediments were stored in plastic containers in the dark at 4 °C for at least 3 weeks to allow the diapausing eggs to experience a refractory period prior to experimentation (see Grice and Marcus 1981; Schwartz and Hebert 1987). After removal from storage, sediments were thoroughly mixed using a sterile spatula. For five of the seven experiments, 40 g sub-samples were distributed into 16 500 ml glass vessels. Four replicates were immediately removed and preserved using 95% ethanol for evaluation of initial diapausing egg densities. The remainder were randomly divided between two treatments: saltwater or freshwater. Two replicates were then randomly selected from each treatment and autoclaved to serve as controls, leaving four experimental replicates per treatment. The absence of organisms in autoclaved controls ensured that taxa observed in experimental replicates originated from sediments and not by some other mechanism. Filtered ocean water (150 ml, 32‰) was added to autoclaved controls and replicates of the saltwater trials, while 150 ml of sterile synthetic pond water (0‰; Hebert and Crease 1980) was added to autoclaved controls and replicates of the freshwater trials. To increase statistical power, in experiments 3 (Lake Erie) and 6 (ballast tank 3) the number of experimental replicates per treatment

was increased to 12 (although one vessel was broken in the saltwater treatment of experiment 3). The experimental and control vessels were then stored in the dark at 4 °C for 10 days, providing a salinity exposure time equivalent to the maximum length of a typical trans-Atlantic voyage (e.g., from the Baltic Sea to the Great Lakes). Because Bailey et al. (2003) observed hatching of zooplankton from ballast tank sediments at 20 °C in the absence of light, we selected a low temperature (4 °C) to prevent organisms from hatching in the freshwater treatment during the exposure period.

To simulate the exposure of sediments to Great Lakes' water, water from all replicates was carefully decanted on day 10 and replaced with synthetic pond water. This replacement procedure was repeated to ensure comprehensive exchange of pre-existing water in the vessels. All decanted media was examined to ensure that there were no live animals in any replicates at the onset of incubation. Vessels were subsequently placed in an environmental chamber at 20 °C with a 16 : 8 light : dark cycle to induce hatching. Organisms were removed from replicates every 48 h by carefully decanting water from each vessel through a 40-µm mesh sieve. Decanted water was replaced in each vessel, and the vessel returned to the environmental chamber. All vessels were examined for 10–20 days, with the experiment terminated when no hatching was observed on any day after the first 10 days. Organisms were enumerated under a dissecting microscope and were identified primarily using the taxonomic keys of Stemberger (1979) and Balcer et al. (1984).

For evaluation of initial diapausing egg densities, replicates were washed through a 45- $\mu\text{m}$  sieve to remove fine sediment. Diapausing eggs were subsequently separated from the remaining sediment using a Ludox<sup>®</sup> HS-40 protocol (Burgess 2001) and enumerated under a dissecting microscope at  $\sim 32\times$  magnification.

Variation in the total abundance and species richness of organisms hatched between freshwater and saltwater treatments was analyzed using two-sample *t*-tests. In experiments where more than one species hatched, *t*-tests were performed on both total abundance and abundance of the numerically dominant species. *P*-values were adjusted using the Dunn–Sidak formula to correct for multiple comparisons. Nested ANOVAs were used to examine variation in the total abundance and species richness of organisms hatched between treatments within sediment types (of natural or ballast origin). Only data for truly planktonic animals were considered in statistical comparisons of abundance, since benthic animals could attach to the substrate or to the vessel during decanting, and subsequently reproduce. However, parthenogenetic eggs were observed attached to *Brachionus angularis* in all replicates of experiment 2. As the appearance of such eggs was not observed in other experiments, we do not believe this was a result of our decanting procedure. Instead, this may be a population adapted for fast development at this temperature, although we do not believe there was sufficient time for these eggs to hatch within this time per-

iod. Both planktonic and benthic species were included in analyses of species richness. Moreover, because the objective of the experiment was to observe the effects of salinity exposure on diapausing eggs, only invertebrate taxa known to produce such eggs were included in analyses (e.g., monogonont rotifers, cladocerans and copepod nauplii). All statistical analyses were performed using Systat 7.0 (SPSS 1997).

## Results

Diapausing egg densities for natural lake/pond sediments were significantly lower than for ballast tank sediments (*t*-test,  $P = 0.007$ ), ranging from 91.5 to 184 eggs/40 g and from 143 to 282 eggs/40 g, respectively (see Table 2). Rotifer eggs dominated both sediment types, although the sediments from western Lake Erie also contained a high density of copepod eggs. Cladoceran eggs were a minor component of the egg bank for all samples, but especially for ballast sediments.

Natural sediments exhibited significantly higher abundances hatched than ballast sediments (ANOVA,  $P = 0.000$ ). There were no significant differences in total abundances of organisms hatched between treated and untreated sediments within any of the experiments (Table 3). Rotifers were the dominant taxon in all experiments: *Brachionus calyciflorus* in experiments 1, 4, 5, 6, and 7, *Brachionus angularis* in experiment 2, and *Synchaeta kitina* in experiment 3. No significant differences were detected when comparing the

Table 2. Mean diapause egg density per 40 g replicate by taxon.

Egg type	Experiment						
	1 NS	2 NS	3 NS	4 BT	5 BT	6 BT	7 BT
<i>Asplanchna</i>	–	0.5	1.0	1.5	0.8	–	3.3
<i>Brachionus</i>	45.0	9.5	6.5	252.3	147.0	162.0	98.3
<i>Filinia</i>	–	–	0.3	0.5	1.0	–	–
<i>Lecane</i>	–	36.5	–	–	–	–	–
<i>Synchaeta</i>	–	–	80.5	–	–	–	–
Unidentified Rotifera	16.8	23.3	14.0	18.8	9.3	1.3	33.5
<i>Bosmina</i>	–	1.8	1.0	1.0	–	–	2.5
Chydoridae	–	21	–	0.3	–	–	–
<i>Daphnia</i>	1.0	0.5	4.8	–	0.3	1.8	2.3
<i>Moina</i>	11.3	–	–	–	–	–	–
Unidentified Cladocera	12.0	15.5	9.3	1.5	–	5.3	1.8
Copepoda	5.5	9.3	66.8	6.3	–	–	1.5
Total	91.5	117.8	184.0	282.0	157.5	170.3	143.0

NS – natural sediment; BT – ballast tank.

Table 3. Mean values with standard deviations (SD) for the total number of organisms hatched, the abundance of the dominant species only, and the number of species per replicate in freshwater and saltwater treatments.

Experiment	Data category	Freshwater		Saltwater		P-value
		n	Mean (SD)	n	Mean (SD)	
1 NS	Total abundance	4	5.50 (3.10)	4	1.00 (1.41)	0.112
	<i>Brachionus calyciflorus</i>	4	2.75 (0.95)	4	1.00 (1.41)	0.236
	Species richness	4	2.00 (1.41)	4	0.75 (0.95)	0.496
2 NS	Total abundance	4	21.75 (4.92)	4	24.00 (18.23)	0.994
	<i>Brachionus angularis</i>	4	17.25 (3.30)	4	23.75 (19.08)	0.894
	Species richness	4	3.00 (0.81)	4	1.25 (0.50)	0.014
3 NS	Total abundance	12	73.33 (30.17)	11	82.72 (15.89)	0.990
	<i>Synchaeta kitina</i>	12	38.08 (18.67)	11	36.45 (7.40)	0.990
	Species richness	12	10.25 (3.51)	11	9.45 (2.73)	0.911
4 BT <sup>a</sup>	Total abundance	4	9.75 (3.40)	4	6.50 (1.29)	0.124
5 BT <sup>a</sup>	Total abundance	4	3.50 (1.73)	4	2.50 (1.91)	0.468
6 BT <sup>a</sup>	Total abundance	12	1.41 (1.18)	12	1.30 (1.30)	0.175
7 BT	Total abundance	4	4.75 (1.50)	4	4.75 (4.50)	1.000
	<i>B. calyciflorus</i>	4	4.75 (1.50)	4	3.75 (4.19)	0.966
	Species richness	4	1.25 (0.50)	4	1.75 (0.95)	0.773

<sup>a</sup> Indicates experiments from which only one species hatched.

P-values obtained from *t*-tests. NS – natural sediment; BT – ballast tank.

abundance of dominant species hatched between freshwater and saltwater-exposed treatments for any of the seven experiments (Table 3).

A combined total of 45 rotifer and cladoceran species emerged from sediments during this study (Table 4). In addition, copepod nauplii hatched from western Lake Erie sediment may represent numerous species, but because they could not be cultured to an identifiable stage, this is difficult to confirm. Significantly more species emerged from natural samples than from ballast tank samples (ANOVA,  $P = 0.000$ ; see Table 4), although this pattern is largely a result of the western Lake Erie sediments (experiment 3). Only one species, *Brachionus calyciflorus*, hatched from ballast sediments in experiments 4, 5 and 6; while three rotifer species and *Daphnia* were hatched from ballast sediment in experiment 7.

Within experiments, a significant difference in species richness between treatments was detected for only one of the seven experiments, with a lower number of species present in the saltwater-exposed treatment (experiment 2,  $P = 0.014$ ; Table 3). In this experiment, four of six rotifer species (*Brachionus havanaensis*, *Epiphanes brachionus*, *Lophocharis salpina* and *Synchaeta tremula*) emerged only from freshwater replicates (Table 4). Overall, 38 species were collected from

freshwater trials, 16 of which emerged exclusively from freshwater treatments. In contrast, only 30 species were collected from sediments exposed to saltwater, eight of which emerged exclusively from the treatments exposed to saltwater. While the appearance of a species in a single replicate may represent a random occurrence driven by low egg density, there were species that appeared to be particularly sensitive to saltwater exposure. For example, in one experiment the rotifers *Euchlanis dilatata* and *E. meneta* were found in 11 and 5 freshwater replicates, respectively, but neither were found in any replicates exposed to saltwater.

## Discussion

Exposing zooplankton diapausing eggs to saline water (32‰) in our study did not reduce total abundances or consistently affect the species richness of hatched invertebrates. Several authors have speculated that euryhaline species pose the principal invasion threat to the Great Lakes because they could potentially survive ballast exchange (e.g., Ricciardi and Rasmussen 1998; Ricciardi and MacIsaac 2000). In fact, the majority of NIS recorded in the Great Lakes since 1959 possess some degree of salinity tolerance (Ricciardi and MacIsaac 2000; Grigorovich et al.

Table 4. List of species that emerged during salinity exposure experiments. Numbers in the FW (freshwater) and SW (saltwater) columns indicate the number of replicates in which each species was found.

Experiment	Group	Species	Treatment		Habitat
			FW	SW	
1 NS	Rotifera	<i>Brachionus angularis</i>	1/4	1/4	P
		<i>Brachionus bidentatus</i>	1/4	0/4	P
		<i>Brachionus calyciflorus</i>	4/4	2/4	P
		<i>Synchaeta oblonga</i>	1/4	0/4	P
		<i>Synchaeta pectinata</i>	1/4	0/4	P
2 NS	Rotifera	<i>Brachionus angularis</i>	4/4	4/4	P
		<i>Brachionus calyciflorus</i>	2/4	0/4	P
		<i>Brachionus havanaensis</i>	1/4	0/4	P
		<i>Epiphanes brachionus</i>	1/4	0/4	P
		<i>Lophocharis salpina</i>	1/4	0/4	B
		<i>Synchaeta tremula</i>	3/4	1/4	P
3 NS	Cladocera	<i>Acantholeberis curvirostris</i>	1/12	0/11	B
		<i>Diaphanosoma</i> sp.	8/12	7/11	P
		<i>Leptodora kindtii</i>	1/12	0/11	P
	Rotifera	<i>Brachionus angularis</i>	10/12	8/11	P
		<i>Brachionus calyciflorus</i>	4/12	3/11	P
		<i>Brachionus quadridentatus</i>	3/12	7/11	B
		<i>Cephalodella auriculata</i>	1/12	0/11	B
		<i>Cephalodella catellina</i>	1/12	0/11	B
		<i>Cephalodella gibba</i>	2/12	5/11	B
		<i>Cephalodella intuta</i>	0/12	1/11	B
		<i>Cephalodella megalcephala</i>	0/12	1/11	B
		<i>Cephalodella misgurnus</i>	3/12	0/11	B
		<i>Cephalodella obvia</i>	9/12	7/11	B
		<i>Colurella obtusa</i>	3/12	4/11	B
		<i>Dicranophorus grandis</i>	1/12	0/11	B
		<i>Encentrum saundersiae</i>	3/12	5/11	B
		<i>Euchlanis dilatata</i>	11/12	0/11	B
		<i>Euchlanis meneta</i>	5/12	0/11	B
		<i>Floscularia cornuta cornuta</i>	1/12	0/11	P
		<i>Filinia terminalis</i>	1/12	1/11	P
		<i>Horaella</i> sp.	0/12	1/11	B
		<i>Lacinularia</i> sp.	0/12	1/11	B
		<i>Lecane lunaris</i>	0/12	1/11	B
		<i>Lepadella rhomboides</i>	10/12	10/11	B
		<i>Lophocharis salpina</i>	1/12	2/11	B
		<i>Macrochaetus subquadratus</i>	1/12	0/11	B
		<i>Polyarthra major</i>	0/12	1/11	P
		<i>Ploesoma truncatum</i>	9/12	8/11	P
		<i>Synchaeta kitina</i>	12/12	11/11	P
		<i>Synchaeta lakowitziana</i>	4/12	7/11	P
		<i>Synchaeta stylata</i>	9/12	7/11	P
		<i>Trichocerca bicristata</i>	0/12	1/11	B
		<i>Trichocerca insignis</i>	1/12	2/11	B
		<i>Trichocerca multirinis</i>	2/12	1/11	B
		<i>Trichocerca pusilla</i>	1/12	1/11	B
<i>Trichocerca similis</i>	1/12	0/11	B		
<i>Trichocerca tigris</i>	1/12	1/11	B		
<i>Trichotria tetractis</i>	3/12	0/11	B		
Copepoda		Unidentified nauplii	12/12	11/11	P
4 BT	Rotifera	<i>Brachionus calyciflorus</i>	4/4	4/4	P
5 BT	Rotifera	<i>Brachionus calyciflorus</i>	4/4	4/4	P
6 BT	Rotifera	<i>Brachionus calyciflorus</i>	12/12	12/12	P

Table 4. Continued.

Experiment	Group	Species	Treatment		Habitat
			FW	SW	
7 BT	Cladocera	<i>Daphnia</i> sp.	0/4	2/4	P
	Rotifera	<i>Brachionus angularis</i>	1/4	0/4	P
		<i>Brachionus calyciflorus</i>	4/4	4/4	P
		<i>Synchaeta oblonga</i>	0/4	1/4	P

P – planktonic; B – benthic; NS – natural sediment; BT – ballast tank.

2003). However, our findings suggest that oligostenohaline zooplankton may also pose an invasion risk because their diapausing eggs are largely resistant to exposure to open-ocean water. Although the adult forms of these taxa may perish if exposed to saltwater, we suggest that ballasting a lens of saline water into the ballast tanks of NOBOB vessels (i.e. partial BWE) might not provide effective protection against diapausing eggs.

Freshwater rotifers were the most abundant taxa to emerge during this study, hatching in both freshwater and saltwater treatments. Rotifers are not typically considered euryhaline, with less than 10% of known species found in saline environments despite adequate dispersal opportunities (see Hammer 1986; Halse et al. 1998). In our study, saltwater exposure generally did not affect the viability of diapausing rotifer eggs, despite the low salinity tolerance of the adult stages of most species. This finding indicates that rotifer diapausing eggs could survive BWE and remain viable when they enter the Great Lakes system. However, nonindigenous rotifers have not previously been recognized in the Great Lakes (e.g., Mills et al. 1993). In this study, a solitary individual from the genus *Horaella* was hatched from sediments collected from Lake Erie. This genus has not been recorded in the Great Lakes, or more broadly in the United States or Canada (Nogrady and Segers 2002). Although we cannot be sure that this taxon is established in the Great Lakes, the recognition of a nonindigenous rotifer indicates that introductions may have already occurred, and will continue to do so. NIS of rotifers have recently been recognized in Southeast Asia (Segers 2001), New Zealand (Duggan et al. 2002a) and elsewhere (see Segers 1996), thus the Great Lakes should seemingly be vulnerable to invasions by this group.

Copepod nauplii and three cladoceran taxa were hatched from Lake Erie sediments, of which both nauplii and *Diaphanosoma* sp. frequently emerged from the sediments exposed to saltwater. A *Daphnia* species was also hatched from one ballast tank sediment after exposure to saltwater. These results indicate that crustacean diapausing eggs also can remain viable following salinity exposure. Indeed, some of the NIS of zooplankton already recorded in the Great Lakes may have entered via this mechanism (e.g., *Bosmina maritima*, *Bythotrephes longimanus* and *Cercopagis pengoi*).

The apparent resistance of diapausing eggs to saltwater exposure may stem from physical or biological characteristics of the eggs themselves or be the product of protection conferred by the sediments in which they are found. Bailey et al. (2004) noted a significant reduction in hatching of zooplankton diapausing eggs of three species (*Brachionus calyciflorus*, *Bosmina liederi* and *Daphnia longiremis*) exposed to saline water after removal from ballast sediments. Thus, the inability of saltwater to reduce the viability of eggs in this study may have resulted from protection provided by the sediments in which they were contained. Alternatively, this disparity may be due to temperature, as our salinity exposure period occurred at 4 °C, vs 20 °C in the above-mentioned study. Interactions between salinity and temperature have been found to impact metabolic rate, ion uptake, and membrane permeability (Lee and Bell 1999), therefore it is possible that greater effects of salinity will be seen at higher temperatures.

Although this study demonstrates that saltwater exposure does not markedly reduce the viability of diapausing eggs in sediments, there are indications that the exposure had an effect on some species. Species richness was significantly

lower for the sediments exposed to saltwater in experiment 2 (*t*-test,  $P = 0.014$ ). In experiment 3, neither *Euchlanis dilatata* nor *E. meneta* were found to hatch from sediments exposed to saltwater, despite the occurrence of both in many of the freshwater replicates. Overall, there were eight fewer species recorded in saltwater-exposed treatments than in freshwater ones (38 species in freshwater vs 30 in saltwater). In addition, there were 16 species found exclusively in freshwater treatments, but only eight that hatched exclusively from sediments that had been exposed to saltwater.

A comparison of the species that emerged from natural sediments vs ballast sediments indicates that species richness in the latter is generally low, and relatively uniform in species composition, despite higher egg densities recorded from the ballast sediments used. Other studies conducted with similar methodologies have also yielded high numbers of species from natural freshwater sediments (e.g., May 1986; Duggan et al. 2002b) indicating that ballast sediments are somehow depauperate. This may suggest that the eggs of fewer species are initially entering the ballast tanks, or that few species entering the tanks are capable of producing resting eggs *in situ*. Alternatively, some factor (e.g., anoxia, predation) during uptake or transport may selectively reduce egg viability, resulting in lowered species richness. Factors reducing viability during transport could include salinity exposure if, for example, the eggs are subjected over longer time periods than examined here, the eggs are exposed at intermittent periods to waters of different salinities, or if exposure coincided with other factors such as sporadic desiccation. The fact that the ballast sediments in this study hatched a lower abundance of organisms than natural sediments, despite having higher egg density, may be an indication of lowered viability post-uptake. However, there are many other factors, such as age or disparity in source environment, which could explain this observation. Further research detailing the species richness and viability of diapausing eggs in sediments from ballast tanks and source habitats is required to assess the factors that may produce this apparent trend.

The majority of invasions recorded in the Great Lakes since 1959 have been attributed to

transoceanic shipping (Mills et al. 1993; Ricciardi and MacIsaac 2000). NOBOB vessels currently comprise about 90% of shipping traffic entering the system (Colautti et al. 2003), and collectively represent the dominant potential shipping vector for introduction of new NIS (MacIsaac et al. 2002). Therefore, finding treatment options to reduce risk posed by these ships should be a priority. This study suggests that saltwater exposure may not be an effective method to reduce diapausing egg viability. Therefore, although ballasting a shallow lens of saltwater into NOBOB ships may reduce the risk of invasion associated with live organisms present in residual ballast, it may not be fully effective for management of diapausing eggs. Our findings also suggest that vessels in BOB status, fully compliant with BWE regulations, may continue to pose an (as yet undefined) invasion risk if NIS are discharged into the lakes as diapausing eggs. Management practises that reduce accumulation of sediments in ballast tanks offer the best solution for reducing risk associated with diapausing eggs in both BOB and NOBOB vessels.

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