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Efficacy of open-ocean ballast water exchange as a means of preventing invertebrate invasions between freshwater ports

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Abstract

Ballast water is a major vector of nonindigenous species invasion globally. Mandatory ballast water exchange (BWE) was implemented for vessels carrying ballast water into the Great Lakes in 1993. Despite the implementation of this policy, few data are available on its effectiveness, and invasions have continued to be reported in the Great Lakes. In this study, we conducted experiments to assess the efficacy of BWE on six operational transoceanic vessels traveling from the Great Lakes to European ports. Each vessel had paired ballast tanks, one of which was designated as a control that remained filled with Great Lakes water, while the other was exchanged with mid-ocean water. Community composition was assessed immediately after tanks were filled and again prior to water discharge in European ports. BWE was verified by ship records and, in two cases, by in situ water quality sensors. BWE was highly effective (>99% loss) for reducing concentrations of freshwater zooplankton. Live sentinel amphipods and oligochaetes deployed in incubator chambers sustained nearly universal mortality in tanks that experienced BWE, but not in unexchanged tanks. Finally, BWE reduced in situ recruitment of zooplankton from diapausing eggs present in ballast sediments in additional incubator chambers deployed in these tanks. Collectively, these studies support the contention that BWE by transoceanic vessels traveling between freshwater ports results in ballast water that would exceed proposed International Maritime Organization (2004) ballast water performance standards if these standards were applied to freshwater species only. Thus, BWE provides strong protection to freshwater ecosystems against invasions by both pelagic and benthic freshwater species.

The transport and release of ballast water has allowed hundreds of nonindigenous species to establish in freshwater, brackish, and marine ecosystems throughout Europe and North America (Mills et al. 1996; Ruiz et al. 2000; Bij de Vaate et al. 2002). Surveys of ballast tank biota reveal that vessels bound for freshwater ports may harbor live planktonic and benthic animals as well as large numbers of viable diapausing invertebrate eggs in accumulated ballast sediment (Locke et al. 1993; Bailey et al. 2005*a*; Duggan et al. 2005). Live individuals can represent an invasion risk if they are discharged from tanks during deballasting. Diapausing eggs could be resuspended during ballasting operations and then discharged from tanks. Alternatively, if animals hatch in situ they may be introduced when the vessel subsequently deballasts to load cargo (Bailey et al. 2005*b*).

Largely in response to the infamous invasion by zebra mussels (*Dreissena polymorpha*) in the mid-1980s, a voluntary procedure was implemented in 1989 and was subsequently made mandatory in 1993; this procedure effectively requires transoceanic vessels bound for the Laurentian Great Lakes from foreign ports to exchange their ballast with mid-ocean water and to attain a salinity of ≥30 for retained ballast water (United States Coast Guard 1993). Despite this requirement, reports of new

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Table 1. Information on vessels used in this study, including departure and destination ports, vessel type, dates of voyage, and the type of ballast tank studied. Ballast water exchange efficiency was assessed on vessels 1, 2, 4, and 5. The effect of exchange on diapausing eggs was evaluated on all voyages. Vessel type: BC, bulk carrier; CT, chemical tanker. Ballast tank type: S, side; DB, double bottom.

Vessel	Departure port	Destination port	Vessel type	Date of voyage	Ballast tank type
1	Hamilton, Ontario	Cartagena, Spain	BC	01 Oct 04–18 Oct 04	S
2	Hamilton, Ontario	Hamburg, Germany	CT	23 Jul 05–09 Aug 05	S
3	Montreal, Quebec	Rotterdam, Holland	BC	29 Sep 05–11 Oct 05	S
4	Hamilton, Ontario	Hamburg, Germany	CT	05 Dec 05–20 Dec 05	S
5	Hamilton, Ontario	Hamburg, Germany	CT	25 Apr 06–09 May 06	S
6	Hamilton, Ontario	Reyðarfjörður, Iceland	BC	01 Sep 06–14 Sep 06	DB

invasions of invertebrate and other taxa have continued to appear, including, most recently, an invasion of *Hemimysis anomala*, a Ponto–Caspian mysid, in December 2006 (Pothoven et al. 2007). One possible explanation for these continued invasions is that ballast water exchange (BWE) may not be fully effective and may not afford the necessary protection to the Great Lakes.

Several studies have assessed the efficiency of BWE for vessels transiting between marine ports (e.g., Wonham et al. 2001; Choi et al. 2005; Ruiz and Smith 2005). These studies have demonstrated that effectiveness of BWE varies greatly and that live planktonic animals often remain in the tanks following exchange. However, a controlled assessment of mid-ocean BWE has not been performed on a transoceanic vessel operating between freshwater ports. The effectiveness of BWE for these voyages may be expected to be greater than that associated with transit between marine ports, since any animals remaining in tanks after BWE would experience a profound osmotic shock effect due to exposure to high-salinity ocean water. Exposure of freshwater zooplankton and benthos to open ocean water (salinity ~35) should be particularly detrimental since these invertebrates are hyperosmotic regulators whose osmoregulatory mechanisms typically fail above 9 (Hart et al. 1991). Diapausing invertebrate eggs may be more resistant, as laboratory experiments indicate that saltwater exposure at salinities greater than 30 does not significantly decrease their subsequent viability (Gray et al. 2005; Bailey et al. 2006).

Knowledge of the effectiveness of BWE for vessels traveling between freshwater ports is vital for two reasons: (1) It is expected to protect freshwater systems like the Great Lakes from ballast-mediated invasions for the foreseeable future (IMO 2004); and (2) data on the postexchange survivorship of planktonic and benthic animals is required to conduct risk assessments for ballast introductions (MacIsaac et al. 2002; Wonham et al. 2005). In this study we evaluate the efficiency of BWE between freshwater ports using data collected from transoceanic vessels traveling from the Great Lakes to European ports.

Methods

BWE efficiency was assessed on cargo vessels traveling from the Great Lakes to European ports of call (Table 1). We initially sought to conduct experiments on vessels traveling in the opposite direction, since doing so would be most applicable to the Great Lakes, but we were constrained by practical considerations. However, considering that our studies involve purging of ballast tanks and exposure of freshwater species to saline water, our results should apply in either direction, even though the species involved may differ. An important caveat, however, is that many recent invaders to the Great Lakes have been brackish species of Ponto–Caspian origin (Ricciardi 2006). Some authors have speculated that these species are more likely to survive BWE and, therefore, may represent a greater threat compared to species that are restricted to freshwater environments (Ricciardi 2006). If this is the case, our results may be a liberal estimate of BWE efficacy for vessels originating from brackish water ports. However, at least two species that can survive in brackish water were included in our experiments (*Bosmina coregoni* and *Echinogammarus ischnus*). Both of these species were either flushed out of the tank or perished as a result of exposure to open ocean water (*see Results; Tables 2, 3*).

All of the vessels reported herein utilized the empty-refill method of BWE. This is an important consideration, as Choi et al. (2005) demonstrated that empty-refill exchange can result in lower zooplankton abundances compared to continuous flow-through exchange. Therefore, when dealing with vessels that have conducted continuous exchange, our results could overestimate efficiency. We could not find data specifying the percentage of vessels entering the Great Lakes or other freshwater ports that have conducted empty-refill versus continuous exchange. However, the limited data we could gather from marine studies indicate that empty-refill is the most prevalent method of exchange. Of the 49 bulk carriers entering the San Francisco Estuary surveyed by Choi et al. (2005), 31 (63%) and 18 (37%) conducted empty-refill and continuous exchange, respectively. Furthermore, a report by Dragsund et al. (2002) suggested that 75% of vessels entering California and Texas ports report conducting empty-refill ballast exchange.

Paired ballast tanks of identical design were utilized on each voyage, one of which was designated as a control that remained filled with Great Lakes water, while the other was exchanged with salt water at mid-ocean. The treatment tank was randomly selected at the outset of the study and underwent BWE during the transatlantic voyage. Prior to

Table 2. List of zooplankton species recovered from ballast tanks at the beginning of the voyage at a Great Lakes port (T_0) and at the recipient European port (T_1). Control tank is listed first in each pair of characters (e.g., X/X), followed by the exchanged tank (i.e., control/treatment). X, species present; O, species absent.

		Vessel (Great Lakes; T_0)						Vessel (Europe; T_1)					
Group	Species	1	2	3	4	5	6	1	2	3	4	5	6
Cladocera	*Bosmina coregoni*	X/X	X/X	—	X/X	X/X	—	X/O	X/O	O/O	X/O	X/O	X/X
	Bosmina liederi	O/O	O/O	—	X/X	X/X	—	O/O	O/O	O/O	X/O	X/O	O/O
	Bosmina longirostris	X/X	X/X	—	O/O	O/O	—	X/O	X/O	O/O	O/O	O/O	X/O
	Daphnia ambigua	O/O	O/X	—	O/O	O/X	—	O/O	O/O	O/O	O/O	X/O	O/O
	Daphnia mendotae	X/X	X/X	—	X/X	X/O	—	X/O	X/X	O/O	X/O	X/O	X/O
	Daphnia retrocurva	X/X	X/X	—	O/O	O/O	—	X/O	O/O	O/O	O/O	O/O	O/O
	Diaphanosoma birgei	X/X	O/X	—	O/O	O/O	—	X/O	O/O	O/O	O/O	O/O	O/O
	Leptodora kindti	O/O	X/X	—	O/O	O/O	—	O/O	O/O	O/O	O/O	O/O	O/O
	Chydorus sp.	O/O	O/O	—	O/O	O/O	—	O/O	O/O	O/O	O/O	X/O	X/X
	Unidentified Chydoridae	X/X	X/X	—	X/O	X/O	—	X/O	X/O	O/O	O/O	X/O	O/O
Copepoda	*Acanthocyclops robustus*	X/X	X/X	—	O/O	O/O	—	X/O	X/O	O/O	O/O	O/O	O/O
	Diacyclops thomasi	X/X	X/X	—	X/X	X/X	—	X/O	X/O	O/O	X/O	X/X	X/O
	Leptodiaptomus siciloides	O/X	X/X	—	X/X	X/X	—	X/O	X/O	O/O	X/O	X/O	X/O
	Mesocyclops edax	X/X	X/X	—	O/O	O/O	—	X/O	X/O	X/O	O/O	X/O	X/O
	Nauplii	X/X	X/X	—	X/X	X/X	—	X/O	X/O	X/X	X/X	X/X	X/X
	Skistodiaptomus oregonensis	X/X	X/X	—	O/O	O/O	—	X/O	X/O	O/O	O/O	O/O	O/O
	Canthocamptus robertcokeri	O/O	O/O	—	O/O	O/O	—	O/O	O/O	X/O	O/O	O/O	O/O
	Marine cyclopoids	O/O	O/O	—	O/O	O/O	—	O/X	O/X	O/X	O/X	O/X	O/X
Rotifera	*Cephalodella* sp.	O/O	O/O	—	X/O	X/O	—	X/O	O/O	O/O	O/O	O/O	O/O
	Dicranophorus sp.	O/O	X/O	—	O/O	O/O	—	O/O	O/O	O/O	O/O	O/O	O/O
	Filinia longiseta	O/O	X/O	—	O/O	O/O	—	O/O	O/O	O/O	O/O	O/O	O/O
	Kellicottia bostoniensis	X/X	X/O	—	X/X	X/X	—	X/O	X/X	O/O	X/O	X/O	O/O
	Kellicottia longispina	X/X	X/X	—	X/X	X/X	—	X/O	X/O	O/O	X/O	X/O	O/O
	Keratella cochlearis	X/X	X/X	—	X/X	X/X	—	X/O	X/X	X/O	X/O	X/X	X/O
	Keratella crassa	X/O	O/O	—	O/O	O/O	—	O/O	O/O	O/O	O/O	O/O	O/O
	Keratella earlinae	O/X	X/X	—	X/X	X/X	—	O/O	X/O	O/O	X/O	X/O	X/O
	Keratella hiemalis	O/O	O/O	—	O/O	X/X	—	O/O	O/O	O/O	O/O	X/O	O/O
	Keratella quadrata	X/X	X/X	—	X/X	X/X	—	X/O	X/O	O/O	X/O	X/O	O/O
	Polyarthra dolichoptera	X/X	X/X	—	O/O	O/O	—	O/O	O/X	X/O	O/O	O/O	O/O
	Polyarthra euryptera	X/X	O/O	—	O/O	O/O	—	O/O	O/O	O/O	O/O	O/O	O/O
	Polyarthra major	X/X	O/O	—	O/O	O/O	—	O/O	O/O	O/O	O/O	O/O	O/O
	Polyarthra remata	O/O	O/O	—	X/X	X/X	—	O/O	O/O	O/O	X/O	X/X	X/O
	Polyarthra vulgaris	O/O	O/O	—	X/X	X/X	—	O/O	O/O	O/O	X/O	X/O	O/O
	Pompholyx sulcata	O/X	X/O	—	X/X	X/X	—	O/O	O/O	X/O	X/O	O/O	X/O
	Synchaeta kitina	O/O	O/O	—	X/X	X/O	—	O/O	O/O	X/O	O/O	O/O	O/O
	Synchaeta sp.	O/O	X/O	—	O/O	O/O	—	O/O	O/O	O/O	O/O	O/O	O/O
	Synchaeta stylata	O/O	O/O	—	X/X	X/X	—	O/O	O/O	O/O	X/O	O/O	O/O
	Trichocerca multicrinis	O/O	X/X	—	O/O	O/O	—	O/O	O/O	O/O	O/O	O/O	O/O
	Trichocerca pusilla	O/O	O/O	—	O/O	O/O	—	O/O	O/O	X/O	O/O	O/O	O/O
	Ascomorpha ecaudis	O/O	O/O	—	O/O	O/O	—	O/O	O/O	X/O	O/O	O/O	O/O
	Brachionus angularis	O/O	O/O	—	O/O	O/O	—	O/O	O/O	O/O	O/O	X/O	O/O
	Cephalodella gibba	O/O	O/O	—	O/O	O/O	—	O/O	O/O	X/O	O/O	O/O	O/O
	Lecane mira	O/O	O/O	—	O/O	O/O	—	O/O	O/O	X/O	O/O	O/O	O/O
	Notholca acuminata	O/O	O/O	—	O/O	O/O	—	O/O	O/O	O/O	O/O	X/O	O/O

ship departure, Great Lakes water from the port of origin was added to fill each tank, as per standard operating procedures. BWE was geo-referenced and was always conducted >320 nautical km from shore in water >200 m in depth using the empty-refill method (Table 1).

In-tank measurements of temperature, dissolved oxygen, and salinity of ballast water were obtained for experiments on vessels 3 and 6 by installing Troll® 9000 multiparameter sondes (In-situ Inc.) equipped with an optical dissolved oxygen sensor. Sensors were secured in the bottom of the tank using a custom-made aluminum mounting tripod and plastic tie-downs. Unfortunately, these instruments were not available for experiments on vessel 1 and could not be operated on chemical tankers as a result of safety concerns (vessels 2, 4, and 5). Ship records were also used to verify occurrence and geographic coordinates of BWE. Postexchange salinity was obtained from the ballast tanks of vessels 1, 2, 4, and 5 using a portable optical refractometer.

To assess BWE exchange efficiency based upon changes in zooplankton density, ballast water in the tanks was sampled prior to the beginning of the voyage from the Great Lakes (T_0) and again upon the ship's arrival at its destination port in Europe (T_1). Three replicate zooplank-

Table 3. Calculated ballast water exchange efficiency based upon density of zooplankton (individuals [ind.] m^{-3}) in matched control and experimental ballast tanks. Copepods and rotifers were found in the exchanged tank at the end of the voyage. Upper 95% confidence limit is provided for all zero measurements, assuming a random (Poisson) horizontal distribution of zooplankton in ballast tanks. SD, standard deviation. Vessel numbers refer to those listed in Table 1.

Vessel	Taxon	Exchange efficiency (%)	Density in exchanged tank at T_0 (ind. $m^{-3} \pm$ SD)	Density in exchanged tank at T_1 (ind. $m^{-3} \pm$ SD or + upper 95% CI*)
1	Copepoda	100.0	4,040.0±645.3	0.0+6.12
	Mesocyclops edax	100.0	2,291.4±483.5	0.0+6.12
	Cladocera	100.0	26,532.9±805.2	0.0+6.12
	Daphnia mendotae	100.0	19,493.2±5,676.7	0.0+6.12
	Rotifera	100.0	15,544.0±1,360.9	0.0+6.12
	Keratella cochlearis	100.0	1,917.3±265.7	0.0+6.12
	All zooplankton	100.0	46,117.0±7,150.5	0.0+6.12
2	Copepoda	100.0	15,533.5±1,731.6	0.0+6.12
	Mesocyclops edax	100.0	10,968.5±638.9	0.0+6.12
	Cladocera	97.8	17,018.6±4,901.6	0.8±0.8
	Daphnia mendotae	95.1	12,474.7±3,544.2	0.8±0.8
	Rotifera	97.9	9,313.2±1,888.4	2.6±1.5
	Keratella cochlearis	99.3	6,358.5±1,778.9	0.8±0.8
	All zooplankton	99.4	41,865.3±6,869.4	3.4±4.0
3	Copepoda	—	—	0.0+5.76
	Mesocyclops edax	—	—	0.0+5.76
	Cladocera	—	—	0.0+5.76
	Rotifera	—	—	0.0+5.76
	Ascomorpha ecaudis	—	—	0.0+5.76
	All zooplankton	—	—	0.0+5.76
4	Copepoda	100.0	3,530.2±560.9	0.0+3.43
	Diacyclops thomasi	100.0	2,957.7±413.0	0.0+3.43
	Cladocera	100.0	6,981.6±995.9	0.0+3.43
	Bosmina coregoni	100.0	6,424.1±941.1	0.0+3.43
	Rotifera	100.0	21,744.8±1,643.2	0.0+3.43
	Synchaeta kitina	100.0	10,703.5±555.6	0.0+3.43
	All zooplankton	100.0	32,256.6±2,603.6	0.0+3.43
5	Copepoda	99.9†	2,313.2±1,106.0	6.3±8.3
	Diacyclops thomasi	99.9†	2,271.4±1,058.3	6.3±8.3
	Cladocera	100.0	211.9±130.1	0.0+3.56
	Bosmina coregoni	100.0	101.7±32.7	0.0+3.56
	Rotifera	99.9†	80,062.9±28,834.6	1.0±1.8
	Polyarthra vulgaris	99.9†	36,675.6±11,633.7	1.0±1.8
	All zooplankton	99.9†	82,588.0±29,668.1	7.3±10.0
6	Copepoda	—	—	0.0+3.72
	Diacyclops thomasi	—	—	0.0+3.72
	Cladocera	—	—	5.6±2.0
	Chydorus sp.	—	—	5.6±2.0
	Rotifera	—	—	0.0+3.72
	Pompholyx sulcata	—	—	0.0+3.72
	All zooplankton	—	—	5.6±2.0

* Included are the exchange efficiencies for copepods, cladocerans, and rotifers, as well as for the most abundant species in each group.

† Copepods and rotifers were found in the exchanged tank at the end of the voyage. However, calculated treatment efficiency was almost 100% due to a large increase in the abundance (reproduction) of animals in the control tank during the voyage.

ton net tows (0.25-m diameter, 30-μm mesh) from each tank were obtained through deck hatch access points, and the animals were preserved in 95% ethanol. To uniformally sample the maximum volume of water (>500 L $tank^{-1}$ in all cases), vertical plankton hauls were drawn from the very bottom of the tanks to the air–water interface. The lengths of zooplankton net tows were recorded so that the volume of water sampled and the density of animals in the tanks could be calculated when samples were returned to the laboratory. Animals were enumerated in the laboratory under a stereomicroscope and were identified with reference to Stemberger (1979), Balcer et al. (1984), and Hudson et al. (1998). Animals were assumed to be alive at the time of sampling if they were recovered from the water column with our plankton nets and appeared to be in good physical condition when examined in the laboratory.

In cases in which no zooplankton were recovered at the end of a voyage, we assumed a random horizontal distribution of animals in the tank to calculate the upper 95% confidence limit, based upon the volume of water

sampled by our vertical net tows. Heterogeneous vertical distribution of zooplankton has been demonstrated in previous studies (e.g., Murphy et al. 2002). However, vertical zooplankton hauls that we performed should have integrated zooplankton throughout the entire water column, minimizing problems associated with vertical stratification (Murphy et al. 2002). As the horizontal distribution of zooplankton has not been demonstrated to deviate from a random distribution (Murphy et al. 2002), we assumed that zooplankton in ballast tanks followed a Poisson distribution. Following this distribution, the upper 95% confidence limit is +3.285 individuals per volume of water sampled (Krebs 1999, p. 24).

We were able to obtain both T_0 and T_1 plankton samples from four of the six vessels used for experiments (Table 1). We were not able to collect T_0 samples from vessel 3 because of draft requirements that prevented the uptake of water in port, while equipment failure prevented the collection of T_0 samples from vessel 6.

We calculated the percent change in zooplankton concentration in each tank as

$$\%r = (T_1/T_0) \times 100 \quad (1)$$

where $\%r$ represents the percent of target taxa remaining in a tank following BWE, T_0 is the initial concentration, and T_1 is the concentration following exchange. Using these values we calculated the exchange efficiency as

$$Ex_{Effic} = ([C_{\%r} - X_{\%r}]/[C_{\%r}]) \times 100 \quad (2)$$

where $X_{\%r}$ is the fraction remaining in the exchanged tank and $C_{\%r}$ is the fraction remaining in the companion control tank. Exchange efficiencies were calculated for copepods, cladocerans, and rotifers on each vessel, as well as for the most abundant species within each group.

The method we used to calculate exchange efficiency assumes that animal abundance was similar in the treatment and control tanks at T_0. To confirm that this was the case, we conducted a nested analysis of variance (ANOVA) (tanks within ships) to test for differences in the total zooplankton density between paired treatment and control tanks at T_0 using data for four ships (vessels 1, 2, 4, and 5). There were no significant differences in total zooplankton abundance between the treatment and control tanks at T_0 (ANOVA; $F_{4,16} = 0.3347$, $p = 0.85$). However, as a result of the small number of replicates available for this analysis ($n = 4$) and the resulting lack of power, the conclusions from this analysis must be interpreted with caution.

Emergence from diapausing eggs— in situ experiments— To assess the effect of BWE on diapausing eggs contained in ballast sediments, we constructed incubation chambers out of polyvinyl-chloride (PVC) piping components (Fig. 1; *see* Bailey et al. 2005*b*). Each chamber was constructed from a 15-cm (inside diameter) pipe cap with a threaded, sealable lid. The chambers were bolted to a rectangular PVC platform, and the bolt holes were sealed with silicone. A total of 12 holes (of 2.5–4-cm diameter) were drilled through the lid (four holes) and approximately half way up the wall (eight holes) of each chamber to allow for the exchange of water between the inside of the chamber and the ballast tank. Sixty-micrometer nitex mesh was affixed to the exterior surface of each chamber body and interior surface of each top to completely cover all holes, and the mesh was secured with PVC cement and 18-cm–diameter hose clamps. The installation of mesh on the exterior rather than the interior of the chambers was performed to reduce contamination from plankton in the ballast water (*see* Bailey et al. 2005*b*). Chambers were submersed in distilled water for 7 d in the lab before use in order to eliminate glue residues.

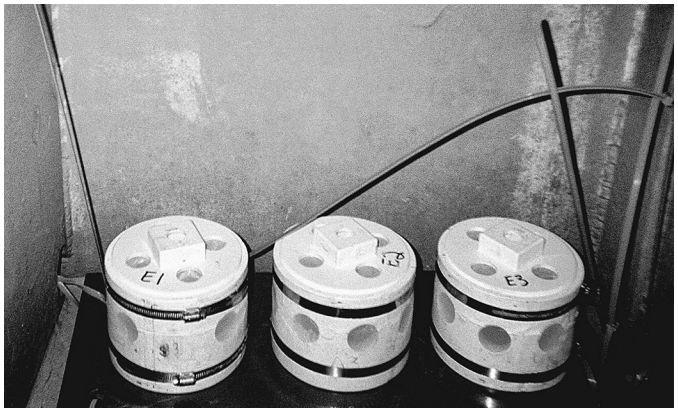

Fig. 1. Polyvinyl-chloride incubation chambers installed in an empty ballast tank. Side and top windows were covered in 60-μm nitex mesh. Inner diameter of chambers is 15 cm.

Two sets of triplicate incubator chambers (*see Fig. 1*) were moored to the bottom of both treatment and control tanks prior to filling of the tanks, and 300 g of previously collected ballast sediment was placed inside each chamber. One of the six chambers received 300 g of autoclaved sediment to serve as a control (i.e., no hatching expected). The presence of animals measuring >60 μm in these control chambers would indicate that contamination from the surrounding ballast water had occurred. Although animals measuring <60 μm were found in the chambers, contamination by larger animals did not occur during experiments. After the sediment had been added to the incubation chambers, the tops were screwed on and the ballast tanks were flooded with Great Lakes water.

Sediment used in the incubation chambers had been collected previously from other transoceanic vessels operating on the Great Lakes. Each experiment used unique sediment collected from separate vessels that entered the Great Lakes between September 2001 and June 2005 (i.e., there were six different sediments). Sediment was stored in a cold room at 4°C. Prior to their use in experiments, sediments were thoroughly mixed using an electric kitchen mixer to ensure that diapausing eggs were homogeneously distributed. The density of eggs in the sediment used for experimentation was doubled to maximize the probability of hatching occurring during the course of the voyage. For each 300-g aliquot of sediment used in a chamber, a 300-g sample from the same sediment had been subjected to a sugar flotation procedure, which isolates but does not

harm eggs (Bailey et al. 2005*b*). The eggs extracted by sugar flotation were then added to the 300-g aliquot to be used in the incubation chambers. The diversity and abundance of diapausing eggs present in the supplemented sediments was characterized prior to their use in experiments using a Ludox® HS40 protocol (Burgess 2001) to isolate them from sediment. Isolated eggs were then enumerated under a stereomicroscope at ~×32 magnification.

At the conclusion of the voyage, hatched animals were collected from the incubation chambers by removing the ~450 mL of water that remained below the drainage holes with a wide-mouth pipette and filtering it through a 30-μm sieve. Retained animals were preserved in 95% ethanol and returned to the lab for enumeration of hatched animals. A paired *t*-test was performed to test for differences in hatching in treatment versus control tanks. The number of hatched individuals from all chambers in a tank was pooled, and each vessel was treated as one replicate for the analysis.

Sentinel benthic invertebrates—To evaluate the effect of BWE on benthic invertebrates, 30 *Echinogammarus ischnus* amphipods and 30 *Brachiura sowerbyi* oligochaetes collected from the Great Lakes were placed with sediments inside one incubation chamber in control and experimental tanks of vessels 4, 5, and 6 at T_0. Incubation chambers used for benthic invertebrates were not used for hatching experiments with diapausing eggs. We considered *E. ischnus* an ideal model species for these experiments since it is euryhaline, is introduced to the Great Lakes, and has a history of transport in ballast (Witt et al. 1997). *B. sowerbyi* were included to test whether salt water would penetrate through residual ballast sediment during exchange and cause mortality of animals below the sediment–water interface. At the conclusion of the voyage, sediment in the live animal chambers was collected and passed sequentially through 4-mm and 1-mm sieves to isolate animals and determine if they survived the voyage.

Results

BWE experiments were conducted on six vessels transiting from North America to Europe between October 2004 and September 2006 (Table 1). Voyages ranged from 13 to 17 d, depending on travel distance, weather conditions, and port delays. All ships exchanged ballast water in experimental tanks at sea, as planned.

Ballast water and zooplankton therein—Calibrated instruments revealed that ballast water conditions were similar at the outset of experiments in control and experimental tanks, though BWE had immediate and profound effects on salinity in flushed tanks. Instrument data gathered from vessel 3 revealed a drop in ballast water temperature from ~18°C to ~6°C between day 3 and day 6 (Fig. 2). From day 6 onward, the temperature of the ballast rose sharply and eventually leveled off at 15–16°C toward the end of the voyage. Dissolved oxygen profiles were similar in the control and treatment tanks and varied between ~8 and 9 mg L^{-1} throughout the voyage (Fig. 2). The salinity of water in both the treatment and control tanks was 2 at T_0, which is likely a result of the vessel filling its ballast tanks while transiting down the St. Lawrence River from Montreal. Ballast had to be taken after the vessel left port because of draft constraints. Salinity in the control tank remained at 2 for the remainder of the voyage. Salinity rapidly increased (to >35) in the exchanged tank on day 7 and then dropped back to 26 for the remainder of the voyage (Fig. 2). We interpret this spike in salinity to be the result of incomplete ballast discharge during the empty-refill process and slow mixing. The incoming saline ballast would have had a higher density than the residual freshwater in the tank, and the two fluids may not have been completely mixed during filling. This would produce the temporary high salinity registered by the instruments that were moored near the bottom of the tank. Subsequent mixing due to the movement of the ship may then have mixed the residual freshwater and the saline water, resulting in the salinity measurement of 26.

Water quality data from vessel 6 revealed a gradual decrease in ballast water temperature, from ~20°C on day 1 to ~9°C on the final day of the experiment (Fig. 2). Dissolved oxygen profiles were similar in both the control and treatment tanks. From day 1 to day 8 the dissolved oxygen declined from ~5 mg L^{-1} to ~3.5 mg L^{-1} (Fig. 2). Thereafter, oxygen levels in both tanks gradually increased to ~10 mg L^{-1} by the end of the voyage, although a brief spike occurred in the exchanged tank during BWE on day 10. Salinity in the exchanged tank rapidly increased on day 10, from ~1.5 to ~37 after BWE had occurred (Fig. 2). Salinity in the control tank remained at ~1.5 for the entire voyage (Fig. 2).

Eight cladoceran, five copepod, and twenty rotifer species were recovered from zooplankton samples collected from control ballast tanks at T_0, while 9, 5, and 15 species of cladocerans, copepods, and rotifers, respectively, were collected from treatment tanks at T_0 (Table 2). Similarly 9, 7, and 19 species of cladocerans, copepods, and rotifers, respectively, were collected from control tanks at T_1 (Table 2). Far fewer species survived BWE, with three, one, and three species of cladoceran, copepod, and rotifer species, respectively, sampled from exchanged tanks at T_1 (Table 2). *Daphnia mendotae, Bosmina coregoni*, and *Bosmina liederi* were the most abundant cladocerans, while *Mesocyclops edax* and *Diacyclops thomasi* were the most abundant copepod species. Abundant rotifer species included *Keratella cochlearis*, several *Polyarthra* species, *Kellicottia bostoniensis*, *Kellicottia longispina*, and *Pompholyx sulcata* (vessel 6). Marine copepods of the family Scolecitrichidae were recovered from the exchanged tanks at the end of each voyage, in addition to many unidentified nauplii.

T_0 and T_1 zooplankton samples were collected on vessels 1, 2, 4, and 5. On vessels 3 and 5 we were only able to obtain T_1 samples. Freshwater zooplankters were completely absent from the exchanged ballast tanks of vessels 1, 3, and 4 at T_1, while freshwater copepods, cladocerans, and rotifers were found at low concentrations in exchanged tanks of vessels 2, 5, and 6 (Table 3; Fig. 3). The abundance of copepods, cladocerans, and rotifers in the

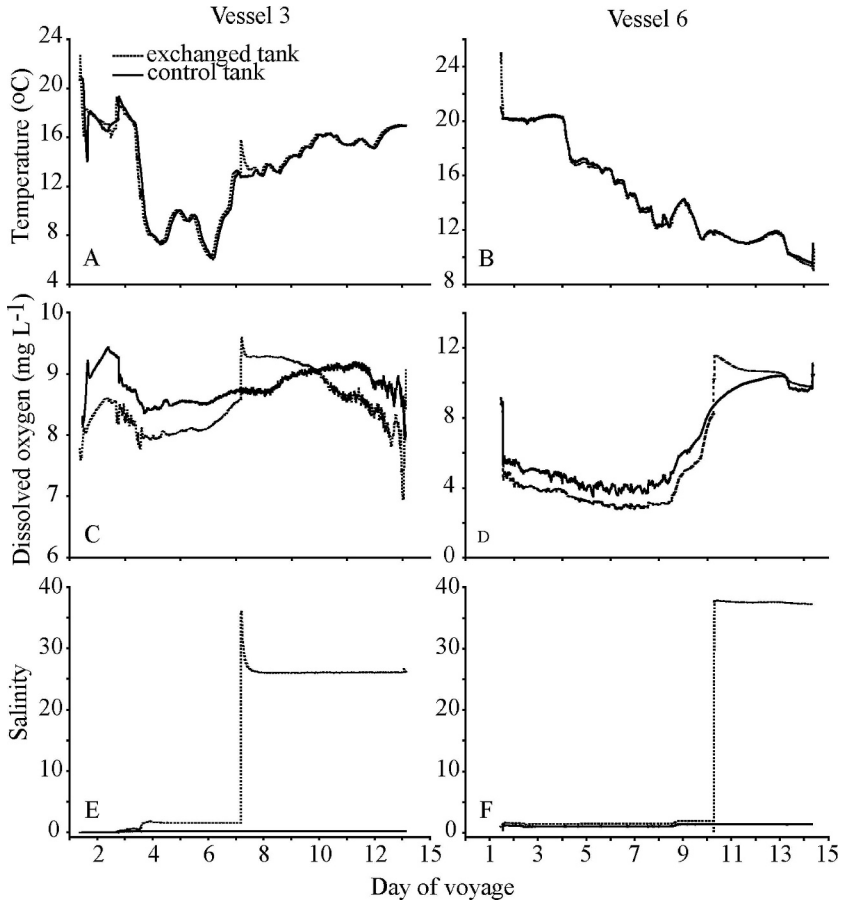

Fig. 2. (A–F) Temperature, dissolved oxygen, and salinity measurements obtained from water quality instruments installed in ballast tanks of vessels 3 and 6. Control tank in panel E is at 0.

control tanks remained high at the conclusion of the voyage for vessels 1, 4, and 5 (Fig. 3). Significant mortality occurred in the control tank of vessel 2, with a decrease in density of >98%, >99%, and >97% for copepods, cladocerans, and rotifers, respectively. However, live individuals of each group were recovered at T_1 at densities of 0.47, 0.04, and 0.17 individuals L^{-1}, respectively (Table 3). The explanation for this mortality in the control tank of vessel 2 is unclear, as we were not able to install water quality instruments in the tanks of this vessel.

BWE was highly effective at removing freshwater zooplankton. For vessels 1, 2, 4, and 5, exchange efficiencies—based upon the reduction in total zooplankton density—ranged from 100% to 99.4%, by ship (Table 3). Exchange efficiencies were also calculated for the most abundant cladoceran, copepod, and rotifer species recovered from each vessel. These efficiencies ranged from 100% for most species to a low of 95.1% for *D. mendotae* on vessel 2 (Table 3). The exchanged ballast tank on vessel 5 contained a low density of animals at the conclusion of the voyage. However, calculated exchange efficiency was 100% owing to the large increase in densities in the control tank during the course of the voyage.

Although exchange efficiencies were not calculated for vessels 3 and 6 because of a lack of T_0 samples, we can glean information regarding BWE efficiency from the T_1 zooplankton samples collected. No freshwater animals were collected from the exchanged tank of vessel 3 at the end of the voyage, while the density of animals in the exchanged tank of vessel 6 was >99.5% lower than that in its companion control tank. The fact that analyses of differences between initial zooplankton densities in control and treatment tanks in other vessels were not significant indicates that BWE was highly effective at reducing zooplankton densities for both ship 3 and ship 6. It is important to note that this conclusion is based on the premise that zooplankton densities were similar in both the treatment and control tanks at the start of the ships' voyages. The nested ANOVA we conducted may have been hindered by a low sample size ($n = 4$), limiting our ability to detect initial differences. However, the difference in animal density between control and treatment tanks of vessels 3 and 6 was >99.5%. If large a priori differences such as this existed for the other vessels (vessels 1, 2, 4, and 5), they should have been detected with a nested ANOVA, despite the low sample size. Therefore, we believe these reductions in density are the result of BWE.

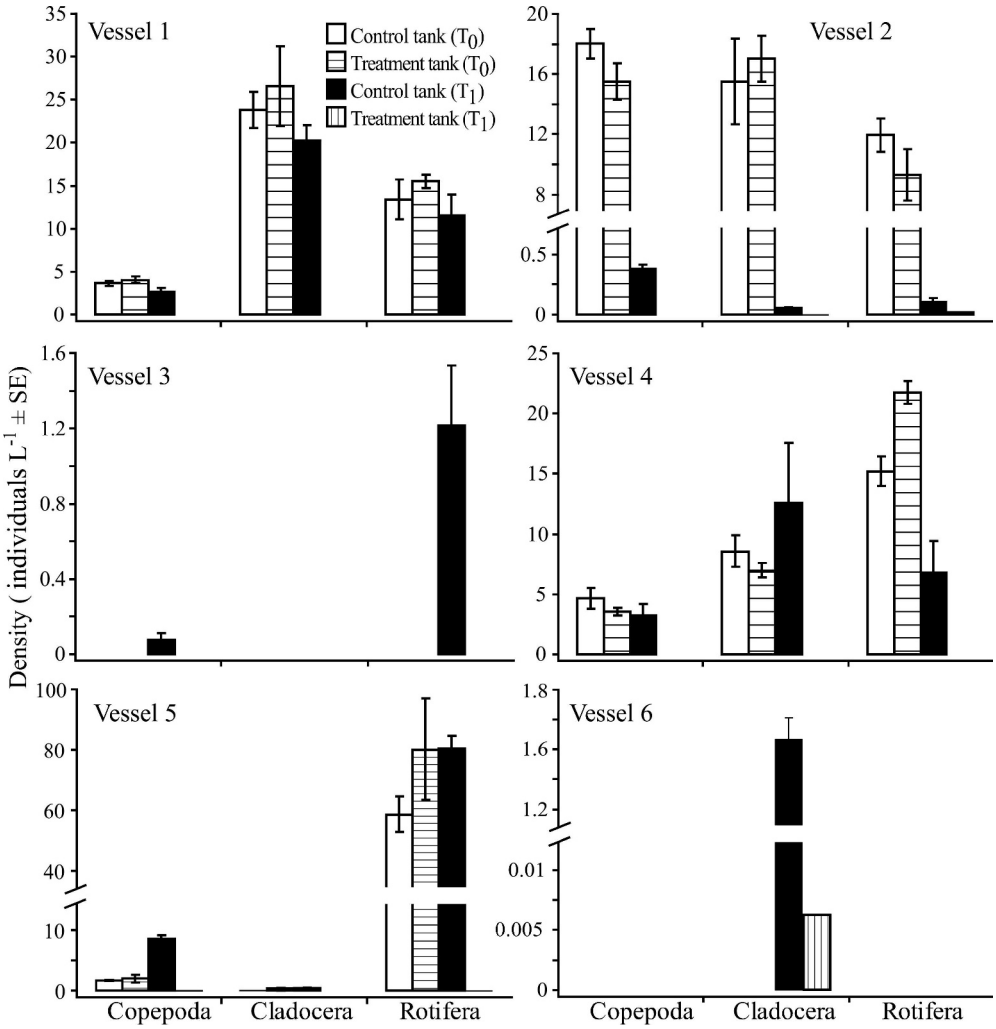

Fig. 3. (A–F) Density (individuals L^{-1} ± standard error [SE]) of copepods, cladocerans, and rotifers sampled from treatment (exchanged) and control (not-exchanged) ballast tanks at the beginning (T_0) and end (T_1) of the ships' voyages. Note breaks in the y-axis for vessels 2, 5, and 6. T_0 samples were not collected for ships 3 or 6 (*see Methods*).

In situ recruitment from diapausing eggs—Egg densities in experimental ballast sediments ranged from 661 to 4,045 eggs 300 g^{-1} (Table 4). Rotifer eggs were numerically dominant in all sediments, comprising between 64% and 97% of eggs. Cladoceran eggs were present in low numbers in all sediments.

Recruitment of animals from diapausing eggs was significantly higher in incubation chambers set in control tanks than in tanks that underwent BWE (Table 5; paired *t*-test, $t = 3.45$, df = 5, $p = 0.018$). Between 0.5 and 3.25 individuals per trap were recovered from chambers in the

Table 4. Mean diapause egg density 300 g^{-1} of supplemented ballast sediment placed in incubation chambers in control and treatment tanks. Numbers (1–6) refer to vessels listed in Table 1.

Egg type	1	2	3	4	5	6
Asplanchna	10.6	—	7.4	4.4	—	—
Brachionus	378	2,958	334.4	784.4	2,253	1,801
Filinia	31.4	7.4	93	48	19.4	10.8
Synchaeta	4.4	604.4	982.4	–	859.4	711.2
Unidentified Rotifera	52.4	336	243	19.4	273	205
Bosmina	—	21	12	22.4	31.4	32
Daphnia	—	—	78	37.4	—	—
Unidentified Cladocera	135	34.4	114	79.4	63	51.2
Copepoda	49.4	84	724.4	4.4	54	24.4
Total No. of eggs	661.2	4,045.2	2,588.6	999.8	3,553.2	2,835.6

Table 5. Mean (±standard deviation [SD]) number of individuals recovered from incubation chambers in control and exchanged ballast tanks. Vessel numbers refer to those listed in Table 1.

Vessel	Individuals recovered, control tank (±SD)	Individuals recovered, exchanged tank (±SD)
1	3.25 (±0.63)	0.25 (±0.25)
2	1.80 (±0.58)	0.00 (±0.00)
3	1.40 (±0.40)	0.20 (±0.20)
4	0.75 (±0.48)	0.00 (±0.00)
5	0.71 (±0.24)	0.00 (±0.00)
6	0.50 (±0.29)	0.00 (±0.00)

Table 6. List of species recovered from incubation chambers at the conclusion of the voyages. X, recovered from exchanged tank; C, recovered from control tank (not exchanged). Vessel numbers refer to those listed in Table 1.

Species	Vessel					
	1	2	3	4	5	6
Rotifera						
Brachionus calyciflorus	—	C	XC	—	C	C
Cephalodella gibba	XC	—	—	—	—	—
Brachionus angularis	C	XC	—	—	—	—
Synchaeta kitina	C	C	—	—	—	—
Brachionus urceolaris	—	C	—	—	—	—
Polyarthra dolichoptera	—	—	C	—	—	—
Synchaeta grandis	—	—	—	—	C	—
Brachionus budapestinensis	—	—	—	C	—	—
Brachionus bidentata	—	—	—	—	C	—
Cladocera						
Diaphanosoma brachyurum	—	—	C	—	—	—

control tanks, while 0–0.25 were recovered from chambers in the exchanged tanks (Table 5). Nine rotifer species and one cladoceran species were recovered from chambers in the control tanks, while only three rotifer species were recovered from those in the exchanged tanks (Table 6). However, this difference in species richness of hatched plankton could simply be a function of the total number of individuals collected (Table 5). Rotifers larger than the nitex mesh on the incubation chambers (60 μm) were not found in the chambers containing autoclaved sediment. In addition, species recovered from experimental incubation chambers were different from those recovered from zooplankton net tows of the outside ballast water (Tables 2, 6). For these reasons, we believe that contamination did not influence the above results.

In situ trials with sentinel invertebrates—B. sowerbyi oligochaetes in incubation chambers in control tanks survived the transoceanic voyages with only moderate mortality (16.6%, 0%, and 20% mortality for vessels 4, 5, and 6, respectively). However, nearly all individuals perished (100%, 100%, and 96.6%) in chambers placed in the exchanged tanks. Mortality of *E. ischnus* in incubation chambers in control tanks was higher (i.e., 40%, 60%, and 53.3%) than that of oligochaetes, while all individuals in tanks that experienced BWE were killed.

Discussion

Currently the Great Lakes are protected from invasions via ships' ballast by regulations that mandate BWE or its equivalent. In the future, similar protections may be provided to other aquatic ecosystems if the International Convention for the Control and Management of Ships' Ballast Water and Sediments comes into force (IMO 2004). This convention would require vessels to meet a ballast water discharge standard (IMO 2004). To comply with the existing BWE standard (D1), vessels must conduct BWE at least 360 nautical km from the nearest land and in water that is at least 200 m in depth, with at least 95% volumetric exchange. The proposed IMO (2004) discharge standard (D2) may be met by conducting ballast management in a manner that results in the release of less than 10 viable organisms m^{-3} for organisms $\geq$50 μm in minimum dimension and less than 10 viable organisms mL^{-1} for organisms $\geq$10 μm but $<$50 μm in minimum dimension. Results from the three different types of studies conducted here indicate that empty-refill, open-ocean BWE on ships transiting between freshwater ports can meet the equivalent of this discharge standard when we consider only planktonic and benthic freshwater invertebrates, which pose a high risk to freshwater systems and some risk to estuarine systems. For example, freshwater animals were absent from samples taken from exchanged tanks on 3 vessels (vessels 1, 3, and 4), while those from the remaining exchanged tanks (vessels 2, 5, and 6) had total densities of 3.4, 7.3, and 5.6 individuals m^{-3} for macroscopic ($\geq$50-μm) invertebrates (Table 3).

It should be noted that proposed IMO (2004) ballast water treatment standards apply to viable organisms discharged with ballast water. Exchanged ballast would be dominated by marine taxa, so without additional treatment it is likely that the total density would exceed the proposed D2 discharge standards. However, in relation to risk of invasion of freshwater ecosystems, only freshwater-tolerant taxa transferred from freshwater areas need be considered. This view is consistent with findings in the Great Lakes, in which no open-ocean invertebrates have colonized the system. Brackish water species including *Cercopagis pengoi* and *Hemimysis anomala* have colonized, although these species occur in coastal areas, where water salinity is far lower than in open-ocean areas (e.g., $<$10 vs. $>$30, respectively).

Zooplankton exchange efficiencies demonstrated in this study are higher than or equivalent to those on ships transiting between marine ports. In this study, sequential (empty-refill) exchange resulted in a decrease in total zooplankton abundance by $>$99% for all four ships for which we were able to assess exchange efficiency (vessels 1, 2, 4, and 5). Studies of sequential exchange between marine ports include those of Wonham et al. (2001) and Ruiz and Smith (2005). Wonham et al. (2001) measured reductions in zooplankton density $>$98% in their assessment of three ballast tanks and a cargo hold on one ship, while Ruiz and

Smith (2005) found reductions in total zooplankton that varied between 51% and 99% for tanks on seven different vessels. The results from our study indicate that the effectiveness of BWE for freshwater organisms is less variable than that for marine organisms (Ruiz and Smith 2005). The reduced variability of BWE effectiveness in our study may result from pronounced osmotic shock experienced by freshwater animals remaining in ballast tanks after BWE. Vessels transiting between marine ports must rely on purging and dilution of ballast water presently in the ballast tanks to eliminate coastal organisms. Vessels transiting between freshwater ports can expect decreases in zooplankton density due both to purging of organisms and to salinity effects.

Although observed densities of freshwater, planktonic invertebrates in tanks that experienced BWE were severely reduced and averaged <10 individuals m^{-3}, the large volume of water discharged by a ballasted vessel—typically between 4×10^6 and 14×10^6 kg (Niimi and Reid 2003)—indicates that substantial numbers of freshwater individuals could be released, even by a vessel that has completed BWE. Assuming the postexchange concentrations of zooplankton found in our experiments are indicative of the typical exchange efficiency for a cargo vessel, postexchange ballast discharge could result in the release of between 0 and 4.8×10^7 live animals, if the ballast was completely discharged. If these organisms belonged primarily to a single species capable of swarming behavior, the resultant lake density could be much higher than that present in discharged ballast water.

While our studies were designed to assess the efficacy of BWE for protecting the Great Lakes, the results presented here are directly applicable to European freshwater ports. Data from our control tanks indicate that vessels traveling with unexchanged Great Lakes' ballast water pose an invasion threat to European freshwater ports. A number of North American species are established in Europe, particularly in the Baltic Sea, and at least one of these species (e.g., *K. bostoniensis*) was recorded in both exchanged and control tanks in our studies.

The high survivorship of zooplankton in control tanks in this study contrasts with the results of other studies that reported a sharp decline in abundance and species richness of plankton in ballast tanks within the first few days of a voyage (e.g., Gollasch et al. 2000*a*,*b*; Olenin et al. 2000). Unfortunately, in situ measurements of water quality could not be performed on vessels 1, 4, and 5 because of lack of equipment for vessel 1 and because of explosion hazards related to vessels 4 and 5. However, the water quality data we obtained from ballast tanks of vessels 3 and 6 (Fig. 2) indicate that dissolved oxygen levels can remain high enough during a voyage to support aerobic, planktonic species. Furthermore, the increase in abundance of copepods and rotifers in the control tank of vessel 5 indicates that physical conditions must have been favorable for reproduction.

Our in situ experiments using incubation chambers indicate that BWE can strongly limit the recruitment of animals from diapausing eggs found in ballast sediments. The number of animals recovered from chambers in exchanged tanks was significantly lower than the number recovered from chambers in control tanks (Table 5). There are three possible explanations for the lower abundance of rotifers and cladocerans in chambers from exchanged tanks. First, saline water exposure may have killed animals that hatched during the pre-exchange period. The pre-exchange period, during which both the control and treatment tanks contained freshwater, ranged from 6 d to 12 d, which was more than sufficient time for species to hatch from diapausing eggs (Bailey et al. 2004). Since salinity in the incubation chambers in exchanged tanks was measured at >26 at the end of the voyages, many freshwater animals that hatched during the pre-exchange period would presumably have perished as a result of osmotic shock. Second, the presence of salt water in the chambers could have prevented further recruitment from diapausing eggs in the sediment, since environmental conditions would not cue hatching. Diapausing eggs often require specific environmental cues to encourage hatching (e.g., Schwartz and Hebert 1987), and the presence of saline conditions may have discouraged development of eggs in exchanged tanks. Previous work has indicated that diapausing eggs of freshwater species will not hatch when exposed to saline conditions, though viability of these eggs would not be adversely affected by such exposure (Bailey et al. 2004). Third, environmental conditions inside incubation chambers deteriorated to less than that required for hatching. We conducted experiments in which instrument sondes were embedded inside separate incubation chambers of the same design used here. Results showed that exchange between ambient water and water trapped in the chamber can be limited, depending on ship motion, and, hence, biochemical oxygen demand from sediment can lead to hypoxic or anoxic conditions inside the chambers (Reid pers. comm.). Such conditions would prevent most diapausing eggs from hatching (Raikow et al. 2007) and could also explain the high mortality of sentinel invertebrates in exchange tank chambers. However, since control tank chambers were set up in a manner similar to that used for their companion exchange tank chambers, and some had significantly higher hatching and survivorship of sentinel invertebrates, it would appear that the potential decline of oxygen inside the chambers cannot explain all the results. Still, the diapausing egg hatch rates observed in our exchange tank chamber experiments must be considered as minima as a result of possible hypoxia or anoxia inside the chambers. Since oxygen levels in the ambient ballast tank water never dropped below 4 mg L^{-1} during the two experiments for which we had instruments in the tanks, the best explanation for the mortality observed in the exchange ballast tanks is death of live individuals resulting from exposure to open-ocean water following BWE.

Experiments with caged, benthic invertebrates (vessels 4, 5, and 6) demonstrated that BWE caused mortality rates that were almost identical to those observed for planktonic species. All but one oligochaete perished in exchanged ballast tanks, while 100% of amphipods perished. Since the same was not true for the control tank caged invertebrates, these results indicate that exposure to saline water following BWE was as lethal for benthic taxa inhabiting

the sediment–water interface as for planktonic species. The survival of one oligochaete in an incubation chamber following BWE highlights a potential problem with using BWE as the sole protective measure to stem invasions. This individual was found at the very bottom of the sediment layer, where exposure to saline water was likely minimal. Thus, the lens of sediment that sometimes accumulates in vessels could potentially harbor live invertebrates, though the likelihood of these individuals being discharged during BWE would seemingly be very low. Future lab or in situ experiments should be conducted to explore the degree to which salinity penetrates through ballast sediments and influences the survival of benthic invertebrates.

Through a combination of sampling of planktonic individuals in control and experimental ballast tanks both before and after BWE, and through the use of in situ incubation chambers to assess the effect of BWE on zooplankton recruitment and benthic invertebrate survival, we were able to demonstrate that BWE provides an effective method to reduce the discharge densities of nonindigenous species into the Great Lakes. Our experiments demonstrate that ships that engage in BWE while moving across the ocean between freshwater ports of call can discharge ballast into freshwater ecosystems that contains densities of freshwater invertebrates below the number of viable organisms that the IMO has proposed as sufficiently protective for aquatic ecosystems (IMO 2004). While our studies were undertaken specifically to address Great Lakes concerns, they may be equally applicable to other instances in which freshwater ballast is exchanged at sea prior to discharge in another freshwater port.

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