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Ballast water treatments to limit the transfer of nonindigenous species between freshwater ports.

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By

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Abstract

Mandatory ballast water exchange (BWE) was implemented for vessels carrying ballast water into the Great Lakes in 1993; however, few data are available on its effectiveness. I conducted BWE experiments aboard six transoceanic vessels traveling from the Great Lakes to European ports. BWE was highly effective (>99% loss) for reducing concentrations of zooplankton, and for causing mortality of sentinel amphipods and oligochaetes (96-100%). BWE also dramatically reduced recruitment of zooplankton from diapausing eggs present in ballast sediment; however it did not affect egg viability when eggs were subsequently returned to freshwater. Laboratory experiments with sodium hypochlorite suggest that ships could employ this treatment to reduce the risk of invasions from diapausing eggs. Estimates of the probability of a cladoceran invasion using data from this study suggest that BWE probably provides sufficient protection for the Great Lakes as an interim measure to prevent future invasions.

Co-authorship Statement

Chapter 2

This chapter is *in review* for the journal *Limnology and Oceanography*. Drs. Hugh MacIsaac, Tom Johengen, and David Reid assisted with fieldwork, contributed intellectually, and edited earlier versions of the manuscript. In addition, Drs. Tom Johengen and David Reid collected water quality data from ballast tanks via their deployment of *in-situ* instruments.

Chapter 3

This chapter has been published in the journal *Marine Pollution Bulletin*. Dr. Ian Duggan assisted with experimental design and data analysis, and edited an earlier draft of this chapter. Dr. Hugh MacIsaac contributed intellectually to this chapter and edited an earlier draft of the manuscript.

For my parents Ron and Shelley

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Chapter 1

Introduction

The natural distribution of many species has been altered due to their intentional or unintentional translocation by humans. While some of these nonindigenous species (NIS) are beneficial, including food crops and livestock, others have had significant environmental and economic impacts (Pimentel et al. 2005; Colautti et al. 2006). Species introductions are recognized as a major threat to endangered species around the globe (Baillie et al. 2004; Dextrase et al. 2006; Lawler et al. 2006), and are expected to be one of the major drivers of biodiversity change within this century (Sala et al. 2000). Nonindigenous species can act as predators, parasites, and pathogens of native species, and their introduction can result in significant habitat changes in ecosystems (Simberloff 2002).

The economic costs of NIS introductions can be tremendous. In the United States, they cause major environmental damage and losses amounting to approximately $120 billion USD annually (Pimentel et al. 2005). Although the costs of NIS are poorly studied in Canada, they have a significant impact in key industries including agriculture, forestry and coastal and marine fisheries and aquaculture (Colautti et al. 2006). The potential damage and control costs for just the 18 notorious NIS considered by Colautti et al. (2006) for Canada ranged between $13.2 billion and $34.8 billion CDN per year.

The establishment of NIS has occurred in many freshwater ecosystems throughout the world (Kaufman 1992; Mills et al. 1993, 1996; Josefsson and Andersson 2001; Bij de Vaate et al. 2002). The problem is particularly acute in the North American Great Lakes where 183 recorded NIS have been found (Ricciardi 2006; Potven et al. 2007). Historically, these introductions have

resulted from a multitude of vectors including deliberate releases by individuals, accidental releases by individuals, the building of canals, and shipping activity (Mills et al. 1993). However, the predominant vector for the introduction of NIS since the opening of the St. Lawrence Seaway in 1959 has been the release of contaminated ballast water of transoceanic ships (Holeck et al. 2004; Ricciardi 2006). Sixty-five percent of introductions since 1959 are thought to have occurred due to discharge of contaminated ballast water (Ricciardi 2006).

In an attempt to slow ballast water introductions of NIS to the Great Lakes, voluntary (1989) and then mandatory (1993) ballast water exchange (BWE) regulations were enacted for ships entering the system with fresh or brackish ballast. These regulations require inbound vessels to treat ballast or perform ballast exchange while on the open-ocean if their ballast is to be discharged while operating on the Great Lakes (Locke et al. 1993; United States Coast Guard 1993). Despite this requirement, reports of new invasions of invertebrate and other taxa have continued to appear, including most recently *Hemimysis anomala*, a Ponto-Caspian mysid in Lakes Ontario and Michigan in 2006 (Pothoven et al. 2007). One possible explanation for these continued invasions is that BWE may not be fully effective and may not afford complete protection to the Great Lakes.

In this thesis I present data from experiments designed to assess the effectiveness of BWE for reducing concentrations of freshwater organisms in ballast tanks. This information is vital to understanding the continuing onslaught of invasions facing the Great Lakes. I also explore the possibility of treating

ballast tanks with sodium hypochlorite (NaOCl) to decrease the viability of diapausing eggs, which, as I demonstrate in laboratory experiments, are resistant to saltwater exposure. Continued research into alternative treatment options, such as NaOCl will be needed if BWE does not effectively halt transfers of NIS in ballast.

Below I include a brief summary of the subsequent chapters. Chapters 2 and 3 of my thesis are presented as stand-alone articles, complete with separate abstracts and introductions. In Chapter 4, I review the results of chapters 2 and 3 while attempting to answer the question: Does ballast water exchange provide adequate protection for the Great Lakes?

Chapter 2 – Ballast water exchange efficiency

Ballast water exchange (BWE) regulations were enacted for ships entering the Great Lakes in 1993 (United States Coast Guard 1993). These regulations are intended to purge non-indigenous species (NIS) from ballast tanks, and kill those remaining in the tanks with high salinity water (Locke et al. 1993). Vessels can conduct BWE by completely emptying a tank and then filling it with ocean water (sequential exchange) or by adding ocean water to the tanks while discharging fresh or brackish water (continuous exchange; Ruiz et al. 2005b). Whichever method is chosen, the ballast water must attain a final salinity ≥30‰ (Locke et al. 1993; United States Coast Guard 1993). Several studies have assessed the efficiency of BWE for vessels transiting between marine and estuarine ports (Rigby and Hallegraeff 1994; Taylor and Bruce 2000; Wonham et al. 2001; Choi

et al. 2005; Ruiz et al. 2005a). These studies have demonstrated that effectiveness of BWE can vary, and that live zooplankton often remain in the tanks following exchange. However, controlled experiments have not been conducted to assess efficacy of mid-ocean BWE on board vessels traveling between freshwater ports. The effectiveness of BWE for these voyages may be expected to be greater than that between marine ports since animals remaining in the tank after exchange will experience a profound osmotic shock effect due to exposure to high salinity ocean water (Locke et al. 1993). To assess the efficacy of sequential BWE for freshwater organisms, I conducted experiments on six operational transoceanic vessels traveling from the Great Lakes to European ports. I utilized an experimental approach, consisting of three simultaneous sets of experiments in ballast tanks of ships while they moved across the ocean. All experiments had otherwise identical control tanks that were not flushed, so observed changes in invertebrates can be attributed to experimental flushing. Results from these experiments provide conclusive evidence that ballast water exchange is highly effective for reducing concentrations of zooplankton and zoobenthos present in ballast tanks. Experiments with emergence chambers also demonstrated that BWE can dramatically reduce recruitment of zooplankton from diapausing eggs present in ballast sediments. However, laboratory experiments conducted with ballast sediment collected following BWE in experimental ships suggests that the viability of diapausing eggs is not decreased by exposure to saline water. Although BWE is effective for live

organisms, another treatment method may be required to decrease the risk of introductions from diapausing eggs.

Chapter 3 – Treatment of ballast with sodium hypochlorite

Owing to design constraints, ballast tanks on vessels typically contain residual sediments that support an abundance and variety of live invertebrate species, and hundreds of thousands of viable invertebrate diapausing eggs, including those of NIS (Bailey et al. 2003; Bailey et al. 2005a; Duggan et al. 2005). Laboratory viability experiments outlined in chapter 2 suggest that ballast water exchange does not affect egg viability, although it can limit in-tank recruitment of zooplankton.

Diapausing zooplankton eggs are present in ballasted ships, as well as ships that declare no-ballast-on-board (NOBOB) status when entering the Great Lakes (Gray et al. 2005). NOBOB vessels are of particular concern because they represent >90% of Great Lakes traffic (Colautti et al. 2003), and carry an average of 15 tonnes of residual sediment and 46.8 tonnes of residual water in their ballast tanks (Duggan et al. 2005). Diapausing eggs or hatched zooplankton could be introduced to a system if a NOBOB vessel loads and then subsequently discharges ballast during multi-port operations (Gray et al. 2005). Some political jurisdictions (Michigan) are concerned by the risk posed by ballast residuals and have enacted legislation requiring treatment of ballast materials for any vessels discharging into state waters (Showalter and Bowling 2006). In this chapter I examine whether exposure to sodium hypochlorite (NaOCl) can reduce the risk

of introductions from diapausing eggs contained in the ballast tanks. I performed acute toxicity experiments by exposing whole ballast sediments containing diapausing eggs of invertebrate species to concentrations of NaOCl ranging between 0 and 10000mg/L (active ingredient) for 24 hours. Results from these experiments suggest that relatively high doses of NaOCl (1000mg/L) are required to effectively reduce viability of eggs. Although the volume of NaOCl required to treat fully-ballast ships is probably unrealistic, NOBOB vessels are prime candidates for this cost-effective treatment.

Chapter 4 – Does ballast water exchange provide sufficient protection?

Experiments I conducted aboard six cargo vessels transiting from the Great Lakes to European ports suggest that ballast water exchange is highly effective for removing live zooplankton and for causing mortality of benthic invertebrates (Chapter 2). However, live animals were collected from tanks after ballast water exchange. To further consider the level of protection offered by BWE, it is important to gather information on the probability of establishment for the remaining live species that can be discharged from ballast tanks. What is the relationship between initial discharge density and the probability of a species establishing? To answer this question, Bailey et al. (in prep.) used a modeling approach that incorporated data gathered from mesocosm experiments testing the impact of propagule density on the establishment success of various cladocerans. Using data from Bailey et al. (in prep.) along with data on BWE efficiency from chapter 2, I evaluated the risk of a cladoceran introduction from a

ship that has conducted BWE. Given the efficiency of BWE demonstrated in my experiments, the model by Bailey et al. (in prep.) suggests that there is no chance (0% probability) of a successful cladoceran introduction. Additional data will be needed to evaluate the risk posed by other groups that were found to survive BWE, including rotifers, copepods, and benthic invertebrates (Chapter 2).

Chapter 2

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

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, I 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 live freshwater zooplankton, exceeding proposed ballast water performance standards. Live sentinel amphipods and oligochaetes deployed in incubator chambers sustained nearly universal mortality in tanks that experienced BWE. Finally, BWE dramatically reduced *in situ* recruitment of zooplankton from diapausing eggs present in ballast sediments in additional incubator chambers deployed in these tanks. Decreased recruitment due to BWE was presumably the result of mortality of hatched individuals, as laboratory viability experiments suggest that diapausing egg viability is not significantly altered by exposure to saline water. Collectively, these studies support the contention that BWE by transoceanic vessels provides good protection against

transfers of live pelagic and benthic species between source and donor freshwater ports.

Introduction

The transport and release of ballast water has allowed hundreds of nonindigenous species (NIS) to establish in freshwater, brackish, and marine ecosystems throughout Europe and North America (Mills et al. 1993, 1996; Ruiz et al. 2000; Josefsson and Andersson 2001; Bij de Vaate et al. 2002). Surveys of ballast tank biota reveal that vessels bound for freshwater ports may harbour live planktonic and benthic animals as well as large numbers of viable diapausing invertebrate eggs in accumulated ballast sediment (Locke et al. 1993; Duggan et al. 2005; Bailey et al. 2005a). 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 while operating on the Great Lakes (Bailey et al. 2005b).

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

standard unit for oceanic salinity is "practical salinity units (psu)," but ballast water exchange regulations specify 30‰, which, for practical purposes, is the same as 30 psu). Despite this requirement, reports of new invasions of invertebrate and other taxa have continued to appear, including most recently *Hemimysis anomala*, a Ponto-Caspian mysid in Lakes Michigan and Ontario during 2006 (Pothoven et al. 2007). One possible explanation for these continued invasions is that BWE may not be fully effective and may not afford the necessary protection to the Great Lakes.

Several studies have assessed the efficiency of ballast water exchange (BWE) for vessels transiting between marine ports (Rigby and Hallegraeff 1994; Taylor and Bruce 2000; Wonham et al. 2001; Choi et al. 2005; Ruiz et al. 2005a). These studies have demonstrated that effectiveness of BWE varies greatly, and that live planktonic animals often remain in the tanks following exchange. Only one study to date has attempted to evaluate BWE efficiency for vessels entering the Great Lakes (Locke et al. 1993). Locke et al. (1993) sampled zooplankton from BOB vessels that had originated in freshwater ports and reported conducting BWE before entering the Great Lakes. However, they relied on the simple presence or absence of freshwater species in the tanks to develop a crude estimate of BWE efficiency, rather than calculating exchange efficiency based on changes in zooplankton density. To provide an accurate measure of BWE efficiency, an assessment that includes comparisons between control (not exchanged) and exchanged tanks is required to ensure that the measured mortality is a result of exchange.

Two methods are available for ships to conduct BWE, termed sequential and continuous exchange. Vessels can completely empty a ballast tank and then refill it with ocean water (sequential exchange) or they can add ocean water to the tanks while simultaneously discharging the fresh or brackish water already found in their tanks (continuous exchange; Ruiz et al. 2005b). Either method allows ships transiting between marine ports to purge coastal zooplankton from their tanks and dilute the concentration of animals remaining in their tanks with the addition of ocean water (Ruiz et al. 2005b). Vessels transiting between freshwater ports can expect to benefit from the purging and dilution effects, as well as reductions in freshwater zooplankton due to exposure to high salinity ocean water. Exposure of freshwater zooplankton and benthos to open ocean water (~35‰) should be particularly detrimental since these invertebrates are hyper-osmotic regulators whose osmoregulatory mechanisms typically fail above 9‰ (Hart et al. 1991). While many invaders to the Great Lakes possess some degree of salinity tolerance (e.g. *Hemimysis*, *Cercopagis*), most freshwater animals are limited to mildly brackish conditions (<9‰; Hart et al. 1991). Diapausing invertebrate eggs may be more resistant, as laboratory experiments suggest saltwater exposure at salinities greater than 30‰ does not significantly decrease their subsequent viability (Gray et al. 2005).

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 post-exchange 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 I evaluate the efficiency of BWE 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, Fig. 1). I initially sought to conduct experiments on vessels traveling in the opposite direction since it would be most applicable to the Great Lakes, but was constrained by practical considerations. However, considering that my studies involve purging of ballast tanks and exposure of freshwater species to saline water, my results should apply in either direction, even though the species involved may differ. All of the vessels reported herein utilized the sequential (empty-refill) method of BWE. Sequential exchange typically results in greater reductions in zooplankton density as compared to continuous exchange (Ruiz et al. 2005b), therefore my results must be viewed as a liberal measure of BWE efficiency.

My studies constitute a replicated (by ship) form of a BACI (before, after, control, intervention) experiment common to field studies of environmental impact assessment (see Stewart-Oaten and Bence 2001). Paired ballast tanks 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 saltwater at mid-ocean. The treatment tank was randomly selected at the outset of the study

and underwent BWE during the transatlantic voyage (see Fig. 1). Prior to 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 always conducted >200 nautical miles from shore in water >2000m in depth using the empty-refill method, as per BWE guidelines (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 In Situ Troll® 9000 multi-parameter sondes 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 due safety concerns (vessels 2, 4, 5). Ships' records were also used to verify occurrence and geographic coordinates of BWE. Post-exchange salinity was obtained from the ballast tanks of vessels 1, 2, 4, and 5 using a portable optical refractometer.

To assess BWE 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 ships' arrival at its destination port in Europe (T_1). Three replicate zooplankton net tows (0.25m diameter, 30μm mesh) from each tank were obtained through deck hatch access points, and the animals were preserved in 95% ethanol. To uniformly sample the maximum volume of water (>500L per tank in all cases), plankton hauls were drawn from the very bottom of the tanks to the air-water interface. Animals were enumerated in the

laboratory under a stereomicroscope and 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 my plankton nets and appeared to be in good physical condition when examined in the laboratory.

I was able to obtain both T_0 and T_1 plankton samples from four of the six vessels used for experiments (Table 1). I was not able to collect T_0 samples from vessel 3 due to draft requirements that prevented the uptake of water in port, while equipment failure prevented the collection of T_0 samples from vessel 6. Therefore, ballast exchange efficiency was calculated using four replicates (ships 1, 2, 4, 5).

I calculated the percent change in zooplankton concentration in each tank as:

$$\%r = (T_1/T_0)*100 \qquad \text{Equation 1}$$

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 I calculated the exchange efficiency as:

$$Ex_{Effic} = [(\%r_C - \%r_T)/(\%r_C)]*100 \qquad \text{Equation 2}$$

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 of each group.

The method I 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, I conducted a nested 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 4 ships (vessel 1, 2, 4, 5). There were no significant differences in total zooplankton abundance between the treatment and control tanks (ANOVA, $F_{4, 16}=0.3347$, $p=0.85$).

Emergence from diapausing eggs – in situ experiments

To assess the impact of BWE on diapausing eggs contained in ballast sediments, I constructed incubation chambers out of PVC piping components (Figure 2; see Bailey et al. 2005b). Each chamber was constructed from a 15cm (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 twelve holes of 2.5-4cm diameter were drilled through the lid (4 holes) and approximately half way up the wall (8 holes) of each chamber to allow for the exchange of water between the inside of the chamber and the ballast tank. 60-µm nitex mesh was affixed to the exterior surface of each chamber body and interior surface of each top to completely cover all holes, and was secured with PVC cement and 18cm 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. 2005b).

Chambers were submersed in water for seven days in the lab before use to eliminate glue residues.

Two sets of triplicate incubator chambers (see Fig. 2) were moored to the bottom of both treatment and control tanks prior to tanks being filled, and 300g of previously collected ballast sediment was placed inside each chamber. One of the six chambers received 300g of autoclaved sediment to serve as a control (i.e. no hatching expected). The presence of animals >60μm in these control chambers would indicate that contamination from the surrounding ballast water had occurred. Although animals <60μm were found in the chambers, contamination of 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. 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 300g aliquot of sediment used in a chamber, a 300g sample from the same sediment had been subjected to a sugar floatation procedure which isolates but does not harm eggs (Bailey et al. 2005b). The eggs extracted by sugar floatation were then added to the 300g 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 ~32X magnification (Table 3).

At the conclusion of the voyage, hatched animals were collected from the incubation chambers by removing ~450mL of water that remained below the drainage holes with a wide-mouth pipette and filtering it through a 30μm sieve. The filtrate was 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.

Diapausing eggs – laboratory viability experiments

Nitex mesh affixed to the incubation chambers allowed the sediment inside to be exposed to saltwater during ballast water exchange (the exchanged tank) or to remain in freshwater (the control tank). At the end of the voyage I collected sediment from the incubation chambers using sterile scoops and spatulas and shipped it back to the laboratory on ice for use in viability experiments.

Viability experiments were conducted following the methodology presented in Gray et al. (2005), and were designed to assess the viability of the eggs under conditions similar to those in the Great Lakes. Sediment collected from each trap was thoroughly mixed using a sterile spatula and a 40g subsample was placed in a 500mL glass vessel. Synthetic pond water (150mL;

Hebert and Crease 1980) was then added to the vessel, and the vessel was agitated gently by hand for approximately five seconds. Sediment was then allowed to settle out of the water (approximately two hours) before the water overlaying the sediment was carefully decanted and replaced with fresh pond water. This exchange of water was performed to ensure that the salinity of the incubation media (pond water) was not affected by residual salt present from BWE. Viability experiments performed with sediment from vessel 1 had to be repeated due to this unforeseen problem. Pond water overlying the sediment collected from the exchanged tanks in this first experiment had a salinity of 4-5 ‰, and consequently fewer freshwater animals hatched during the incubation period. The extra exchange of incubation media allowed me to conduct subsequent viability experiments without confounding due to differences in salinity between treatments.

After the incubation media was exchanged, the vessels were placed in an environmental chamber at 20°C with a 16:8 light:dark cycle. I checked for hatched animals every 48 hours by carefully decanting the water through a 30μm sieve and examining the contents under a stereomicroscope. Decanted water was then replaced in each vessel, and the vessel was 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. To test for a difference in egg viability between eggs collected from exchanged versus control tanks I performed a two-sample *t*-test using the total number of animals that hatched from each vessel during the course of the

experiments. *P*-values were adjusted using the Dunn–Sidak formula to correct for multiple comparisons.

Sentinel benthic invertebrates

To evaluate the impact of BWE on benthic invertebrates, 30 *Echinogammarus ischnus* amphipods and 30 *Branchiura sowerbyi* oligochaetes collected from the Great Lakes were placed inside one incubation chamber in control and experimental tanks of vessels 4, 5, and 6 at T_0. I 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* oligochaetes were included to test if saltwater 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 4mm and 1mm sieves to isolate animals and determine if they survived the voyage.

Results

Ballast water exchange 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 days depending on the 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. For example, instrument data gathered from vessel 3 revealed a drop in ballast water temperature from ~18° to ~6°C between day 3 and 6 (Fig. 3). From day 6 onward the temperature of the ballast rose sharply and eventually leveled of at 15 to 16°C by the end of the voyage. Dissolved oxygen profiles were similar in the control and treatment tanks and varied between ~8-9mg/L throughout the voyage (Fig. 3). The salinity of water in both the treatment and control tank was measured at 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 due to 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. 3). I interpret this spike in salinity as a 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 26‰ measurement.

Water quality data from vessel 6 revealed a gradual decrease in ballast water temperature from ~20° on day 1 to ~9°C on the final day of the experiment (Fig. 3). Dissolved oxygen profiles were similar in both the control and treatment tanks. From day 1 to 8 the dissolved oxygen declined from ~5 to ~3.5mg/L (Fig. 3). Thereafter, oxygen levels in both tanks gradually increased to ~10mg/L 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. 3). Salinity in the control tank remained at ~1.5‰ for the entire voyage (Fig. 3).

Eight cladoceran, 5 copepod and 20 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 (Appendix 1). Similarly 9, 7, and 19 species of cladocerans, copepods, and rotifers, respectively, were collected from control tanks at T_1 (Appendix 2). Far fewer species survived BWE, with 3, 1, and 3 cladoceran, copepod, and rotifer species, respectively, sampled from exchanged tanks at T_1 (Appendix 2). *Daphnia mendotae*, *Bosmina coregoni*, and *Bosmina liederi* were the most abundant cladocerans at T_0, while *Mesocyclops edax* and *Diacyclops thomasi* were the most abundant copepod species. Abundant rotifer species at T_0 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 copepod nauplii.

T_0 and T_1 zooplankton samples were collected on vessels 1, 2, 4, and 5. On vessels 3 and 5 I was 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 2, Fig. 4). The abundance of copepods, cladocerans, and rotifers in the control tanks remained high at the conclusion of the voyage for vessels 1, 4, and 5 (Fig. 4). 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, respectively (Table 2). The explanation for this mortality in the control tank of vessel 2 is unclear, as I was not able to install water quality instruments in the tanks of this vessel.

Ballast water exchange 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 2). 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 *Daphnia mendotae* on vessel 2 (Table 2). The exchanged ballast tank on vessel 5 contained a low density of animals at the conclusion of the voyage. However, calculated

exchange efficiency was almost 100% (>99.95% for copepods, cladocerans, and rotifers) 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, due to a lack of T_0 samples, I can infer 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. Because analyses of differences between initial zooplankton densities in control and treatment tanks in other vessels was not significant, this suggests that BWE was highly effective at reducing zooplankton densities for both ships 3 and 6.

In situ recruitment from diapausing eggs

Egg densities in experimental ballast sediments ranged from 661 to 4045 eggs per 300 g (Table 3). 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 4; 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 control tanks, while 0 to 0.25 individuals per trap were recovered from chambers in the exchanged tanks

(Table 4). Nine rotifer species and one cladoceran species were recovered from chambers in the control tanks, while only 3 rotifer species were recovered from those in the exchanged tanks (Table 5). However, this difference in species richness of hatched plankton could simply be a function of the total number of individuals collected (Table 4). Rotifers larger than the nitex mesh pore size (60μm) were not found in the incubation chambers containing autoclaved sediment, indicating that contamination did not influence the above results.

Diapausing eggs – laboratory viability experiments

Twenty-four rotifer species, three cladoceran species, and unidentified copepod nauplii hatched during laboratory viability experiments (Table 6). Neither total abundance of hatched individuals nor species richness of hatched individuals differed significantly between sediments collected from incubation chambers in the exchanged versus control ballast tanks (two-sample *t*-tests, Table 7).

In situ trials with sentinel invertebrates

Branchiura sowerbyi oligochaetes in incubation chambers in control tanks survived the transoceanic voyages with only moderate mortality (16.6, 0, 20%, for vessels 4, 5, and 6, respectively). However, nearly all individuals perished (100, 100, 96.6%) in chambers placed in the exchanged tanks. Mortality of *Echinogammarus ischnus* in incubation chambers in control tanks was higher

(i.e. 40, 60 and 53.3%) than that of *B. sowerbyi*, 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 either a ballast water exchange standard or a ballast water treatment performance standard (IMO 2004). To comply with the ballast water exchange standard, vessels must conduct BWE at least 200 nautical miles from the nearest land and in water at least 200m in depth, with at least 95% volumetric exchange. The performance standard may be met by conducting ballast management in a manner that results in the release of less than 10 viable organisms per m^3 ≥ 50μm in minimum dimension and less than 10 viable organisms per mL for organisms ≥10μm but <50μm in minimum dimension. Results from the three different types of studies conducted here suggest that empty-refill open-ocean BWE can meet the performance standard for both planktonic and benthic invertebrates. For example, freshwater animals were absent from sample taken from exchanged tanks on 3 vessels (1, 3, 4), while those from the remaining exchanged tanks (vessels 2, 5, 6) had total densities of 3.4, 7.3, and 5.6 individuals per m^3 for macroscopic (≥ 50μm) invertebrates (Table 2).

Zooplankton exchange efficiencies demonstrated in this study are higher than, or equivalent to, those conducted with ships transiting between marine ports (Wonham et al. 2001; Ruiz et al. 2005a). In this study, sequential (empty-refill) exchange resulted in a decrease in total zooplankton abundance by >99% for all four ships for which I was able to assess exchange efficiency (vessels 1, 2, 4, 5). Other studies that have assessed sequential exchange with vessels transiting between marine ports include Wonham et al. (2001) and Ruiz et al. (2005a). 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 et al. (2005a) found reductions in total zooplankton that varied between 51% and 99% for tanks on seven different vessels. The results from my study suggest that the effectiveness of BWE for freshwater organisms is probably more consistent than that for marine organisms (e.g. Ruiz et al. 2005a). This increased consistency may be a result of higher mortality due to 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 to both purging of organisms and salinity effects.

Although observed densities of planktonic invertebrates in tanks that experienced BWE exceed the proposed IMO performance standard, the large volume of water discharged by a ballasted vessel - typically between 4000 and 14000 tonnes (Niimi and Reid 2003) - indicates that substantial numbers of

individuals could be released even by a vessel in compliance. Assuming the post-exchange concentrations of zooplankton found in my experiments are indicative of the typical exchange efficiency for a cargo vessel, ballast discharge could result in the release of between 0 and 4.8 X 10^7 live animals, if the ballast was completely discharged. While my studies were designed to assess the efficacy of BWE for protecting the Great Lakes, the studies conducted here are also directly applicable to European freshwater ports. Results from my control tanks indicate that vessels traveling with Great Lakes' ballast water pose an invasion threat to European freshwater ports unless they first engage in open-ocean BWE. A number of North American species are invasive in Europe, particularly in the Baltic Sea, and at least one of these species (*Kellicottia bostoniensis*) was recorded in both exchanged and control tanks in my studies.

The high survivorship of zooplankton in control tanks contrasts with the results of other studies that observed a sharp decline in abundance and species richness of plankton in ballast tanks within the first few days of a voyage (Rigby and Hallegraeff 1993, 1994; Gollasch et al. 2000a,b; Olenin et al. 2000). Unfortunately, *in situ* measurements of water quality could not be performed on vessels 1, 2, 4, and 5 due to lack of equipment for vessel 1 and explosion hazards on vessels 2, 4 and 5. However, the water quality data I obtained from ballast tanks of vessels 3 and 6 suggests that dissolved oxygen levels can remain high enough during a voyage to support aerobic, planktonic species (Fig. 3). Furthermore, the increase in the abundance of copepods and rotifers in the

control tank of vessel 5 suggests that physical conditions must have been favorable for reproduction.

My *in situ* experiments using incubation chambers suggest 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 that of those in control tanks (Table 4). 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 to12 days, which was more than sufficient time for species to hatch from diapausing eggs (Bailey et al. 2004). Since water 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 owing to osmotic shock. Second, the presence of saltwater 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 saline conditions may have discouraged development of the eggs in the exchanged tanks (Bailey et al. 2004). 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 the incubation chambers deteriorated to less than that required for hatching. Separate studies in which instrument sondes were embedded inside incubation chambers of the same design used here showed that water exchange between the chambers and the ballast tank can be retarded, and biochemical oxygen demand from sediment used in the experiments can lead to hypoxic or anoxic conditions inside the chambers (D. Reid, pers. comm.). Such conditions would prevent diapausing eggs from hatching, and could also explain the high mortality of the sentinel invertebrates in the exchange tank chambers. However, since the control tank chambers were set-up the same as their companion exchange tank chambers yet some had significantly higher hatching and survivorship of sentinel invertebrates, it would appear that the observed decline of oxygen inside the chambers cannot explain all the results. Still, the diapausing egg hatch rates observed in my exchange-tank chamber experiments must be considered as minima due to possible hypoxia/anoxia inside the chambers. Since oxygen levels in the ambient ballast tank water never got below 3.5mg/L during the two experiments for which I 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.

The results of my laboratory viability experiments suggest that diapausing invertebrate eggs may be largely resistant to saltwater exposure.Ne ither the total abundance of hatched individuals nor the species richness of hatched individuals differed significantly between sediments collected from incubation chambers in

the exchanged versus control ballast tanks (Table 7). Similar results were presented in Gray et al. (2005), although those experiments simulated BWE in the lab rather than performing the saltwater exposure under operational conditions in ballast tanks. In those experiments with both natural and ballast sediment, Gray et al. (2005) found no significant differences in the abundance of hatched animals or the species richness of animals hatched between sediments that had been exposed to saltwater for ten days versus those that were not.

My results suggest that saltwater exposure has minimal effect on diapausing eggs; however, the relationship between saltwater exposure and viability is not straightforward. Bailey et al. (2004) found significant differences in viability when they performed salinity exposure experiments with the eggs of the cladocerans *Bosmina liederi* and *Daphnia longiremis* and the rotifer *Brachionus calyciflorus*. Similarly, Bailey et al. (2006) found significant differences in the abundance of hatched animals among treatments exposed to salinities of 0, 8, and 32‰ water. However, in both of the aforementioned studies the eggs had been isolated from sediment with sugar floatation prior to saltwater exposure, whereas Gray et al. (2005) and the current study conducted whole sediment incubation experiments. The inability of saltwater to reduce the viability of eggs in the latter studies may have resulted from protection provided by the sediments in which they were contained. If this is the case, then BWE exchange will not be effective under operational conditions when eggs in the ballast tanks are embedded in sediments. It is unclear if the eggs embedded in sediment have a high probability of being ejected from the tanks during deballasting, as residual

sediments are often very compact (D. Gray, personal observation). However, if eggs are occasionally ejected from tanks they could still represent a risk to the Great Lakes, despite exposure to saltwater.

Experiments with caged benthic invertebrates (vessels 4, 5, 6) demonstrated that BWE caused mortality rates almost identical to those observed for planktonic species. All but one oligochaete individual perished in exchanged ballast tanks, while 100% of amphipods perished. Since the same was not true for invertebrates caged in the control tank, these results suggest that saltwater exposure during BWE is likely to be 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 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 harbour live invertebrates, though the likelihood of these individuals being discharged during BWE could be very low. Future lab or *in situ* experiments could be conducted to explore the degree to which salinity penetrates through ballast sediments, and the survival of a variety of benthic invertebrates in these sediments.

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 impact of BWE on zooplankton recruitment and benthic invertebrate survival, I was able to demonstrate that

BWE provides a high degree of protection for the Great Lakes. My experiments demonstrate that ships that engage in BWE while moving across the ocean between freshwater ports of call, can discharge ballast into freshwater that equals or exceeds proposed performance standards (IMO 2004). However, exposure of diapausing eggs to saline water during BWE did not alter their viability. A risk of invasions *via* diapausing eggs remains if they are discharged into freshwater habitats (Gray et al. 2006). While my 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.

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: UW- upper wing; DB – double bottom.

Vessel	Departure port	Destination port	Vessel type	Date of voyage	Ballast tank type
1	Hamilton, Ontario	Cartagena, Spain	BC	10/01/04 – 10/18/04	UW
2	Hamilton, Ontario	Hamburg, Germany	CT	07/23/05 – 08/09/05	UW
3	Montreal, Quebec	Rotterdam, Holland	BC	09/29/05 – 10/11/05	DB
4	Hamilton, Ontario	Hamburg, Germany	CT	12/05/05 – 12/20/05	UW
5	Hamilton, Ontario	Hamburg, Germany	CT	04/25/06 – 05/09/06	UW
6	Hamilton, Ontario	Reyðarfjörður, Iceland	BC	09/01/06 – 09/14/06	UW

Table 2. Calculated ballast water exchange efficiency based upon density of zooplankton (individuals/m^3) in matched control and experimental ballast tanks. 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. Vessel numbers refer to those listed in Table 1.

Vessel	Taxon	Exchange efficiency (%)	Density in exchanged tank at T_1 (ind. per m^3 ± SD)
1	Copepoda	100.0	-
	Mesocyclops edax	100.0	-
	Cladocera	100.0	-
	Daphnia mendotae	100.0	-
	Rotifera	100.0	-
	Keratella cochlearis	100.0	-
	All zooplankton	100.0	-
2	Copepoda	100.0	-
	Mesocyclops edax	100.0	-
	Cladocera	97.8	0.8 ± 0.8
	Daphnia mendotae	95.1	0.8 ± 0.8
	Rotifera	97.9	2.6 ± 1.5
	Keratella cochlearis	99.3	0.8 ± 0.8
	All zooplankton	99.4	3.4 ± 4.0
3	Copepoda	-	-
	Mesocyclops edax	-	-
	Cladocera	-	-
	Rotifera	-	-
	Ascomorpha	-	-
	All zooplankton	-	-
4	Copepoda	100.0	-
	Diacyclops thomasi	100.0	-
	Cladocera	100.0	-

	Bosmina coregoni	100.0	-
	Rotifera	100.0	-
	Synchaeta kitina	100.0	-
	All zooplankton	100.0	-
5	Copepoda	99.9*	6.3 ± 8.3
	Diacyclops thomasi	99.9*	6.3 ± 8.3
	Cladocera	100.0	-
	Bosmina coregoni	100.0	-
	Rotifera	99.9*	1.0 ± 1.8
	Polyarthra vulgaris	99.9*	1.0 ± 1.8
	All zooplankton	99.9*	7.3 ± 10.0
6	Copepoda	-	-
	Diacyclops thomasi	-	-
	Cladocera	-	5.6 ± 2.0
	Chydorus sp.	-	5.6 ± 2.0
	Rotifera	-	-
	Pompholyx sulcata	-	-
	All zooplankton	-	5.6 ± 2.0

Table 3. Mean diapause egg density per 300g of supplemented ballast sediment placed in incubation chambers.

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	2958	334.4	784.4	2253	1801
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 Number of Eggs	661.2	4045.2	2588.6	999.8	3553.2	2835.6

Table 4. Mean (± 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 5. 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.

	Vessel					
Species	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	-	-	-

Table 6. List of species that emerged during laboratory incubation experiments. Exchanged tank refers to sediments collected from incubation chambers in the tank that underwent ballast water exchange, while control tank represents sediment collected from incubation chambers in the tank that did not undergo exchange. Experiment number corresponds to the vessel numbers listed in Table 1. X – species hatched from this sediment; O – species did not hatch.

Experiment	Group	Species	Exchanged tank	Control tank
1	Rotifera	*Brachionus angularis*	O	X
		Brachionus bidentata	X	O
		Brachionus budapestinensis	O	X
		Brachionus calyciflorus	O	X
		Filinia longiseta	X	O
		Keratella valga	O	X
	Cladocera	*Daphnia* sp.	O	X
		Diaphanosoma birgei	X	X
	Copepoda	Unidentified nauplii	X	O
2	Rotifera	*Brachionus angularis*	X	X
		Brachionus bidentata	O	X
		Brachionus budapestinensis	X	X
		Brachionus calyciflorus	X	X
		Brachionus urceolaris	X	O
		Filinia longiseta	X	O
		Polyarthra major	X	X
		Pompholyx sulcata	X	X
		Trichocerca pusilla	X	X
	Copepoda	Unidentified nauplii	X	O
3	Rotifera	*Ascomorpha* sp.	X	O
		Asplanchna sp.	O	X
		Brachionus angularis	X	X
		Brachionus bidentata	X	X
		Brachionus budapestinensis	X	O
		Brachionus calyciflorus	X	X
		Brachionus caudatus	X	O
		Brachionus havanaensis	X	X
		Brachionus quadridentatus	X	O

		Filinia longiseta	X	X
		Keratella quadrata	X	X
		Polyarthra dolichoptera	X	X
		Proales sp.	X	X
		Synchaeta grandis	X	X
		Synchaeta kitina	X	O
		Trichocerca pusilla	X	X
	Cladocera	*Diaphanosoma brachyurum*	X	X
	Copepoda	Unidentified nauplii	X	X
4	Rotifera	*Brachionus bidentata*	X	X
		Brachionus calyciflorus	X	X
		Filinia longiseta	X	X
		Trichocerca pusilla	X	X
		Brachionus budapestinensis	X	X
5	Rotifera	*Asplanchna brightwelli*	O	X
		Brachionus angularis	X	X
		Brachionus bidentata	X	O
		Brachionus budapestinensis	X	X
		Brachionus calyciflorus	X	X
		Brachionus urceolaris	O	X
		Collotheca pelagica	X	O
		Filinia longiseta	X	X
		Keratella testudo	X	X
		Keratella valga	X	X
		Polyarthra dolichoptera	X	X
		Pompholyx sulcata	O	X
		Synchaeta sp.	X	X
		Trichocerca pusilla	X	X
	Cladocera	*Diaphanosoma* sp.	X	O
	Copepoda	Unidentified nauplii	X	O
6	Rotifera	*Brachionus angularis*	X	X
		Brachionus calyciflorus	X	X

Table 7. Mean values with standard deviations (SD) for the total number of individuals that hatched during incubation experiments for sediments collected from incubation chambers in the tank that underwent ballast water exchange (exchanged tank) and the tank that did not undergo exchange (control tank). *P*-values display the results of *t*-tests performed to test for a difference in hatching between exchanged and control tanks. The Dunn-Sidak corrected p-value for 0.05 level of significance is 0.009.

Experiment	Data Category	Exchanged tank		Control tank		*P*-value
		n	Mean (SD)	*n*	Mean (SD)	
1	Total hatching	4	1.50 (0.58)	4	2.00 (1.41)	0.549
	Species richness	4	1.75 (0.96)	4	1.75 (0.96)	1.000
2	Total hatching	4	8.00 (0.84)	4	4.40 (1.2)	0.033
	Species richness	4	3.00 (1.22)	4	2.60 (1.95)	0.708
3	Total hatching	4	19.40 (10.26)	4	14.60 (7.44)	0.422
	Species richness	4	6.40 (2.51)	4	6.60 (1.67)	0.866
4	Total hatching	4	3.50 (0.58)	4	2.00 (0.82)	0.024
	Species richness	4	2.00 (0.86)	4	1.25 (0.50)	0.168
5	Total hatching	4	12.50 (5.51)	4	19.25 (4.50)	0.106
	Species richness	4	5.40 (3.58)	4	6.40 (3.65)	0.673
6	Total hatching	4	2.00 (0.82)	4	2.50 (0.58)	0.356
	Species richness	4	1.25 (0.50)	4	1.25 (0.50)	1.000

Figure 1. Map of pathways taken by six transoceanic vessels from the Laurentian Great Lakes to European ports. Locations of mid-ocean exchange are identified with a star pattern. Numbers 1-6 refer to the vessels listed in Table 1. Note that vessels 2 and 4 shared the same terminal port.

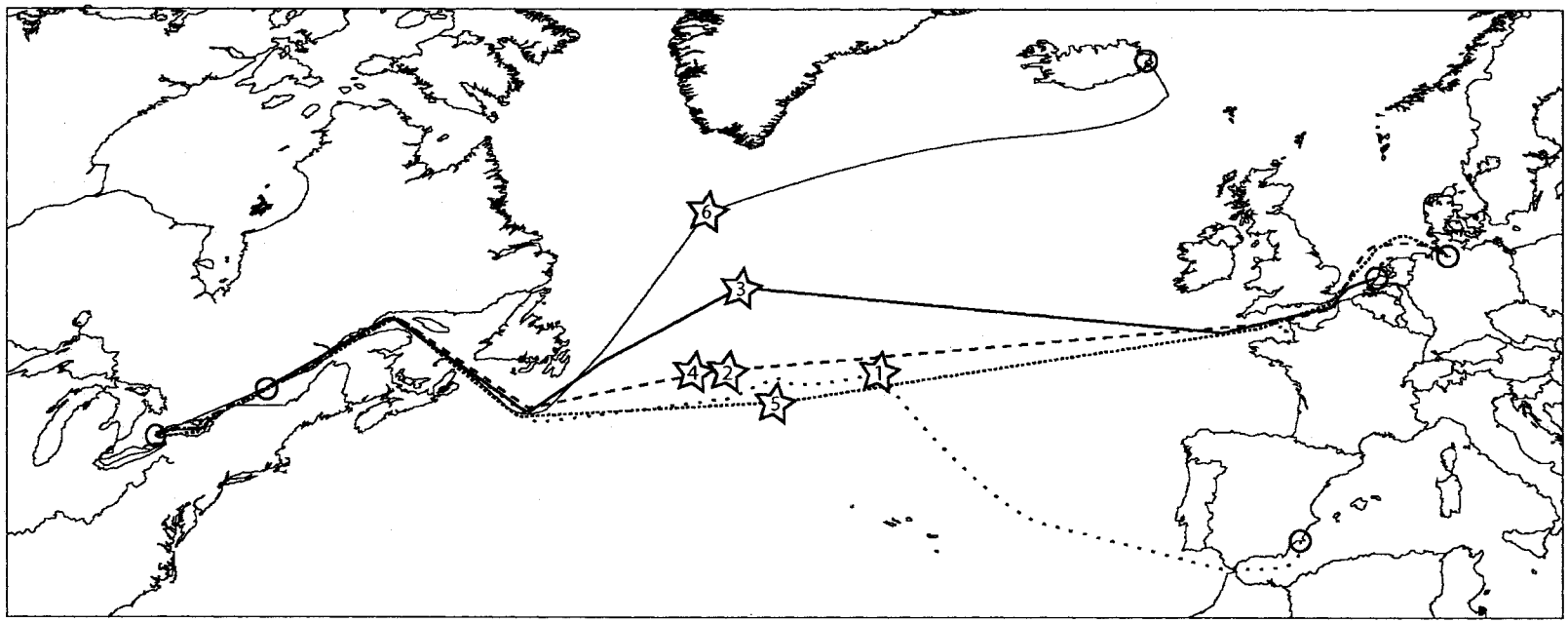


Figure 2. Polyvinyl chloride incubation chambers installed in an empty ballast tank. Side and top windows were covered in 60µm nitex-mesh.

Figure 3. Temperature, dissolved oxygen, and salinity measurements obtained from water quality instruments installed in ballast tanks of vessels 3 and 6.

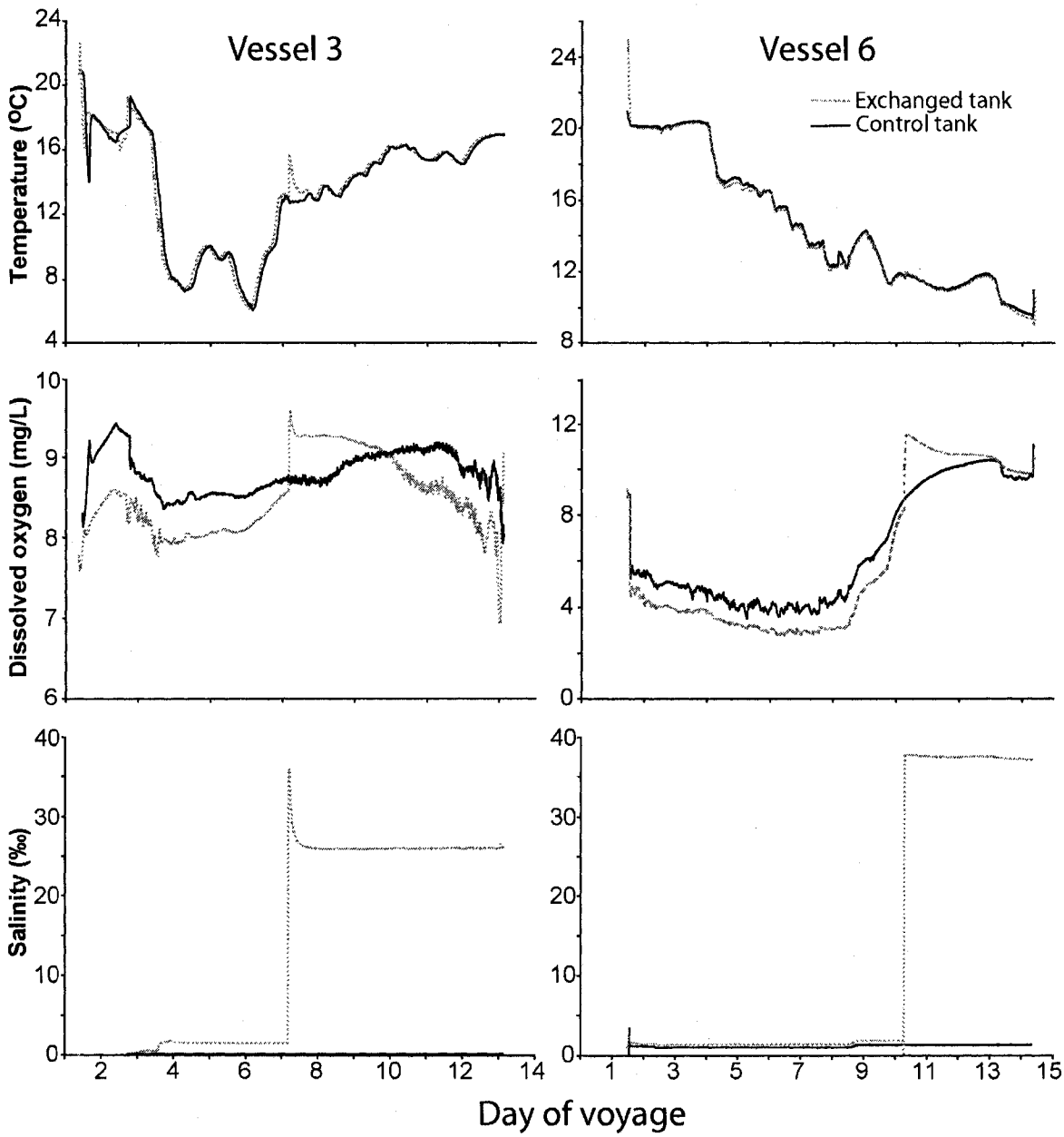
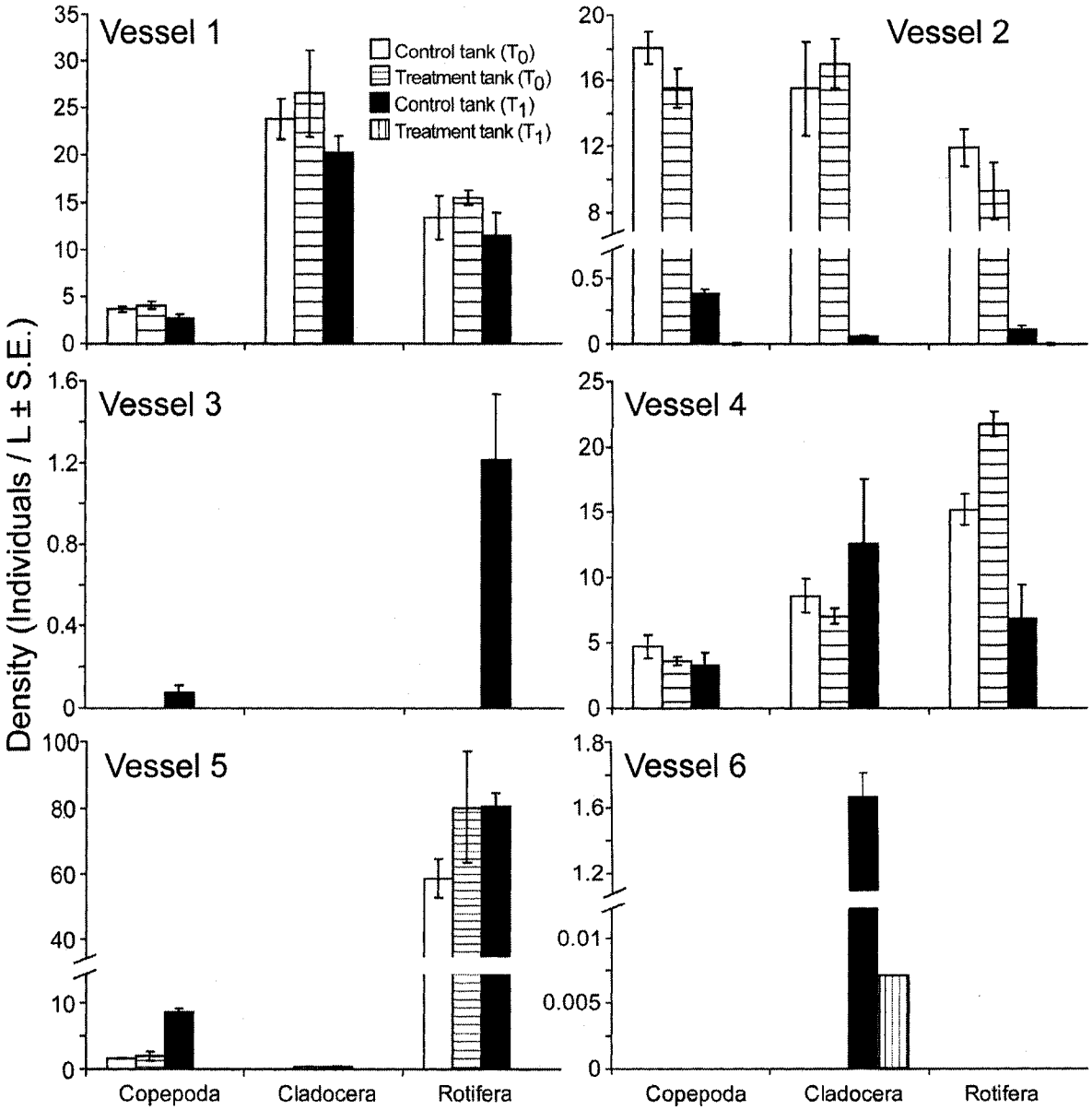


Figure 4. Density (Individuals/L ± SE) of copepods, cladocerans, and rotifers sampled from treatment (exchanged) and control (not exchanged) ballast tanks at the beginning (T_0) and end (T_1) of the ships' voyage. Note breaks in the y-axis for vessels 2, 5, and 6. Despite these breaks, values for the exchanged tanks of vessels 2, 5, and 6 at T_1 are not visible on this graph. Please refer to Table 2 for this data. No T_0 samples were not collected for ships 3 or 6 (see Methods).



Chapter 3

Can sodium hypochlorite reduce the risk of species introductions from diapausing invertebrate eggs in non-ballasted ships?

Abstract

Many transoceanic vessels enter the Great Lakes carrying residual ballast water and sediment that harbours live animals and diapausing eggs. In this study, I examine the potential for sodium hypochlorite (NaOCl) to reduce the risk of species introductions from diapausing invertebrate eggs in residual ballast sediment. Icollec ted sediment from three transoceanic vessels and from Lake Erie and exposed samples to NaOCl concentrations between 0 and 10000mg/L for 24 hours. Hatching success was reduced by >89% in all four experiments at 1000mg/L relative to unexposed controls. Fewer species hatched at high than at low NaOCl concentrations. Based on an average residual ballast of 46.8 tonnes, the volume of NaOCl required to treat inbound vessels is 374L. Impacts of NaOCl use could be minimized by neutralization of treated residuals with sodium bisulfite. Further research is needed, however, to evaluate the effect of NaOCl on ballast tank corrosion.

Introduction

Greater than 90% of inbound shipping traffic to the Great Lakes is comprised of vessels carrying cargo (Colautti et al. 2003). These vessels declare no-ballast-on-board (NOBOB) status when entering the Great Lakes, and are exempt from existing ballast exchange regulations (U.S. Coast Guard 1993). Such regulations in ballasted ships are intended to purge non-indigenous species (NIS) from ballast tanks, and kill those remaining in the tanks with high salinity water (U.S.

Coast Guard 1993). Owing to design constraints, ballast tanks on NOBOB vessels typically contain residual water and sediment that support an abundance and variety of live invertebrate species, and hundreds of thousands of viable invertebrate diapausing eggs, including those of NIS (Bailey et al. 2003; Bailey et al. 2005a; Duggan et al. 2005). Such species can be introduced to the Great Lakes when a NOBOB vessel loads and then subsequently discharges ballast during multi-port operations within the system. Diapausing eggs may enter the system either directly by disturbance of the residual sediment during deballasting, or, more likely, as live individuals after hatching within the ballast tanks (Bailey et al. 2005b).

Designing treatment strategies to reduce the risk posed by diapausing invertebrate eggs could prove difficult because they are contained within the ballast sediments and are not easily flushed from the tanks (Bailey et al. 2005a), and because the eggs are resistant to a wide array of adverse conditions including freezing, desiccation, disinfection, and anoxia (Gilbert 1974; Hairston 1996; Williams 1998). Laboratory experiments indicate that salt water exposure, as might be achieved with open ocean ballast tank flushing, is an ineffective treatment method for this life stage but effective for water-borne active stages (Gray et al. 2005; Bailey et al. 2006). Here I examine whether exposure to sodium hypochlorite (NaOCl) can reduce the risk of introductions from diapausing eggs contained in NOBOB vessels entering the Great Lakes. This chemical oxidizes live tissue, and is also effective on cysts of parasitic

tapeworms (Zehnder et al. 2002; Karaoglanoglu et al. 2004). At low concentrations (e.g. 1.0mg/L), NaOCl increases hatching success of diapausing eggs or cysts, owing to its antibacterial activity and scarification effect on egg coverings (Balompapueng et al. 1997; Douillet 1998). Biocidal effects of NaOCl when used at high concentrations on diapausing eggs have been investigated, though studies have been conducted for a limited number of species and only on eggs isolated from sediment (Pati and Belmonte 2003; Sano et al. 2004). Consequently it is difficult to determine the effectiveness of sodium hypochlorite as a biocide for ships' ballast. To evaluate this potential, I performed acute toxicity experiments by exposing whole sediments containing diapausing eggs of invertebrate species to concentrations of NaOCl ranging from 0 to 10000mg/L (active ingredient) for 24 hours.

Methods

Sample collection

Ballast sediment for experimentation was obtained from three transoceanic vessels inbound to the Great Lakes on November 2001, July 2003, and June 2005 (sediments 1, 2, and 3, respectively). Sediment was collected from at least five areas of the ballast tanks using sterile scoops and spatulas. An additional, natural sediment sample was collected in March 2005 from Lake Erie at the marina in Amherstburg, Ontario, Canada using a 15cm x 15cm ponar grab (Sediment 4). Sediment samples were stored in the dark at 4°C for at least three

weeks prior to their use in experiments in order to allow diapausing eggs to experience a refractory period (Marcus and Lutz 1998; Jo and Marcus 2004). To evaluate the density of diapausing eggs present in the sediments, three 40g replicates from each sediment source were washed through a 45μm sieve to remove fine sediment. The eggs were then separated from the sediment using a Ludox® HS-40 protocol (Burgess 2001) and were enumerated under a dissecting microscope at ~32X magnification. Organic carbon content was measured using loss-on-ignition methods (K. Drouillard, Great Lakes Institute for Environmental Research, Windsor, Ontario).

NaOCl Exposure experiments

Studies testing NaOCl as a biocide for live invertebrates in ballast have demonstrated that the quantity of sediment present can affect residual chlorine levels, and hence treatment success (BMT Fleet Technology Ltd. 2002). To ensure that the sediment:water ratio used for experiments was representative of conditions in NOBOB vessels, experimental sediment and water volumes equated those measured from NOBOB vessels entering the Great Lakes. Data indicate that NOBOB vessels entering the Great Lakes average 15 tonnes of residual sediment and 46.8 tonnes of residual water, representing ~1:3 sediment:water ratio (Duggan et al. 2005). Consequently, I used 150mL of NaOCl solution and 40g of sediment to give a sediment:water ratio similar to that expected for vessels entering the Great Lakes.

After removal from storage, sediment was thoroughly mixed and 40 g was distributed into 500mL glass vessels. NaOCl solution (150mL) ranging in concentration from 0 to 10000mg/L was added to each vessel, which were then agitated by hand for approximately five seconds. Each of five treatment levels of NaOCl concentration was replicated three times. NaOCl solutions were prepared by mixing quantities of commercial Javex® bleach (5.25% NaOCl by volume) with synthetic pond water (Hebert and Crease 1980). NaOCl concentrations used for the first experiment (Sediment 1) were 0, 50, 100, 1000 and 10000mg/L. Based on results from this experiment, which suggested lower concentrations (50, 100mg/L) were ineffective, I altered the treatment concentrations for the subsequent experiment (Sediment 4) to 0, 500, 1000, 5000, and 10000mg/L. Significant reductions in hatching seemed to occur at NaOCl concentrations between 1000 and 5000mg/L, thus the final two experiments (Sediments 2 and 3) included a 2500mg/L treatment.

After adding NaOCl solution to the vessels, they were placed in an environmental chamber at 20°C with a 16:8 light:dark cycle. Sediment was exposed to NaOCl solution for 24 hours, after which time water from each vessel was carefully decanted and replaced with fresh synthetic pond water. This exchange procedure was performed twice in succession with each vessel - allowing time between water changes for sediment to settle - to ensure that any residual NaOCl solution left after the first exchange was adequately diluted. Each exchange left ≤10mL of the media within the sediment, resulting in an

NaOCl concentration of approximately 62.5 and 666mg/L after the first exchange for vessels treated at 1000 and 10000mg/L, respectively. After the second exchange this solution would be diluted to 3.9 for 1000mg/L treatments and to 44 for 10000mg/L treatments. This is likely an overestimate of the residual chlorine levels since the sediment chlorine demand would have lowered the available chlorine levels after the 24 hour exposure period. However, even at concentrations of 3.9 and 44mg/L I would not expect a significant effect on hatching success as it was not significantly altered at NaOCl levels ≤100mg/L in this study.

Vessels were checked for hatching every 48 hours by carefully decanting the water through a 30μm sieve. Decanted water was replaced in each vessel, and the vessel returned to the environmental chamber. Vessels were checked for up to 20 days, and the experiment was terminated when no hatching occurred on any day after the first 10 days. Individuals were enumerated under a dissecting microscope and species identified using Stemberger (1979) and Balcer et al. (1984).

Data analysis

I calculated the reduction in hatching success in treatments relative to controls as:

$$\% \text{ reduction} = 100((HC-HT)/HC)$$

where HC and HT are the mean number of individuals hatched in control (0% NaOCl) and experimental (≥50mg/L NaOCl) treatments. Use of this index precludes problems that could occur when comparing results obtained from sediments with differing densities of diapausing eggs. Total abundance and species richness data was non-normal (Lilliefors' test; $p<0.05$), and transformation of the data failed to yield a normal distribution. As a result, nonparametric Kruskal-Wallis Analysis of Variance (ANOVA) tests were performed to determine if the abundance or species richness of hatched individuals differed significantly among treatments exposed to different NaOCl concentrations.

Results and Discussion

Egg densities for the sediments used for experimentation ranged from 41.6 eggs / 40g in sediment 2 to 269.7 eggs / 40g in sediment 3 (Table 1). Rotifer eggs were numerically dominant in all sediments, representing between 63% and 96% of eggs. However, copepod eggs were highly abundant in the sediment collected from Lake Erie, and cladoceran eggs were present in low numbers in all sediments. Organic carbon content was high in sediment 3 and in Lake Erie Sediment at 22.8% while sediments 1 and 2 had 11.6% and 10.5% organic carbon, respectively.

A total of 20 rotifer and cladoceran species hatched from diapausing eggs during this study (Table 2). Nineteen species hatched from sediment collected

from Lake Erie (sediment 4), six species hatched from sediments 2 and 3, and two hatched from sediment 1. Copepod nauplii hatched from sediments 2 and 4, but could not be cultured to an identifiable stage.

The abundance of hatched individuals differed significantly among treatments with differing NaOCl concentrations for all four experiments (ANOVA's, $p<0.05$ in all cases). Declines in the abundance of hatched individuals were especially prominent in treatments of NaOCl solution of ≥500mg/L (Fig. 1). 500mg/L NaOCl exposure resulted in a 24%, 59%, and 93% reduction in hatching compared to controls with sediments 4, 3, and 2, respectively (Fig. 2). The marked reduction in hatching at 500mg/L in sediment 2 could be related to its lower organic carbon content. While sediment 2 had 10.53% organic carbon, sediments 3 and 4 would presumably have exerted a higher chlorine demand with organic carbon values more than twice as high at 22.8%. Exposure to 1000 mg/L NaOCl reduced hatching by approximately 89, 94, and 90% with sediments 4, 1 and 3, respectively, and completely inhibited hatching with sediment 2 (Fig. 2).

The amount of NaOCl solution (usually 500 or 1000mg/L) required to reduce hatching success in this study was much higher than that observed in previous trials that used diapausing eggs or cysts isolated from sediment. For example, exposure to 53mg/L NaOCl solution for 24 hours killed 90% of isolated *Artemia* cysts (Sano et al. 2004), while ~77mg/L killed 99% of *Daphnia magna* ephippia (BMT Fleet Technology Ltd. 2002). The presence of sediment is likely

responsible for the high concentrations required in this study, as it serves as a physical barrier preventing or limiting exposure of the eggs to the biocide. As well, sediment generates a high chlorine demand, reducing the effective concentration of NaOCl to which the diapausing eggs are exposed (Sano et al. 2004; BMT Fleet Technology Ltd. 2002).

In addition to a reduction in the abundance of hatched individuals, NaOCl exposure significantly reduced the number of species that emerged in experiments with sediments 3 and 4 (Fig. 3; ANOVAs, $p<0.05$ in both cases). Experiments with sediment 4 averaged 11.0 species in control replicates, but only 5.3 species in the 1000mg/L treatment. Similarly, experiments with sediment 3 revealed an average of 2.3 species in controls but only 0.6 species in the 1000mg/L treatment. In experiments with sediments 1 and 2, too few individuals hatched at concentrations above zero to meaningfully compare species richness among treatments. The reduction in species richness found with sediments 3 and 4 could be due to interspecific differences in tolerance to NaOCl exposure, or it may reflect the reduced probability of observing rare species when their abundances are low.

The high NaOCl concentration required to prevent hatching of diapausing eggs in this study indicates that NaOCl treatment would not be feasible for vessels with filled ballast tanks, which can contain between ~4000 and 14000 tonnes of ballast (Niimi and Reid 2003). However, NOBOB cargo vessels typically contain relatively small volumes of residual water (average 46.8, range

0.0-153.0 metric tonnes per vessel; Duggan et al. 2005). To achieve a 90% or more reduction in hatching, water in ballast tanks would need to be exposed to a solution of NaOCl of at least 1000mg/L for 24 hours before being deballasted. Assuming the use of industrial grade NaOCl (12.5% NaOCl by volume), between 0 and 1224L (average 374L) would be required to treat the full range of NOBOB ships surveyed recently on the Great Lakes (Duggan et al. 2005). Considerably less volume would be required for vessels that did not intend to discharge water from all of their tanks while operating within the system, or for ships that contain design features, such as a dedicated stripping system, intended to limit sediment and water accumulation in tanks.

Use of NaOCl for treating diapausing invertebrate eggs in NOBOB vessels could have several advantages. First, the NaOCl concentration needed to eliminate many live invertebrates and algae is far lower than the 1000mg/L treatment level suggested in this study (BMT Fleet Technology Ltd. 2002; Sano et al. 2004). This suggests that the use of NaOCl would not only reduce the invasion risk from diapausing invertebrate eggs, but it would kill live individuals present in ballast residuals. Second, the chlorine in treated ballast water could be treated with sodium bisulfite before discharge into the Great Lakes, thereby minimizing its environmental impacts (BMT Fleet Technology Ltd. 2002). The dose of sodium bisulfite required to neutralize residual chlorine could itself act as a biocide before reacting with the residual chlorine since this compound has been shown to be toxic to *Daphnia magna*, resulting in immobility and mortality at

solutions greater than 80mg/L (Freeman and Fowler 1953; Dowden and Bennett 1965). Third, the cost of using NaOCl would be relatively low. Using an average contract price of ~$0.13US/L industrial grade NaOCl (Kirschner 2003) costs of purchase for the average vessel would only be ~$50US. Additional hardware or administrative costs would be necessary for the acquisition of NaOCl, its safe storage, and for safe dosing of ballast tanks. As well, regulatory hurdles pertaining to discharge of chlorinated residuals would have to be addressed.

Despite the encouraging results from this study, further research is required before the use of NaOCl in NOBOB vessels can be seriously considered. First, a method to measure the amount of chlorine present in the tanks over time is needed to ensure that an effective concentration is maintained, and that the correct amount of neutralizing agent can be added before discharge (see Gracki et al. 2002). The dose of NaOCl needed for treatment could differ considerably among ships due to differing chlorine demands of the residual water and sediments. In cases where significant amounts of sediment are present, the amount of organic matter in the sediment will be of paramount importance (BMT Fleet Technology Ltd. 2002). Levels of dissolved organic carbon and ammonia could also have an impact when sediment loads are low (Lin and Evans 1974; Reckhow and Inger 1990). Dissolved organic carbon and sediment loads differ among vessels (Duggan et al. 2005; Johengen et al. 2005), emphasizing the importance of measuring residual chlorine levels. Unfortunately, colorimetric methods (Hach Kits) to measure chlorine were not employed in this study,

limiting my ability to evaluate the role of these factors in determining chlorine demand. Presumably residual chlorine in ballast tanks could be measured by colorimetric methods; however a safe and efficient method of extracting water from the tanks would be required. Second, for NaOCl treatment to work effectively it would have to be well mixed within the tanks. This might be facilitated by the movement of the vessel during normal operations, but would probably require that at least 8-10cm of residual water be present in the tanks. Even if the NaOCl is well mixed, it may not penetrate through deep residual sediment. Residual sediment is often very compacted (personal observation), and may provide a refuge for invertebrates, such as nematodes, which were occasionally found alive in this study after treatment with 1000mg/L NaOCl for 24 hours. However, the effect of these refugia on treatment success is probably minimal since diapausing eggs and invertebrates deep within the sediments would probably not be available for discharge during ballasting operations. Organisms close to the sediment surface that could be resuspended during ballasting operations would have presumably been exposed to the NaOCl treatment and perished as a result. Third, studies on the impact of NaOCl on ballast tank and piping corrosion at 1000mg/L or higher is imperative since hypochlorous acid can increase corrosion of steel in water under some conditions (Gracki et al. 2002). BMT Fleet Technology Ltd. (2002) noted accelerated corrosion at 10mg/L NaOCl, suggesting that dosing with 1000mg/L could be a major concern. I contacted individuals familiar with ballast treatment

technology and ballast coatings. All suggested that dosing with 1000mg/L bleach could have a negative impact on ballast coatings and tank corrosion (T. Wilkins, INTERTANKO; D. Stocks, BMT Fleet Technology Ltd.; Capt. P.T. Jenkins, Philip T. Jenkins & Associates Ltd.; J.A. van Marle, Valvoline EMEA, personal communications). However, the typical vessel would only enter the Great Lakes a maximum of five times each shipping season (P.T. Jenkins, Philip T. Jenkins and Associates Ltd., personal communication), requiring a maximum of five 24 hour NaOCl treatments. It should be noted that quantitative data on the impact of this treatment regime are lacking. Therefore, I propose that additional work is needed on this topic before reaching any conclusions. If damage is significant, NaOCl treatment may not be a viable option unless residual sediment loads are decreased, which would allow for lower NaOCl dosages. Currently, chlorine treatment is required for vessels entering Buenos Aires, Argentina from areas in which cholera is endemic, and Chile provides vessels with the option of using powdered NaOCl as a substitute for ballast exchange (100 mg/L; INTERTANKO, http://www.intertanko.com/tankerfacts/environmental/ ballast/ballastreq.htm). Thus, a precedent exists with respect to use of chlorine for ballast water treatment.

In conclusion, my results suggest that the use of NaOCl in vessels could represent an effective method for reducing the risk of future species introductions via invertebrate resting stages contained in NOBOB ships' residual sediments. Although NOBOB ballast residuals have been studied most intensively in the

Great Lakes, the risk of introductions from these vessels is probably widespread. NaOCl has the potential to be a cheap, effective, and environmentally benign treatment for all areas with regular shipping traffic and therefore warrants consideration by researchers and policy-makers.

Table 1. Mean diapause egg density per 40g by taxon. NS - natural sediment; BT - ballast tank.

Egg Type	1 BT	2 BT	3 BT	4 NS
Asplanchna	0.3	0.8	--	0.5
Brachionus	85.6	23.8	197.2	22.3
Filinia	--	1.5	0.5	2.3
Synchaeta	--	--	40.3	65.5
Unidentified Rotifera	1.3	3.2	22.4	16.2
Bosmina	1.5	--	1.4	0.8
Daphnia	2.5	--	--	5.2
Unidentified Cladocera	5.3	9	2.3	7.6
Copepoda	0.3	3.3	5.6	48.3
Total	96.8	41.6	269.7	168.7

Table 2. List of species that emerged during hatching experiments after 24 hours exposure to various NaOCl concentrations. NS- Natural sediment (L. Erie); BT- Ballast sediment; X- species present, O - species absent, - treatment not run at this concentration.

Sediment	Group	Species	Treatment (mg/L NaOCl)							
			0	50	100	500	1000	2500	5000	10000
1 BT	Rotifera	*Brachionus angularis*	X	X	X	-	O	-	-	O
		Brachionus calyciflorus	X	X	X	-	X	-	-	O
2 BT	Rotifera	*Brachionus angularis*	X	-	-	O	O	O	O	-
		Brachionus bidentata	X	-	-	O	O	O	O	-
		Brachionus calyciflorus	X	-	-	O	O	O	O	-
		Keratella quadrata	X	-	-	O	O	O	O	-
		Proales sp.	X	-	-	O	O	O	O	-
		Trichocerca pusilla	X	-	-	O	O	O	O	-
	Copepoda	Copepod nauplii	X	-	-	X	O	O	O	-
3 BT	Rotifera	*Brachionus calyciflorus*	X	-	-	X	X	O	O	-
		Brachionus urceolaris	O	-	-	O	X	O	O	-
		Keratella cochlearis	O	-	-	X	O	O	O	-
		Pompholyx sulcata	X	-	-	O	O	O	O	-
		Synchaeta kitina	X	-	-	O	O	O	O	-
		Trichocerca pusilla	X	-	-	X	O	O	O	-
4 NS	Rotifera	*Asplanchna brightwelli*	X	-	-	X	O	-	O	O
		Brachionus angularis	X	-	-	X	X	-	O	O
		Brachionus bidentata	O	-	-	X	O	-	O	O
		Brachionus budapestinensis	X	-	-	O	O	-	O	O

	Brachionus calyciflorus	X	-	-	X	O	-	O	O
	Brachionus caudatus	X	-	-	O	O	-	O	O
	Brachionus urceolaris	O	-	-	X	O	-	O	O
	Filinia longiseta	X	-	-	X	X	-	O	O
	Hexarthra mira	X	-	-	X	O	-	O	O
	Keratella cochlearis	X	-	-	O	O	-	O	O
	Keratella quadrata	X	-	-	X	X	-	O	O
	Polyarthra dolichoptera	X	-	-	X	X	-	O	O
	Proales sp.	X	-	-	O	O	-	O	O
	Synchaeta grandis	X	-	-	X	X	-	O	O
	Synchaeta kitina	X	-	-	X	X	-	O	O
	Synchaeta lakowitziana	X	-	-	X	O	-	O	O
	Trichocerca pusilla	X	-	-	X	X	-	O	O
Cladocera	*Bosmina longirostris*	O	-	-	X	O	-	O	O
	Diaphanosoma birgei	X	-	-	X	X	-	O	O
Copepoda	Copepod nauplii	X	-	-	X	O	-	O	O

Figure 1. Mean number of individuals (± S.E) hatched from sediments after exposure to various NaOCl concentrations. A- Sediment 1; B- Sediment 2; C- Sediment 3; D – sediment 4 (Lake Erie). Sediments 1-3 were obtained from ballast tanks of NOBOB vessels. Note differences in values on the x and y axes.

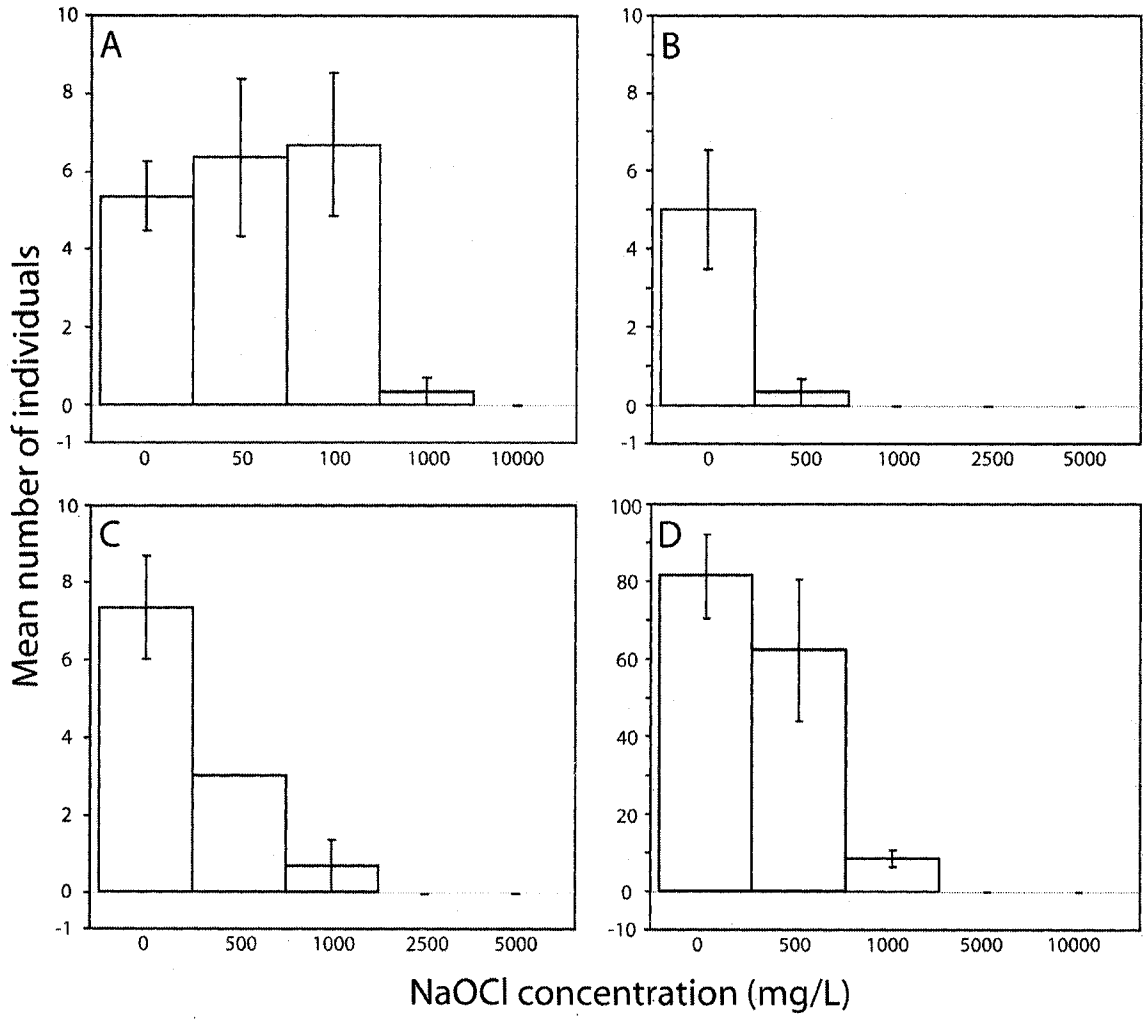


Figure 2. Mean reduction in hatching (± S.E.) observed at various NaOCl concentrations compared to controls. Percent reduction compared to controls was calculated as 100((HC-HT)/HC), where HC and HT are the mean number of individuals hatched in control and experimental treatments, respectively. Values shown are the means for all experiments in which the treatments were run. Values in parentheses indicate the number of experiments in which the NaOCl concentrations were tested.

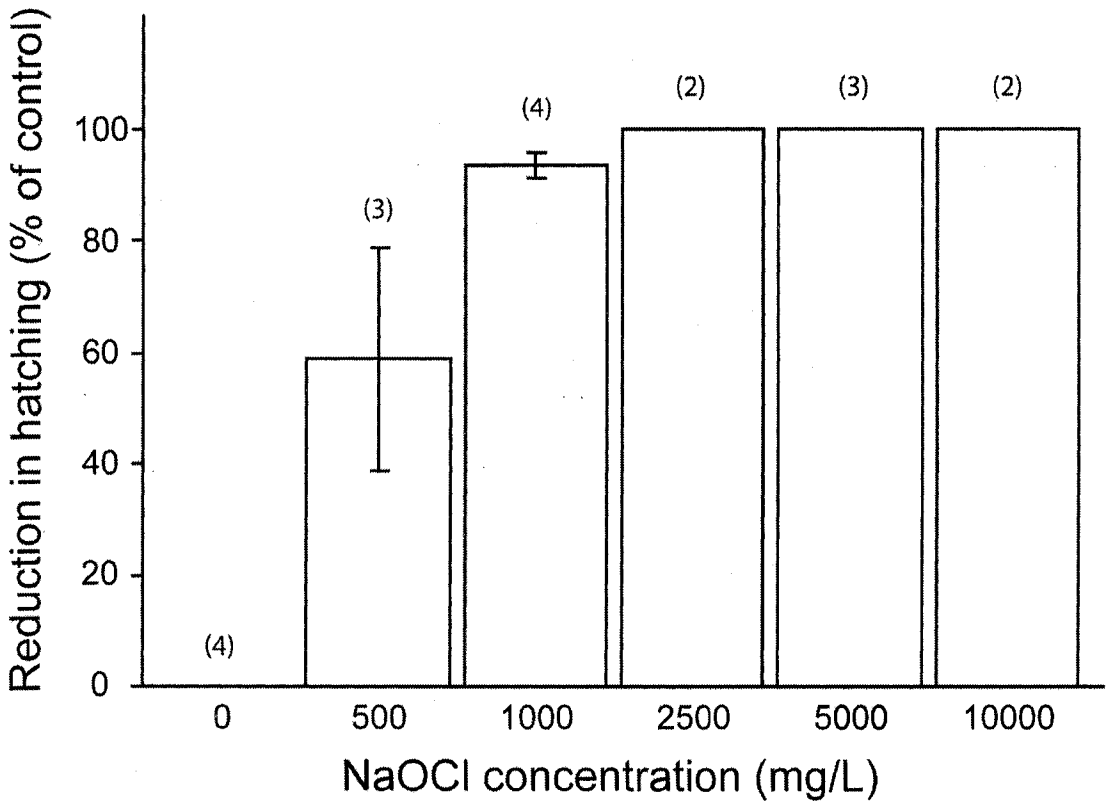
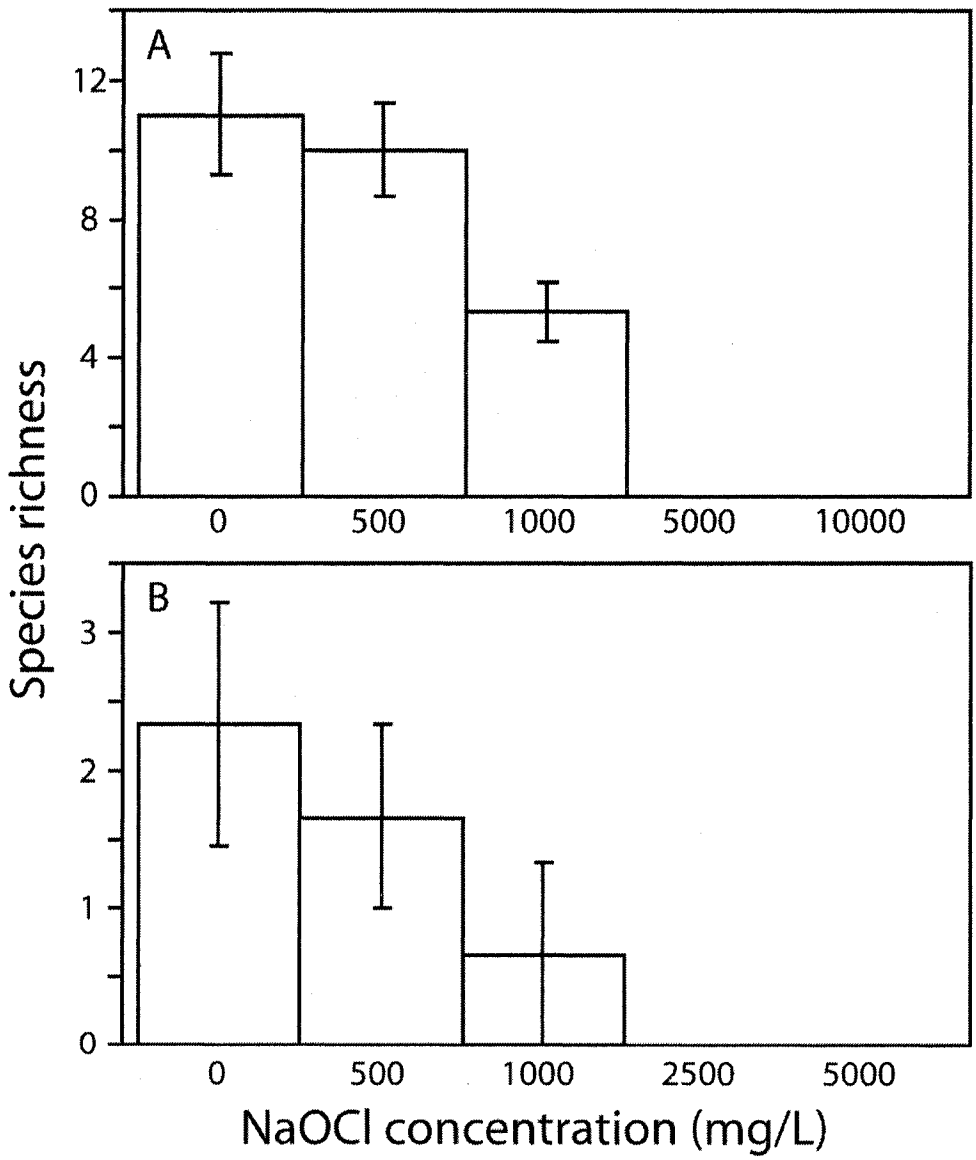


Figure 3. Mean number of species hatched from sediments (± S.E.) after exposure to various NaOCl concentrations. A – Sediment 4; B- Sediment 3. Sediment 1 and 2 data were omitted from the figure since too few individuals hatched at concentrations above zero to meaningfully compare species richness among treatments. Note differences in scales on the y axes.



Chapter 4

Does ballast water exchange provide adequate protection for the Great Lakes?

To protect the Great Lakes from nonindigenous species (NIS) introductions, voluntary (1989) and then mandatory (1993) ballast water exchange (BWE) regulations were enacted for ships entering the system with fresh or brackish ballast. These regulations require inbound vessels to treat or change their ballast while on the open-ocean if they plan to discharge it during subsequent operations on the Great Lakes (United States Coast Guard 1993; Transport Canada 2006). Despite this requirement, reports of new invasions of invertebrate and other taxa have continued to appear (Ricciardi 2006; Pothoven et al. 2007). How are these species getting to the Great Lakes? Does BWE provide adequate protection, or are other treatment methods required for vessels entering the system?

Providing answers to the these questions is complicated by the fact that two classes of cargo vessel enter the Great Lakes, termed no-ballast-on-board (NOBOBs) and ballast-on-board (BOBs) vessels. From their inception in 1993, ballast water exchange regulations have only applied to ships carrying ballast tanks filled with water (BOBs; United States Coast Guard 1993). Ships that enter the Great Lakes filled with cargo often have empty ballast tanks, as ballast is used to compensate for the weight of a load when the ship is not carrying cargo. NOBOB vessels are not required to flush their ballast tanks with saltwater: the United States has put in place a voluntary program that encourages saltwater flushing (United States Coast Guard 2005), while Canadian regulations give NOBOB vessels the option of exchanging their tanks or meeting the Canadian Shipping Federation's Code of Best Practices (Transport Canada 2006). However, NOBOB vessels represent >90% of traffic entering the Great Lakes

(Colautti et al. 2003), and owing to design constraints, each NOBOB vessel contains an average of 15 tonnes of residual sediment and 46.8 tonnes of residual water in their ballast tanks (Duggan et al. 2005). This residual water and sediment often contains an abundance of live invertebrates and diapausing eggs that can be introduced if ballast is taken on and then discharged during multi-port operations within the Great Lakes (Bailey et al. 2003; Duggan et al. 2005).

The lack of mandatory regulations for NOBOB vessels means that it is difficult to determine how effective BWE has been for protecting the Great Lakes. Have the majority of invaders since 1993 been entering in the residual ballast of NOBOB vessels, or are they continuing to arrive in BOB vessels despite the use of BWE? Most recent studies suggest that NOBOBs represent the greatest risk for continued introductions (MacIsaac et al. 2002; Bailey et al. 2003; Gray et al. 2005, 2006; Ricciardi 2006). However, it is impossible to identify which class of vessel (BOBs vs. NOBOBs) has been responsible for recent introductions. Furthermore, it is difficult to establish the exact introduction date for a NIS, as significant time could pass between the introduction of a species and its identification by investigators (Ricciardi 2006). At the moment a species is introduced, the number of individuals discharged into the new habitat is likely to be very small compared to the volume of the receiving waters. Therefore, detection may not occur until: 1) significant population growth has occurred, resulting in a reasonable probability that the animal will be captured if the invaded habitat is surveyed; and 2) Investigators actually perform some type of field survey in the invaded area (Costello and Solow 2003). Some investigators

argue that the majority of recent NIS discoveries in the Great Lakes can be explained by a time lag between invasion and detection (Costello et al. 2006). As a result of these complexities, continuing introductions should not be cited as evidence that BWE has been an ineffective method for preventing introductions to the Great Lakes.

To determine if BWE can provide a reasonable level of protection for the Great Lakes it is necessary to gather information on: 1) the effectiveness of BWE for reducing the concentration of freshwater animals in ballast; and 2) the probability of establishment of animals that survive BWE, should they be released into the Great Lakes. In this thesis I have presented data on the effectiveness of BWE for reducing the concentration of animals in ballast tanks. A recent study by Bailey et al. (in prep.) will provide some information on the probability of species establishment based on discharge densities.

Experiments I conducted aboard six cargo vessels transiting from the Great Lakes to European ports suggests that ballast water exchange is highly effective for removing live zooplankton (>99%) and for causing mortality of benthic invertebrates (>96%; Chapter 2). Final concentrations of zooplankton in exchanged ballast tanks on all six vessels met International Maritime Organization (IMO) criteria for organisms ≥50μm in minimum dimension (IMO 2004), with densities ranging from 0 to 7.3 individuals/m^3. However, the survival of some animals suggests that a small introduction risk could remain after a vessel has conducted BWE.

To further consider the level of protection offered by BWE, it is important to gather information on the probability of establishment for species discharged from ballast tanks. Accurately forecasting which species will invade a given habitat can be a complex task, as a lengthy list of physical, chemical, and biotic factors may need to be incorporated into a predictive model (Drake et al. 2006; Moyle and Marchetti 2006; Williamson 2006). However, within a particular species or group the probability of establishment can be directly related to propagule supply characteristics (i.e., the density and frequency of inoculation events; Grevstad 1999a, 1999b; Lonsdale 1999; Levine 2000; Forsyth and Duncan 2001). Given the relationship between establishment success and propagule supply, the importance of BWE efficiency becomes clear. What is the relationship between initial discharge density and the probability of zooplankton species establishing in the Great Lakes?

To answer this question, Bailey et al. (in prep.) used a modeling approach that incorporated data gathered from mesocosm experiments conducted in the Great Lakes with various cladocerans, including *Bosmina* sp., *Eubosmina* sp., *Ceriodaphnia* sp., *Chydoridae* sp., *Daphnia retrocurva*, and *Daphia galeata*. To successfully establish in a temperate climate, cladoceran species must reach a critical density required for the onset of sexual reproduction (Drake 2004). If the species does not reach this critical density then diapausing egg production will not occur, and the species will fail to survive through the winter (Drake 2004). The model constructed by Bailey et al. (in prep.) provides an estimate of the probability of reaching the critical density required for sexual reproduction (i.e.

probability of establishment) given a range of initial population densities (0-80000 individuals/m^3).

To calculate the probability density of reaching the critical threshold required for sexual reproduction over a short period of time (t), Bailey et al. (in prep.) used the inverse Gaussian distribution:

$$g(t \mid \mu, \sigma^2, d) = (d / [2\pi \sigma^2 t^3]^{1/2}) \exp(-[d + \mu t]^2 / [2\sigma^2 t]) \quad (1)$$

where d is the difference between the log of the critical reproductive threshold density and the log of the inoculum density (N_0) (i.e., d = log $CRTD$ – log N_0), and μ and σ^2 are the mean and variance of log population growth in which day-to-day variation in the deterministic population growth rate and unpredictability can be expressed as probabilities in a stochastic process. They then integrated equation 1 from time t to a specific time horizon (T), resulting in the cumulative distribution function:

$$G(T \mid d, \mu, \sigma^2) = \Phi([-d- \mu T] / [\sigma T^{1/2}]) + \exp(-2\mu d / \sigma^2) \Phi([-d- \mu T] / [\sigma T^{1/2}]) \quad (2)$$

where $\Phi(y)$ is the standard normal distribution:

$$\Phi(y) = (1/2\pi) \int_{-\infty}^{y} \exp(-z^2/2)\, dz \quad (3)$$

Preliminary results from this model developed by Bailey et al. (in prep.) suggest that the IMO regulations (<10 individuals/m^3 ≥50μm in minimum dimension) are

very conservative, resulting in low probabilities of establishment for the cladoceran species they considered.

If the results from Bailey et al. (in prep.) are evaluated in combination with the results of my BWE experiments (Chapter 2), it is possible to estimate the risk of cladoceran introductions to the Great Lakes from ships that have conducted BWE. Of the six vessels that I assessed during my experiments, two contained live cladoceran species in their exchanged ballast tanks at the conclusion of their voyages. These cladocerans were *Daphnia mendotae* at 0.8 individuals/m^3 and *Chydorus* sp. at 5.6 individuals/m^3. According to the model simulations run for these particular species by Bailey et al. (in prep.) there is no chance (0% probability) of establishment given the aforementioned discharge densities. Assuming that my data accurately reflects BWE efficiency for vessels entering the Great Lakes, it would seem that little risk exists for the introduction of freshwater cladocerans.

It is important to note that the model constructed by Bailey et al. (in prep.) constitutes a limited first step toward a more comprehensive methodology incorporating additional complexities intrinsic to the establishment of nonindigenous species. In particular, the model does not take into account total inoculum size, seasonality, and introduction rate, including the timing of multiple introductions. While the density of introduced population will be the prime factor determining the onset of sexual reproduction and establishment, total inoculum size has also been identified as an important factor determining invasion success (Lonsdale 1999; Levine 2000; Forsyth and Duncan 2001), and clearly it should

be considered for future studies. In addition, the timing (seasonality) of an introduction is important, as sufficient time is needed for an introduced population to reach the critical density required for sexual reproduction before the onset of unfavourable environmental conditions (Drake 2004). The rate of introductions should also be considered, as propagule pressure theory predicts that the probability of a successful invasion will increase with multiple attempts (Allendorf and Lundquist 2003, Lockwood et al. 2005).

Additional work is also needed to determine establishment probabilities for copepods, rotifers, and benthic invertebrates, as they were also recovered from tanks at low densities following ballast water exchange (Chapter 2). In addition, further experiments should be conducted to determine if the BWE efficiencies demonstrated in my experiments are typical of all cargo ships entering the Great Lakes. Although a sample size of four vessels may be adequate to estimate exchange efficiency, I do not believe that my assessment was able to capture all of the variability inherent in the BWE process. In particular, most of my experiments were performed using upper wing ballast tanks, which typically accumulate less sediment and are easier to exchange compared to large double-bottom ballast tanks (Johengen et al. 2005). This could have conceivably skewed the results to provide higher overall exchange efficiencies than may be possible for all ballast tanks on a ship. However, I have no reason to believe that this is the case, as BWE on the single pair of double-bottom ballast tanks I assessed resulted in 100% exchange efficiency (Chapter 2).

Combining the use of BWE with other treatment options

A further unknown in the evaluation of the effectiveness of BWE is the risk posed by diapausing invertebrate eggs found in residual ballast sediments. Experiments outlined in chapter 2 demonstrated that BWE dramatically reduced *in situ* recruitment from diapausing eggs, however, exposure to saline water did not change the viability of the eggs should they be returned to fresh water. These eggs could be introduced to the Great Lakes if the residual sediments are disturbed during ballast activities (Gray et al. 2006). It is unclear if eggs embedded in sediment have a high probability of being ejected from the tanks during deballasting, as residual sediments are often very compacted (D. Gray, personal observation). However, if eggs are occasionally ejected from tanks they could still represent a risk to the Great Lakes, despite exposure to saltwater.

The risk of additional NIS introductions from diapausing eggs could be reduced by combining BWE with another treatment. In chapter 3, I presented data evaluating the potential to use sodium hypochlorite (NaOCl) to reduce the viability of diapausing invertebrate eggs. This treatment at 1000mg/L reduced the viability of eggs by > 89% relative to controls. Due to the high concentration required for effective treatment, however, NaOCl would not be practical for fully ballasted (BOB) ships. However, NOBOB vessels entering the Great Lakes are prime candidates for this type of treatment given the small volume of their ballast residuals (Gray et al. 2006). In addition, BOB vessels that have discharged their saltwater ballast could use NaOCl treatment if they anticipate conducting subsequent ballasting activities within the Great Lakes.

Treating ballast water with NaOCl may become more common, as the state of Michigan has approved hypochlorite as an acceptable treatment for vessels to meet discharge standards required by Michigan Senate Bill 332 (Showalter and Bowling 2006). Regulations under this law took effect January 1, 2007 and require that vessels apply for a ballast discharge permit and use ballast treatments approved by the Michigan Department of Environmental Quality (MDEQ) if they intend to discharge ballast in Michigan waters (Showalter and Bowling 2006). Under MDEQ guidelines, treatment is to be conducted at 10ppm (~10mg/L) free chlorine for 19 hours before discharge (Showalter and Bowling 2006). Although this 10ppm treatment can be effective for live organisms (BMT Fleet Technology Ltd. 2002), it may not deactivate diapausing eggs as a significantly higher initial dosage was required for meaningful reductions in egg viability (chapter 3). In addition, further research on the safety of NaOCl treatments may be needed if treating ballast tanks with high concentrations of NaOCl, as there is a potential for ballast tank and piping corrosion (Gray et al. 2006).

Other ballast treatment options are also being explored, including various chemical biocides, such as chlorine, ozone, menadione, and peracetic acid, as well as UV light, filtration, deoxygenation, electrolysis, sonication, and heat (Sutherland et al. 2001; Rigby 2004; Faimali et al. 2006; Herwig et al. 2006; Raikow 2006; Veldhuis et al. 2006). A salt brine treatment currently under investigation by the Great Lakes Environmental Research Laboratory (GLERL) in Ann Arbor, Michigan in collaboration with the Smithsonian Environmental

Research Center in Edgewater, Maryland is particularly promising. It may offer a cheap, effective tool by which to treat ballast tanks on NOBOB vessels that have fresh or low-salinity residual ballast water and that were unable to flush their tanks with seawater before entering the St. Lawrence Seaway (Dr. David Reid, GLERL, personal comm.). All of the above treatments could be combined with ballast water exchange to achieve optimal results.

Conclusions

Given the effectiveness of BWE and the resulting low probability of establishment for most species (Bailey et al. in prep.), I propose that BWE provides adequate protection for the Great Lakes, at least with respect to the groups studied (i.e. planktonic and benthic invertebrates). However, more research is required before reaching a definitive conclusion, as it is possible that other taxa may behave dissimilarly to the invertebrates studied here. In particular, additional data should be collected for phytoplankton, bacteria, viruses, and fish, to determine if BWE functions as well for these groups as it does for invertebrates.

To ultimately reach a conclusion on the need for ballast treatment systems, a cost-benefit analysis should be conducted to determine if the benefits of installing ballast treatment systems in transoceanic vessels -- in terms of risk reduction and the prevention of future economic and environmental impacts -- outweigh the costs involved with research, development, and deployment of these systems. To conduct this analysis, the risk of introductions for some

species (cladocerans) can be estimated from data in Bailey et al. (in prep.) in combination with data from Chapter 2 of this thesis. However, much more research will be needed to estimate invasion risk for other groups of organisms. Data on the environmental and economic costs of future invaders may need to be estimated from information found in the literature (Pimentel et al. 2005; Colautti et al. 2006; Dextrase and Mandrak 2006). Data from this type of cost-benefit analysis could allow investigators to replace or supplement BWE regulations with ballast treatment options that are both environmentally sound and economically feasible.

References

Allendorf, F. W. and L. L. Lundquist. 2003. Population biology, evolution, and control of invasive species. *Cons. Biol.*17: 24-30.

Bailey, S. A., I. C. Duggan, C. D. A van Overdijk, P. T. Jenkins and H. J. MacIsaac. 2003. Viability of invertebrate diapausing eggs collected from residual ballast sediment. *Limnol. Oceanogr.* 48: 1701-1710.

Bailey, S. A., I. C. Duggan, C. D. A. van Overdijk, T. H. Johengen, D. F. Reid, and H. J. MacIsaac. 2004. Salinity tolerance of diapausing eggs of freshwater zooplankton. *Freshwat. Biol.* 49: 286-295.

Bailey, S. A., I. C. Duggan, P. T. Jenkins, and H. J. MacIsaac. 2005a. Invertebrate resting stages in residual ballast sediment of transoceanic ships. *Can. J. Fish. Aquat. Sci.* 62: 1090-1103.

Bailey, S. A., K. Nandakumar, I. C. Duggan, C. D. A. van Overdijk, T. H. Johengen, D. F. Reid, and H. J. MacIsaac. 2005b. *In situ* hatching of invertebrate diapausing eggs from ships' ballast sediment. *Divers. Distrib.* 11: 453-460.

Bailey, S. A., K. Nandakumar, and H. J. MacIsaac. 2006. Does saltwater flushing reduce viability of diapausing eggs in ship ballast sediment? *Divers. Distrib.* 12: 328-335.

Bailey, S. A., L-A Velez-Espino, O. E. Johannsson, M. A. Koops and C. J. Wiley (in preparation). Estimating probability of establishment of freshwater Cladocera following introduction at low population density. To be submitted to *Ecol. Appl.*

Balcer M. D., N. L. Korda and S. I. Dodson. 1984. *Zooplankton of the Great Lakes*. University of Wisconsin Press, Madison, WI.

Balompapueng, M. D., N. Munuswamy, A. Hagiwara, and K. Kirayama. 1997. Effects of disinfectants on the hatching of marine rotifer resting eggs *Brachionus plicatilis* Müller. *Aquac. Res.* 28: 559-565.

Bij de Vaate, A., K. Jazdzewski, H. A. M. Ketelaars, S. Gollasch, and G. Van Der Velde. 2002. Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. *Can. J. Fish. Aquat. Sci.* 59: 1159-1174.

BMT Fleet Technology Ltd. 2002. *Ballast water treatment evaluation using copper and sodium hypochlorite as ballast water biocides*. Report to the Michigan Department of Environmental Quality, Surface Water Quality Division, Lansing, MI. October 2002.

Baillie, J. E. M., C. Hilton-Taylor and S. N. Stuart (Editors). 2004. *2004 IUCN Red List of threatened species. A global species assessment*. IUCN, Gland, Switzerland and Cambridge, UK. 191 pp.

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

Choi, K. H., W. Kimmerer, G. Smith, G. M. Ruiz and K. Lion. 2005. Post-exchange zooplankton in ballast water of ships entering the San Francisco Estuary. *J. Plankton Res.* 27: 707-714.

Colautti, R.I., A. J. Niimi, C. D. A. van Overdijk, E. L. Mills, K. Holeck and H. J. MacIsaac. 2003. Spatial and temporal analysis of transoceanic shipping

vectors to the Great Lakes. In *Invasion pathways: analysis of invasion patterns and pathway management*, eds. G. Ruiz, J.T. Carlton and R.N. Mack. Washington: Island Press.

Colautti, R. I., S. A. Bailey, C. D. A. Van Overdijk, K. Amundsen, and H. J. MacIsaac. 2006. Characterised and projected costs of nonindigenous species in Canada. *Biol. Invas.* 8: 45-59.

Costello, C., J. M. Drake and D. M. Lodge. 2006. Evaluating the effectiveness of an environmental policy: ballast water exchange and invasive species in the North American Great Lakes. Ecological Applications *in press.*

Dextrase, A. J., and N. E. Mandrak. 2006. Impacts of alien invasive species on freshwater fauna at risk in Canada. *Biol. Invas.* 8: 13-24.

Douillet, P. 1998. Disinfection of rotifer cysts leading to bacteria-free populations. *J. Exp. Mar. Biol. Ecol.* 224: 183-192.

Dowden, B. F. and H. J Bennett. 1965. Toxicity of selected chemicals to certain animals. *J. Water Pollut. Control Fed.* 37:1308-1316.

Drake, J. M., K. L. S. Drury, D. M. Lodge, A. Blukacz, N. D. Yan and G. Dwyer. 2006. Demographic stochasticity, environmental variability, and windows of invasion risk for *Bythotrephes longimanus* in North America. *Biol. Invas.* 8: 843-861.

Duggan, I. C., C. D. A. van Overdijk, S. A. Bailey, P. T. Jenkins, H. Limen, and H. J. MacIsaac. 2005. Invertebrates associated with residual ballast water and sediments of cargo-carrying ships entering the Great Lakes. *Can. J. Fish. Aquat. Sci.* 62: 2463-2474.

Faimali, M., F. Garaventa, E. Chelossi, V. Piazza, O. D. Saracino, F. Rubino, G. L. Mariottini and L. Pane. 2006. A new photodegradeable molecule as a low impact ballast water biocide: efficacy screening on marine organisms from different trophic levels. *Mar. Biol.* 149: 7-16.

Forsyth, D. M. and R. P. Duncan. 2001. Propagule size and the relative success of exotic ungulate and bird introductions to New Zealand. *Am. Nat.* 157: 583-595.

Freeman, L. and I. Fowler. 1953. Toxicity of combinations of certain inorganic compounds to *Daphnia magna* Straus. *Sewage Ind. Wastes* 25: 1191-1195.

Gilbert J. J. 1974. Dormancy in rotifers. *Trans. Am. Microsc. Soc.* 93: 490-513.

Gollasch, S., J. Lenz, M. Dammer, and H-G Andres. 2000a. Survival of tropical ballast water organisms during a cruise from the Indian Ocean to the North Sea. *J. Plankton Res.* 22: 923-937.

Gollasch, S., H. Rosenthal, H. Botnen, J. Hamer, I. Laing, E. Leppakoski, E. Macdonald, D. Minchin, M. Nauke, S. Olenin, S. Utting, M. Voigt, and I. Wallentius. 2000b. Fluctuations of zooplankton taxa in ballast water during short-term and long-term ocean-going voyages. *Int. Rev. Hydrobiol.* 85: 597-608.

Gracki, J. A., R. A. Everett, H. Hack, P. F. Landrum, D. T. Long, B. J. Premo, S. C. Raaymakers, G. A. Stapleton and K. G. Harrison. 2002. *Critical review of a ballast water biocides treatment demonstration project using copper and sodium hypochlorite*. Report of the Michigan Environmental Science Board, Lansing, MI, October 2002.

Gray, D. K., S. A. Bailey, I. C. Duggan and H. J. MacIsaac. 2005. Viability of invertebrate diapausing eggs exposed to saltwater: implications for Great Lakes' ship ballast management. *Biol. Invas.* 7: 531-539.

Gray, D. K., I. C. Duggan and H. J. MacIsaac. 2006. Can sodium hypochlorite reduce the risk of species introductions from diapausing invertebrate eggs in non-ballasted ships? *Mar. Pollut. Bull.* 52: 689-695.

Grevstad, F. S. 1999a. Experimental invasions using biological control introductions: the influence of release size on the chance of population establishment. *Biol. Invas.* 1:313-323.

Grevstad, F. S. 1999b. Factors influencing the chance of population establishment: implications for release strategies in biocontrol. *Ecol. Appl.* 9:1439-1447.

Hairston, N. G., Jr. 1996. Zooplankton egg banks as biotic reservoirs in changing environments. *Limnol. Oceanogr.* 41: 1087-1092

Hart, B. T., P. Bailey, R. Edwards, K. Hortle, K. James, A. McMahon, C. Meredith, and K. Swadling. 1991. A review of the salt sensitivity of the Australian freshwater biota. *Hydrobiologia* 210: 105-144.

Hebert P. D. N. and T. J. Crease. 1980. Clonal existence in *Daphnia pulex* (Leydig): another planktonic paradox. *Science* 207: 1363-1365.

Herwig, R. P., J. R. Cordell, J. C. Perrins, P. A. Dinnel, R. W. Gensemer, W. A. Stubblefield, G. M. Ruiz, J. A. Kopp, M. L. House, W. J. Cooper. Ozone treatment of ballast water on the oil tanker S/T Tonsina: chemistry, biology and toxicity. *Mar. Ecol. Prog. Ser.* 324: 37-55.

Hudson, P. L., J. W. Reid., L. T. Lesko, and J. H. Selgeby. 1998. *Cyclopoid and harpacticoid copepods of the Laurentian Great Lakes*. Ohio Biological Survey Bulletin, New Series, 12: 1-50.

IMO (International Maritime Organization). 2004. International convention for the control and management of ships ballast water and sediments. Adopted 13 February 2004.

Jo, S.-G. and Marcus, N. H. 2004. The effect of temperature and duration of incubation on the hatching of diapause eggs of *Centropages hammatus* (Lilljeborg) (Copepoda, Calanoida). *Aquac. Res.* 35: 432-435.

Johengen, T., D. F. Reid, G. Fahnenestiel, H. J. MacIsaac, F. Dobbs, M. Doblin and P. T. Jenkins. 2005. *Assessment of transoceanic NOBOB vessels and low-salinity ballast water as vectors for non-indigenous species introductions to the Great Lakes*. A report to the Great Lakes Protection Fund. Available online at http://www.glerl.noaa.gov/res/projects/nobob /products/

Josefsson, M. and B. Andersson. 2001. The environmental consequences of alien species in the Swedish lakes Mälaren, Hjälmaren, Vänern and Vättern. *Ambio* 30: 514-521.

Karaoglanoglu, M., O. F Akinci, S. Bozkurt, S. Deniz, G. Karatas, A. Coskun and S. Z. Ziylan. 2004. Effect of different pharmacologic and chemical agents on the integrity of hydatid cyst membranes. *Am. J. Roentgenol.*183: 465-469.

Kaufman, L. 1992. Catastrophic change in species-rich fresh-water ecosystems. *Bioscience* 42: 846-858.

Kearns, C. M., N. G. Hairston, Jr., and D. H. Kesler. 1996. Particle transport by benthic invertebrates: its role in egg bank dynamics. *Hydrobiologia* 332: 63-70.

Kirschner, M. 2003. Chemical profile, sodium hypochlorite. *Chemical Market Reporter* 263: 31.

Lawler, J. J. and others. 2006. Conservation science: a 20-year report card. *Front. Ecol. Environ.* 4: 473-480.

Levine, J. M. 2000. Species diversity and biological invasions: relating local process to community pattern. *Science* 288: 852-854.

Lin, S. and R.L Evans. 1974. A chlorine demand study of secondary sewage effluents. *Water Sewage Works* 121: 35-44.

Locke, A., D. M. Reid, H. C. Vanleeuwen, W. G. Sprules, and J. T. Carlton. 1993. Ballast water exchange as a means of controlling dispersal of fresh-water organisms by ships. *Can. J. Fish. Aquat. Sci.* 50: 2086-2093.

Lockwood, J. L., P. Cassey and T. Blackburn. 2005. The role of propagule pressure in explaining species invasions. TREE 20: 223-228.

Lonsdale, W. M. 1999. Global patterns of plant invasions and the concept of invasibility. *Ecology* 80: 1522-1536.

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.

Marcus, N. H. and R. V. Lutz. 1998. Longevity of subitaneous and diapause eggs of *Centropages hamatus* (Copepoda: Calanoida) from the northern Gulf of Mexico. *Mar. Biol.* 131: 249-257.

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. Great Lakes Res.* 19: 1-54.

Mills, E. L., D. L. Strayer, M. D. Scheuerell and J. T. Carlton. 1996. Exotic species in the Hudson River Basin: A history of invasions and introductions. *Estuaries* 19: 814-823.

Moyle, P. B. and M. P. Marchetti. 2006. Predicting invasion success: Freshwater fishes in California as a model. *Bioscience* 56: 515-524.

Niimi, A. J. and D. M. Reid. 2003. Low salinity residual ballast discharge and exotic species introductions to the North American Great Lakes. *Mar. Pollut. Bull.* 46: 1334-1340.

Olenin, S., S. Gollasch, S. Jonusas and I. Rimkutf. 2000. En-route investigations of plankton in ballast water on a ship's voyage from the Baltic Sea to the open Atlantic coast of Europe. *Int. Rev. Hydrobiol.* 85: 577-596.

Pati, A. C. and G. Belmonte. 2003. Disinfection efficacy on cyst viability of *Artemia franciscana* (Crustacea), *Hexarthra fennica* (Rotifera) and *Fabrea salina* (Ciliophora). *Mar. Biol.* 142: 895-904.

Pimentel, D., R. Zuniga and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol. Econ.* 52: 273-288.

Pothoven, S. A., I. A. Grigorvich, G. L. Fahnenstiel and M. D. Balcer. 2007. Introduction of the Ponto-Caspian bloody-red mysid *Hemimysis anomala* into the Lake Michigan basin. *J. Great Lakes Res.* (in press).

Raikow, D. F., D. F. Reid, E. E. Maynard and P. E. Landrum. 2006. Sensitivity of aquatic invertebrate resting eggs to SeaKleen (R) (menadione): A test of potential ballast tank treatment options. *Environ. Toxicol. Chem.* 25: 552-559.

Reckhow, D. A. and P. C. S. Inger. 1990. Chlorination by-products in drinking waters: From formation potentials to finished water concentrations. *J. Am. Waterworks Assoc.* 82: 173-180.

Ricciardi, A. 2006. Patterns of invasion in the Laurentian Great Lakes in relation to changes in vector activity. *Div. Distrib.* 12: 425-433.

Rigby, G. 2004. Ballast water treatment technology: choosing the best options. *Maritime Studies*, March-April, pages 22-30.

Rigby, G. and G. M. Hallegraeff. 1993. *Ballast water exchange trials and marine plankton distribution on the MV "Iron Whyalla"*. Vol. 2, Australian Government Publishing Service, Canberra.

Rigby, G. and G. Hallegraeff. 1994. The transfer and control of harmful marine organisms in shipping ballast water: Behaviour of marine plankton and ballast water exchange trials on the MV Iron Whyalla. *J. Mar. Env. Eng.* 1: 91-110.

Ruiz, G. M., T. K. Rawlings, F. C. Dobbs, L. A. Drake, T. Mullady, A. Huq and R. R. Colwell. 2000. Global spread of microorganisms by ships. *Nature* 408: 49-50.

Ruiz G. M. and others. 2005a. *Biological study of container ships arriving to the Port of Oakland. Part b - ballast water exchange efficacy: results of tests on eight container ships. A pilot study.* Available online at http://www.serc.si. edu/labs/ marine_invasions/publications/PortOakfinalrep.pdf

Choi, K. H., W. Kimmerer, G. Smith, G. M. Ruiz and K. Lion. 2005. Post-exchange zooplankton in ballast water of ships entering the San Francisco Estuary. *J. Plankton Res.* 27: 707-714.

Sala, O. E., F. S. Chapin III, J. J. Armesto, *et al.* 2000. Global biodiversity scenarios for the year 2100. *Science* 287: 1770-1774.

Sano, L. L ., M. A. Mapili, A. Krueger, E. Garcia, D. Gossiaux, K. Phillips and P. F. Landrum. 2004. Comparative efficacy of potential chemical disinfectants for treating unballasted vessels. *J. Gt. Lakes Res.* 30: 201-216.

Schwartz S. S., and P. D. N. Hebert. 1987. Methods for the activation of the resting eggs of *Daphnia*. *Freshwat. Biol.* 17: 373–379.

Showalter, S. and T. Bowling. 2006. *Michigan's New Ballast Water Regime: Navigating the Treacherous Waters of States' Rights, Federal Preemption, and International Commerce.* A whitepaper prepared by the National Sea Grant Law Center. Available online at: http://www.seagrant.umn.edu/ downloads/ballast.pdf

Simberloff, D. 2002. *Ecological and economic impacts of alien species: a phenomenal global change.* Pages 29-39 *in* R. Claudi, P. Nantel, and E. Muckle-Jeffs (eds.). Alien invaders in Canada's waters, wetlands, and forests. Natural Resources Canada, Canadian Forest Service, Ottawa, Ontario.

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, Cincinnati, Ohio.

Stewart-Oaten, A. and J. R. Bence. 2001. Temporal and spatial variation in environmental impact assessment. *Ecol. Monogr.* 71:305–339.

Sutherland, T. F., C. D. Levings, C. C. Elliott and W. W. Hesse. 2001. Effect of a ballast water treatment system on survivorship of natural populations of marine plankton. *Mar. Ecol. Prog. Ser.* 2010: 139-148.

Taylor, M. D. and E. J. Bruce. 2000. *Mid ocean ballast water exchange: shipboard trials of methods for verifying efficiency.* Cawthron Report No. 524, Nelson.

Transport Canada. 2006. A guide to Canada's ballast water control and management regulations. Report number TP 131617 E. Available online at: http://www.tc.gc.ca/MarineSafety/tp/Tp13617/Tp13617E.pdf

United States Coast Guard. 1993. *Ballast water management for vessels entering the Great Lakes.* Code of Federal Regulations 33-CFR Part 151.1510.

United States Coast Guard. 2005. Ballast water management for vessels entering the Great Lakes that declare no ballast onboard. Federal Register 70: 51831

Veldhuis, M. J. W., F. Fuhr, J. P. Boon, C. C. Ten Hallers-Tjabbers. 2006. Treatment of ballast water; How to test a system with a modular concept? *Env. Tech.* 27: 909-921.

Williams, D. D. 1998. The role of dormancy in the evolution and structure of temporary water invertebrate communities. *Arch. Hydrobiol. Spec. Issues Advanc. Limnol.* 52: 109-124.

Williamson, M. 2006. Explaining and predicting the success of invading species at different stages of invasion. *Biol. Invas.* 8: 1561-1568.

Witt, J. D. S., P. D. N. Hebert and W. B. Morton. 1997. *Echinogammarus ischnus*: another crustacean invader in the Laurentian Great Lakes basin. *Can. J. Fish. Aquat. Sci.* 54: 264-268.

Wonham, M. J., W. C. Walton, G. M. Ruiz, A. M. Frese, and B. S. Galil. 2001. Going to the source: role of the invasion pathway in determining potential invaders. *Mar. Ecol. Prog. Ser.* 215: 1-12.

Wonham, M. J., S. A. Bailey, H. J. MacIsaac, and M. A. Lewis. 2005. Modelling the invasion risk of diapausing organisms transported in ballast sediments. *Can. J. Fish. Aquat. Sci.* 62: 2386-2398.

Zehnder, M., D. Kosicki, H. Luder, B. Sener and T. Waltimo. 2002. Tissue-dissolving capacity and antibacterial effect of buffered and unbuffered hypochlorite solutions. *Oral. Surg. Oral. Med. O.* 94: 756-762.

Appendix 1. List of zooplankton species recovered from ballast tanks at the beginning of the voyage at Great Lakes' ports (T_0). 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.

Group	Species	Vessel					
		1	2	3	4	5	6
Cladocera	*Bosmina coregoni*	X/X	X/X	-	X/X	X/X	-
	Bosmina liederi	O/O	O/O	-	X/X	X/X	-
	Bosmina longirostris	X/X	X/X	-	O/O	O/O	-
	Daphnia ambigua	O/O	O/X	-	O/O	O/X	-
	Daphnia mendotae	X/X	X/X	-	X/X	X/O	-
	Daphnia retrocurva	X/X	X/X	-	O/O	O/O	-
	Diaphanosoma birgei	X/X	O/X	-	O/O	O/O	-
	Leptodora kindti	O/O	X/X	-	O/O	O/O	-
	Unidentified Chydoridae	X/X	X/X	-	X/O	X/O	-
Copepoda	*Acanthocyclops robustus*	X/X	X/X	-	O/O	O/O	-
	Diacyclops thomasi	X/X	X/X	-	X/X	X/X	-
	Leptodiaptomus siciloides	O/X	X/X	-	X/X	X/X	-
	Mesocyclops edax	X/X	X/X	-	O/O	O/O	-
	nauplii	X/X	X/X	-	X/X	X/X	-
	Skistodiaptomus oregonensis	X/X	X/X	-	O/O	O/O	-
Rotifera	*Cephalodella* sp.	O/O	O/O	-	X/O	X/O	-
	Dicranophorus sp.	O/O	X/O	-	O/O	O/O	-
	Filinia longiseta	O/O	X/O	-	O/O	O/O	-
	Kellicottia bostoniensis	X/X	X/O	-	X/X	X/X	-
	Kellicottia longispina	X/X	X/X	-	X/X	X/X	-
	Keratella cochlearis	X/X	X/X	-	X/X	X/X	-
	Keratella crassa	X/O	O/O	-	O/O	O/O	-
	Keratella earlinae	O/X	X/X	-	X/X	X/X	-
	Keratella hiemalis	O/O	O/O	-	O/O	X/X	-
	Keratella quadrata	X/X	X/X	-	X/X	X/X	-
	Polyarthra dolichoptera	X/X	X/X	-	O/O	O/O	-
	Polyarthra euryptera	X/X	O/O	-	O/O	O/O	-
	Polyarthra major	X/X	O/O	-	O/O	O/O	-
	Polyarthra remata	O/O	O/O	-	X/X	X/X	-
	Polyarthra vulgaris	O/O	O/O	-	X/X	X/X	-
	Pompholyx sulcata	O/X	X/O	-	X/X	X/X	-
	Synchaeta kitina	O/O	O/O	-	X/X	X/O	-
	Synchaeta sp.	O/O	X/O	-	O/O	O/O	-
	Synchaeta stylata	O/O	O/O	-	X/X	X/X	-

Trichocerca multicrinis	O/O	X/X	-	O/O	O/O	-

Appendix 2. List of zooplankton species recovered from ballast tanks at the end of the ships' voyage at European ports (T_1). X – species present; O – species absent. Control tank is listed first in each pair of characters (e.g. X/X), followed by the exchanged tank (i.e. Control/Treatment).

Group	Species	Vessel					
		1	2	3	4	5	6
Cladocera	*Bosmina coregoni*	X/O	X/O	O/O	X/O	X/O	X/O
	Bosmina liederi	O/O	O/O	O/O	X/O	X/O	O/O
	Bosmina longirostris	X/O	X/O	O/O	O/O	O/O	X/O
	Chydorus sp.	O/O	O/O	O/O	O/O	X/O	X/X
	Daphnia ambigua	O/O	O/O	O/O	O/O	X/O	O/O
	Daphnia mendotae	X/O	X/X	O/O	X/O	X/O	X/O
	Daphnia retrocurva	X/O	O/O	O/O	O/O	O/O	O/O
	Diaphanosoma birgei	X/O	O/O	O/O	O/O	O/O	O/O
	Unidentified Chydoridae	X/O	X/O	O/O	O/O	X/O	O/O
Copepoda	*Acanthocyclops robustus*	X/O	X/O	O/O	O/O	O/O	O/O
	Canthocamptus robertcokeri	O/O	O/O	X/O	O/O	O/O	O/O
	Diacyclops thomasi	X/O	X/O	O/O	X/O	X/X	X/O
	Leptodiaptomus siciloides	X/O	X/O	O/O	X/O	X/O	X/O
	Marine cyclopoids	O/X	O/X	O/X	O/X	O/X	O/X
	Mesocyclops edax	X/O	X/O	X/O	O/O	X/O	X/O
	nauplii	X/O	X/O	X/X	X/X	X/X	X/X
	Skistodiaptomus oregonensis	X/O	X/O	O/O	O/O	O/O	O/O
Rotifera	*Ascomorpha ecaudis*	O/O	O/O	X/O	O/O	O/O	O/O
	Brachionus angularis	O/O	O/O	O/O	O/O	X/O	O/O
	Cephalodella gibba	O/O	O/O	X/O	O/O	O/O	O/O
	Cephalodella sp.	X/O	O/O	O/O	O/O	O/O	O/O
	Kellicottia bostoniensis	X/O	X/X	O/O	X/O	X/O	O/O
	Kellicottia longispina	X/O	X/O	O/O	X/O	X/O	O/O
	Keratella cochlearis	X/O	X/X	X/O	X/O	X/X	X/O
	Keratella earlinae	O/O	X/O	O/O	X/O	X/O	X/O
	Keratella hiemalis	O/O	O/O	O/O	O/O	X/O	O/O
	Keratella quadrata	X/O	X/O	O/O	X/O	X/O	O/O
	Lecane mira	O/O	O/O	X/O	O/O	O/O	O/O
	Notholca acuminata	O/O	O/O	O/O	O/O	X/O	O/O
	Polyarthra dolichoptera	O/O	O/X	X/O	O/O	O/O	O/O
	Polyarthra remata	O/O	O/O	O/O	X/O	X/X	X/O
	Polyarthra vulgaris	O/O	O/O	O/O	X/O	X/O	O/O
	Pompholyx sulcata	O/O	O/O	X/O	X/O	O/O	X/O
	Synchaeta kitina	O/O	O/O	X/O	O/O	O/O	O/O

Synchaeta stylata	O/O	O/O	O/O	X/O	O/O	O/O
Trichocerca pusilla	O/O	O/O	X/O	O/O	O/O	O/O

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