Journal of Great Lakes Research 38 (2012) 72–77

Contents lists available at SciVerse ScienceDirect

Journal of Great Lakes Research

journal homepage: www.elsevier.com/locate/jglr

Efficacy of NaCl brine for treatment of ballast water against freshwater invasions

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ARTICLE INFO

Article history:
Received 23 March 2011
Accepted 18 September 2011
Available online 26 October 2011

Communicated by Joseph Makarewicz

Keywords:
Biocide
Laurentian Great Lakes
Invasive species
Sodium chloride brine
Vector management
Zooplankton

ABSTRACT

Currently, all transoceanic vessels entering the Great Lakes must perform ballast water exchange or saltwater flushing, procedures designed to reduce the risk of new biological invasions from ballast water. Vessels not in compliance with these regulations presently have limited, often costly, and/or time-consuming alternatives available. Treatment with sodium chloride brine at an initial concentration of 230‰ has been proposed as an emergency ballast water management option and is examined here. Six shipboard trials were conducted under operational conditions to determine the efficacy of brine ballast water treatment. Trials were conducted on three vessels with full ballast tanks and on three vessels with only residual ballast water. Brine distribution in tanks was adequate, noting that vessel movement was essential to ensure mixing into ballast water or ballast residuals. Once mixing has occurred, approximately 25 hour exposure to 45‰ brine or 1 hour exposure to 115‰ brine is required to effectively exterminate freshwater zooplankton. Brine appears to be a cost-effective and relatively safe procedure that could be implemented immediately for emergency treatment of non-compliant ballast water to reduce risk of new invasions in the Great Lakes.

Introduction

Up to five billion cubic meters of ballast water, carrying an estimated 7000 species, are transported daily around the world by commercial vessels (Carlton, 1999; Tsolaki and Diamadopoulos, 2010). These vessels may transport 1000 s to 100,000 s of zooplankton (plus other taxa) per m^3 in ballast water (Wonham et al., 2005; Verling et al., 2005; McCollin et al., 2008). Propagule pressure theory predicts that the probability of successful establishment of introduced nonindigenous species (NIS) is positively related to the number of viable individuals introduced (Lockwood et al., 2005, 2007), thus it is not surprising that ships' ballast has been a principal source of species invasions. Even though attenuation of propagule number is common for many species during transit in a vessel's ballast tanks, commercial shipping and ballast water release have played a strong role in the introduction of NIS to novel habitats worldwide (e.g., Ruiz et al., 1997; Wonham et al., 2005). For example, 34 of 56 (61%) aquatic NIS discovered in the Laurentian Great Lakes since the St. Lawrence Seaway opened in 1959 were attributed to shipping activities, including at least 10 zooplankton species (Kelly et al., 2009; NOAA, 2010). Zooplankton are a major invasion concern considering that the group is taxonomically diverse, is generally capable of rapid reproduction, and can exert strong ecological effects (Machida et al., 2009).

Ballast water management systems, utilizing filtration, deoxygenation, biocides, and/or ultraviolet treatment, can reduce the risk of ship-mediated aquatic invasions by reducing propagule pressure. At least 59 ballast water treatment systems are in various stages of development and approval (Lloyd's Register, 2011). However, these systems will not be widely deployed until approximately 2016, and then only if a convention adopted by the International Maritime Organization (IMO) is ratified globally (Tsolaki and Diamadopoulos, 2010; Lloyd's Register, 2011). Until then, ballast water exchange (BWE) and saltwater flushing are mandatory management practices required by both Canada and the U.S. to prevent aquatic NIS introductions in the Great Lakes (Government of Canada, 2006; SLSDC, 2008). BWE involves replacing coastal water in ballast tanks with oceanic saltwater, achieving a final salinity of ≥30‰. Saltwater flushing is similar to BWE but involves much smaller volumes of water and is used for tanks containing only residual ballast water (i.e., "no-ballast-on-board" or "NOBOB" tanks).

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doi:10.1016/j.jglr.2011.10.001

Biological efficacy of these management methods is variable for coastal marine habitats (Ruiz and Reid, 2007; McCollin et al., 2008), though they appear to be highly effective (>99%) against freshwater zooplankton (Gray et al., 2007; Bailey et al., 2011).

BWE and saltwater flushing reduce invasion risk by decreasing the number of individuals (propagule pressure) and number of species (colonization pressure) in ballast tanks by physical removal. In addition, exposure to mid-ocean water may enhance protection via osmotic stress to fresh- and brackish-water taxa that remain in the tank after purging (Santagata et al., 2008; Ellis and MacIsaac, 2009). Though compliance with current requirements for BWE and saltwater flushing is high, on an annual basis up to 10% of ballast tanks may be non-compliant upon arrival to the Great Lakes (L. Jean, Great Lakes Seaway Ballast Water Working Group, personal communication). Currently, vessels can retain non-compliant ballast water on board throughout their operations on the Great Lakes or can return to an approved offshore location to repeat BWE and/or saltwater flushing; the first option can be unworkable if ballast water must be discharged to offset cargo operations, while the second can result in costly time delays — indicating a need for emergency, or 'back-up', treatment methods.

Here we explore the use of sodium chloride (NaCl) brine, hereafter called 'brine', as an emergency treatment method for non-compliant tanks. Brine, at 230‰ salinity, is presently used for deicing roads during winter around the Great Lakes; it also is relatively inexpensive and readily available (Jenkins, 2007). Laboratory experiments have demonstrated that brine kills a broad array of freshwater and oceanic zooplankton over a short time exposure (hours) when applied at a minimum concentration of 77‰ (Santagata et al., 2009; Bradie et al., 2010). We extend these studies by conducting shipboard experiments to determine if ballast water treatment with brine is effective under operational conditions. We examine the extent of mixing of brine and the survivability of ambient freshwater invertebrates in both fully ballasted tanks and those containing only residual ballast water.

Methods

Ballasted tanks

Three trials were conducted with filled (ballast-on-board) tanks of transoceanic commercial bulk carriers during voyages from Toronto, ON, to Thunder Bay, ON, between June and October 2009 (Table 1). For each of the trials, two upper-stool ballast tanks having approximately 200,000-L water capacity were filled with Great Lakes freshwater (0‰) at port in Toronto. One tank served as a control, which was filled completely and had no brine addition. The second (treatment) tank was filled to roughly 90% capacity, leaving sufficient head space to allow subsequent addition of brine. Owing to the large volume of water in the tanks, the highest salinity that could reasonably be achieved with addition of brine under normal operating conditions was 45‰. After initial (time 0) samples were collected, brine (230‰) was delivered to vessels by tanker truck (Road Maintenance Equipment & Services Inc., Cobourg, ON) and added to treatment tanks through opened deck hatches (first two vessels) or sounding tube (third vessel) at Port Weller, ON. A sufficient volume of brine was added to the tank to achieve the target concentration, assuming complete mixing, based on estimates of ballast volume provided by the ships' crew. It took approximately 1 h to apply the brine for each experiment, and the volume added is indicated in Table 1.

Table 1
Description of experimental voyages, including date (in 2009, unless indicated), location and details of brine treatment. Location refers to initial and final ports for ballasted tank experiments, while trials with residual ballast occurred at a single location. BOB refers to fully ballasted tanks, NOBOB to tanks containing only ballast residuals.

Experiment	Date	Location	Target concentration (‰)	Brine volume applied (L)
BOB 1	June 16–20	Toronto, ON to Thunder Bay, ON	45	~20,000
BOB 2	Sept. 30–Oct. 2	Toronto, ON to Thunder Bay, ON	45	24,445
BOB 3	Oct. 22–24	Toronto, ON to Thunder Bay, ON	45	20,000
NOBOB 1	Nov. 22, 2008	Sarnia, ON	115	~10,000
NOBOB 2	May 27	Sarnia, ON	115	~10,000
NOBOB 3	Dec. 16	Sarnia, ON	115	~10,000

To examine the extent of mixing, five self-recording programmable sondes were secured in the treatment tank, each with temperature, conductivity, optical dissolved oxygen and depth sensors. Sondes were positioned in distal tank bays away from the location of brine application and at a range of depths; however, three sondes malfunctioned during the first experiment which precluded examination of mixing in the upper portion of the tank. One sonde was installed in the control tank to monitor the same parameters. Dissolved oxygen sensors were calibrated against air saturation for each sonde prior to deployment. Because the sensors are not factory calibrated specifically to NaCl and because expected conductivity levels were outside the manufacturer's specifications and normal calibration range, the sensors were calibrated with NaCl solutions ranging from 0–120‰ prior to each deployment. Measured specific conductance could then be converted to equivalent NaCl concentration (‰) using sonde-specific empirically derived third-order polynomial equations generated between specific conductivity and NaCl measured in the lab. In addition, specific conductance output of each sonde was checked post-experiment against a 44 ± 1‰ NaCl solution measured with a precision hand-held NaCl refractometer. All sonde measurements were within 1‰ of the expected concentration. Vertical salinity profiles were also taken in the treatment tank (mid-tank) with a hand-held YSI unit at each time point that zooplankton samples were collected.

Zooplankton samples were collected to assess the abundance of live individuals in ballast water in both treatment and control tanks prior to the addition of brine and at time points approximately 1 h, 10 h, 25 h, and 45 h post-treatment. Vertical plankton net tows were taken using a 30-cm diameter, 40-μm mesh conical net through an opened deck hatch located near the center of the tank for the first two vessels. The net tow covered the depth of the tank directly below the access hatch (1.8 m); the deepest location of the wedge-shaped tank (4 m) was not accessible. The number of net tows taken was based on the expected density of zooplankton in the ballast water; thus, sample volume increased over time as the density of live animals in treatment tanks decreased (target of ≥25 individuals per sample). Since the tanks of the third vessel did not have deck hatches, samples were collected by lowering 1.27-cm inner diameter high density polyethylene tubing, fitted with a stainless steel check valve, into the tank through the sounding tube. Approximately 50 L of water was manually pumped to the deck surface at each sample time point and filtered through the 40-μm mesh plankton net for subsequent assessment of viability (see later section).

Tanks with residual ballast

Three trials were conducted in tanks of domestic commercial tankers containing only residual ballast while the vessels were moored in Sarnia, ON, between November 2008 and December 2009 (Table 1). Paired double-bottom ballast tanks having approximately 800,000-L water capacity, but containing only residual Great Lakes freshwater (0‰), were utilized for each trial. One tank served as a control, while the second was treated with brine. After initial assessment of salinity and zooplankton density, brine (230‰) was pumped by tanker truck directly into treatment ballast tanks. The volume of residual ballast to be treated (~10,000 L) was estimated in consultation with the ships' crew so that brine could be added to the treatment tank in a 1:1 ratio to achieve a final target concentration of 115‰, based upon results of previous laboratory experiments [see Bradie et al. (2010) and recommendations by

Jenkins (2007)]. Using an intrinsically safe air-driven pump, which was slower than the diesel pump used to add brine for ballasted experiments, it took approximately 1 h to add the brine for each residual trial. A flowmeter on the pump indicated the amount of brine pumped into tanks.

The multi-parameter sondes could not be used to examine the extent of mixing during residual ballast experiments due to safety issues related to battery-powered instruments around volatile cargo. Instead, water samples were taken from at least the three tank corner locations farthest from the site of brine addition for subsequent measurement of salinity using a digital salinity refractometer. Samples were collected approximately hourly from both the top and bottom layer of ballast residuals using a plastic pipette, at the same time zooplankton samples were collected. When stratification was observed, as many as 60 additional samples were taken to examine variability in salinity across the tank.

Zooplankton samples were collected in both treatment and control tanks prior to the addition of brine and following treatment, with sampling conducted approximately hourly until no live individuals were observed. Zooplankton were collected by physically entering ballast tanks. A manual bilge pump was used to collect a measured volume of water in 25-L plastic pails prior to filtration through a 40-μm mesh plankton net for assessment of viability (see next section). The volume sampled for each trial depended upon the initial density of zooplankton in residual ballast water, with a target of ≥25 individuals per sample; a 1-L sample was initially collected to determine zooplankton density in tanks before each trial began, and a constant volume was sampled for all time points for both control and treatment tanks for each trial. For treated tanks, samples were collected at the location most distal to that of the brine addition, where mixing was expected to be most restricted.

Viability assessment

Viability of zooplankton was assessed on board the vessel immediately following sample collection. Zooplankton samples were examined under a microscope using a combination of physical stimulation with a dissection probe and vital staining with 10-g L^{-1} neutral red (Tang et al., 2006). Samples were filtered through a 40-μm sieve to remove excess water and transferred to a 250-mL glass beaker for staining. One mL of neutral red solution was added to 100-mL of zooplankton sample volume and left for 15 min. Following staining, samples were repeatedly washed with tap water over a 40-μm sieve to remove excess stain and transferred to a small, gridded petri dish for viability assessment. Neutral red stained most live zooplankton, making organisms much easier to find and check for body movement; however, as the stain was not 100% accurate, care was taken to assess all non-motile organisms that did not stain by gentle probing. Zooplankton that showed movement in antenna, thoracic appendages, or body when stimulated by probe were considered live.

Viability assessments were completed within 30 min of sample collection. Live and dead zooplankton were divided into separate sample jars and preserved in 95% ethanol for later enumeration in the laboratory; to do so efficiently, the smallest fraction (live or dead) in each sample dish was enumerated and removed by pipette while the larger fraction was rinsed directly into a sample jar. Subsequently, both live and dead sample fractions were enumerated in the laboratory to determine abundance and proportion of live zooplankton in both control and treatment replicates. Owing to large numbers of zooplankton in some control samples, three 0.5-mL subsamples were taken from 50-mL total sample volume to estimate abundance. Subsamples were drawn with replacement by Hensen–Stemple pipette, following thorough mixing to ensure uniform distribution of organisms. Samples with less than 1000 individuals were counted in entirety.

Statistical analysis

Paired sample t-tests were conducted to ensure similarity of initial zooplankton density in control and treatment tanks for both BOB and NOBOB experiments. One-way analysis of variance with repeated measures (RM-ANOVA) was utilized to examine differences in abundance of live zooplankton between control and treatment tanks through time following brine treatment. Zooplankton abundance data was square-root transformed before analysis because variance was correlated with the mean (Sokal and Rohlf, 1995; all statistical analyses were conducted with SPSS 11.5).

Results

Ballasted tanks

The final concentration of brine achieved in fully ballasted tanks was within 77–98% of the target value, reflecting inaccurate estimates of ballast water volume inside tanks; in all three trials, approximately 30-cm empty head space remained in treatment tanks after brine addition. Vertical salinity profiles indicated brine was well mixed in the center of the tank (at the location of brine addition) within 10 h (data not shown); however, records from sondes installed in tanks showed that stratification occurred at tank extremities initially, and tanks were not consistently well mixed until 30 h after brine addition (Fig. 1a, c, e). Tank mixing appeared to be augmented by rolling action after the vessels were underway. Environmental conditions (temperature, dissolved oxygen) in control and treatment tanks remained relatively constant except for the conductivity increase in treatment tanks following the addition of brine (data not shown).

Initial zooplankton abundance varied widely between trials, ranging from 1.7 to 80.3 individuals·L^{-1}; however, paired t-tests confirmed that initial abundance did not differ between control and treatment tanks within trials ($t = -0.234$, $p = 0.837$). Ambient zooplankton consisted mainly of rotifers, copepods, and cladocerans (32.8%, 31.3%, and 26.4%, respectively). Other taxa, including annelids and mollusks, were found in lesser number (Table 2). The abundance of live zooplankton in control tanks remained relatively constant through time during the first two trials, while increasing three fold for the final experiment (Fig. 2a). In contrast, total abundance in treatment tanks rapidly decreased following brine application, with complete mortality consistently observed at 25 h post-treatment (Fig. 2a). Correspondingly, the RM-ANOVA indicated that treatment, time and the interaction between the two factors were all statistically significant (Table 3).

Tanks with residual ballast

Brine appeared to mix rapidly with residual ballast water during the first trial, while stratification of brine and freshwater residuals was observed during the second and third trials (Fig. 1b, d, f). Observations in the field indicated that cargo operations caused shifts in vessel list or trim during trial 1, while the vessels remained virtually static for trials 2 and 3. Salinity did not change in control tanks for the duration of the trials.

As for ballasted tank trials, wide variability in initial zooplankton abundance was observed between residual ballast trials (0.22 to 43.0 individuals·L^{-1}) but was not statistically different between control and treatment tanks within trials (paired *t*-test, $t = 0.638$, $p = 0.589$). Ambient zooplankton consisted mainly of rotifers (44.8%), followed by cladocerans (26.9%), copepods (21.5%), and other taxa (6.7%) (Table 2). The abundance of live zooplankton in control tanks remained relatively constant through time in all three trials but responded directly to brine concentration in treatment tanks; when brine concentration at the location of sample collection approached 115‰, complete mortality was observed within 1 h (Fig. 2b). Again, the RM-ANOVA indicated that treatment, time, and the interaction between the two factors were all statistically significant (Table 3).

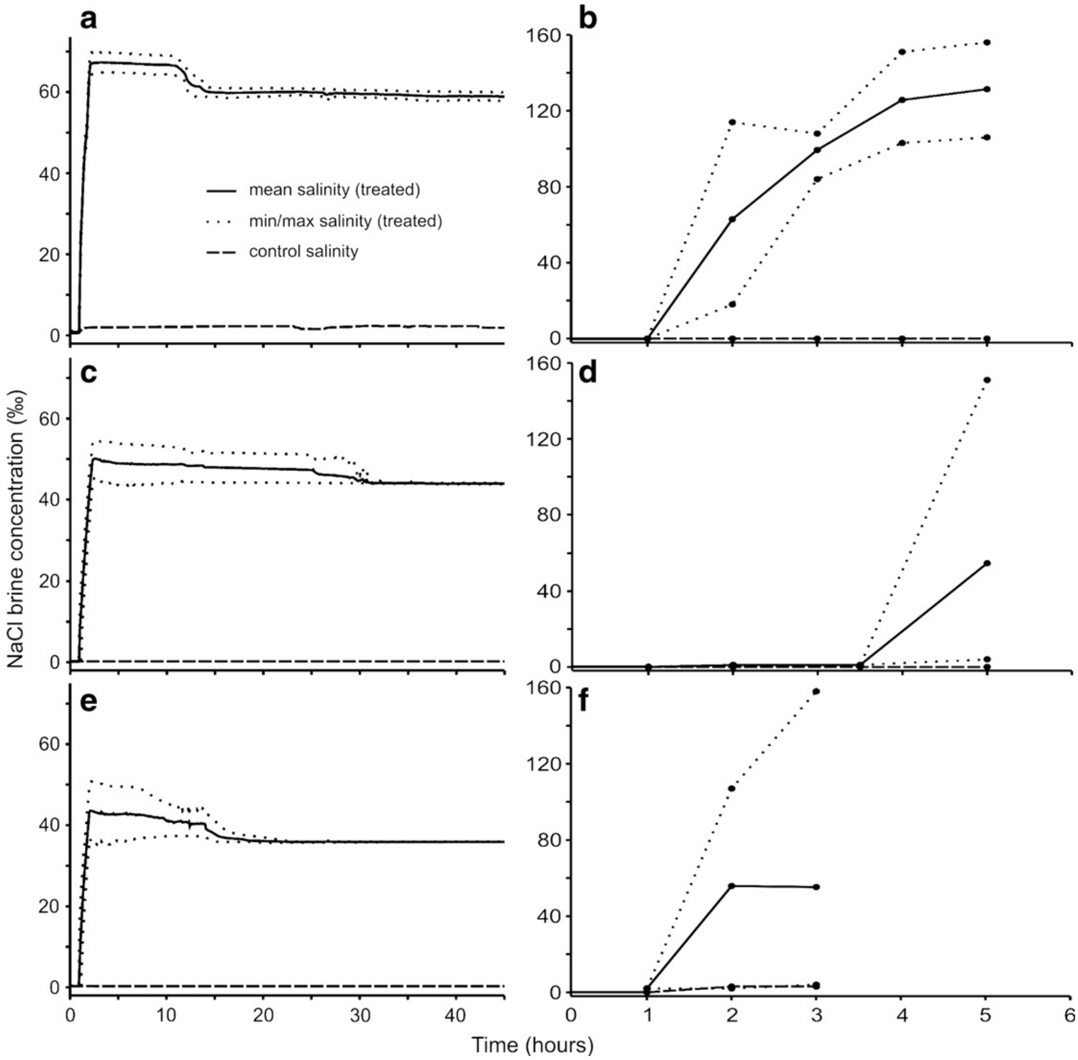

Fig. 1. NaCl brine concentration measured across time in ballasted tanks (a, c, e), and tanks with residual ballast (b, d, f), during treatment trials. Brine addition occurred at hour one. Note scale differences between ordinates, and that the x-axis has been lowered to make control data visible.

Discussion

The addition of brine appears to be a highly effective and operationally practical means for treatment of invertebrates in freshwater ballast, contingent on the ability to achieve comprehensive mixing inside tanks. It appears that vessel motion is an essential element of thorough mixing, particularly for treatment of residual ballast water using highly concentrated brine. Once mixing has occurred, approximately 25 hour exposure to 45‰ brine or 1 hour exposure to 115‰ brine is required to effectively exterminate freshwater zooplankton. Given these conditions, brine appears to be a viable interim treatment for non-compliant vessels entering the Great Lakes that could be implemented immediately to reduce invasion risk, although several operational and biological challenges remain.

Table 2
Percent occurrence and total abundance of zooplankton recorded from control samples during three ballasted and three residual ballast experiments, by taxon.

	Ballasted tanks		Tanks with residual ballast	
	% Occurrence	% Total abundance	% Occurrence	% Total abundance
Copepoda				
Calanoida	100	14.00	33.34	3.03
Cyclopoida	100	11.08	66.67	5.39
Harpacticoida	100	6.25	66.67	3.70
Nauplii	100	2.72	100	9.43
Cladocera				
Daphnia spp.	66.67	1.09	66.67	4.04
Bosmina spp.	100	23.32	33.34	4.04
Diaphanosoma spp.	33.34	0.68	66.67	1.35
Bythotrephes longimanus	66.67	1.29	33.34	17.51
Rotifera	100	32.83	66.67	44.78
Other	100	6.73	66.67	6.73

Internal tank structures, such as longitudinal members and bulkheads, restrict natural mixing of brine into recipient ballast water for tanks that are completely filled as well as those containing only residual ballast water. We observed that adjustments to vessel trim and/or list, either as a result of sailing or cargo activities, greatly facilitated mixing of brine in ballasted and residual ballast tanks, respectively. Similarly, Reynolds et al. (2009) reported ship motion alone may take between 20 and 48 h to completely mix a ballast tank. The effectiveness of ship-motion mixing will depend on several factors, including sea state, tank configuration, and free-surface energy. As a result, care must be taken to ensure complete mixing with recipient ballast water to ensure maximal efficacy. Furthermore, when treating filled ballast tanks, the volume of brine needed to achieve the target concentration must be considered since sufficient space must be available in tanks to accommodate the addition of brine. In fact,

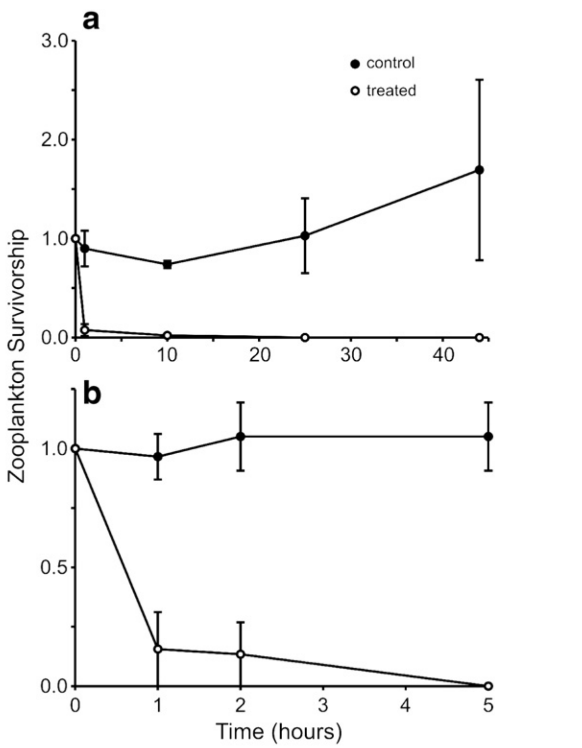

Fig. 2. Mean (±SE) proportion of zooplankton surviving over time in (a) ballasted tanks, and (b) tanks with residual ballast, during NaCl brine treatment trials. Note scale differences between ordinates.

provision of empty head space in tanks after brine addition might also be necessary to facilitate mixing. Ballast water needing treatment may therefore need to be divided among several tanks within a vessel to receive sufficient brine volume without overflow of tanks. Since residual ballast is treated while tanks are nearly empty, having sufficient space to accommodate brine is not an issue.

Jenkins (2007) estimated that costs for treatment of residual ballast water including brine, transportation, and labor could range from $5200 to $7200 per vessel, depending on size. In the field experiments conducted here, brine was delivered by tanker truck. This method of delivery may not be cost effective on a larger scale due to many factors that could delay or prevent brine trucks from reaching their destination. During our experiments, we encountered incidents where the brine truck broke down en route or was significantly delayed in heavy traffic. To solve this problem, it may be possible to set up a number of brine stations at major Great Lakes' ports or at strategic locations, such as the Welland Canal. Furthermore, if vessels were outfitted with a system to inject brine into tanks via the ballast piping system, treatment efficacy might be increased through enhanced mixing and/or an ability to treat ballast water that may be trapped within ballast pumps and pipes which would not be effectively managed when adding brine directly to tanks.

While brine appears to be a broadly effective biocide exterminating at least 60 invertebrate taxa from an array of habitats (Santagata et al., 2009; Bradie et al., 2010; this study), additional tests examining efficacy against bacteria, viruses, and phytoplankton, which may also pose an invasion risk, are warranted. We also acknowledge that taxa associated with ballast sediments, such as invertebrate dormant stages, are of concern (Bailey et al., 2004, 2005; Briski et al., 2010). Previous studies have demonstrated that dormant stages of freshwater zooplankton can withstand exposure to full strength seawater and other chemical treatments (Bailey et al., 2004; Gray et al., 2006; Raikow et al., 2007a, b; Gray and MacIsaac, 2010), suggesting that brine treatment would likely be an ineffective management method; however, the risk posed by dormant stages may be offset by high retention rates within tanks, as dormant stages are not easily dislodged and discharged with ballast (Bailey et al., 2006).

Table 3
Analysis of variance with repeated measures demonstrating the effect of brine treatment on abundance of live zooplankton. Significance levels for F-values: * ($p<0.05$), **($p<0.10$). MS = Mean Square, MSE = Mean Square Error.

Effect	Ballasted tank experiment			Residual ballast experiment		
	F values (df)	MS	MSE	F values (df)	MS	MSE
Treatment	335.02* (1,2)	3.50	0.01	168.05* (1,2)	2.89	0.017
Time	6.69* (4,8)	0.39	0.06	9.93* (3,6)	0.29	0.030
Treatment*Time	3.57** (4,8)	0.24	0.07	20.72* (3,6)	0.34	0.016

Finally, we acknowledge concerns regarding the environmental impact of discharging brine-treated ballast water at Great Lakes' ports. The relatively high concentration of brine used to treat residual ballast water (115‰) should be diluted to approximately 5.5‰ by filling ballast tanks with Great Lakes water prior to discharge. In contrast, ballasted tanks treated to 45‰ would have to be discharged directly, since there would be no head space available to load additional fresh water into tanks for dilution purposes. In both scenarios, a further immediate dilution of 100x is expected with discharge to a freshwater harbor (see Wells et al., 2011), resulting in brine concentrations of ~55 to 450 mg L^{-1} (0.10‰–0.81‰). Potential impacts might be further reduced by discharging treated ballast water while the vessel is underway, rather than into an enclosed port environment. Furthermore, potential impacts could be regarded as relatively minor, comparing the small volume of discharges expected annually (~500 tonnes) to the amount of salt already entering the system as run-off from winter road treatment; American states bordering the Great Lakes and the province of Ontario use about 5.2 million tonnes of road salt annually (Transportation Research Board, 1991; Government of Canada, 2001).

A method to treat non-compliant ballast tanks may be required for the foreseeable future, as ballast water management systems utilizing filtration, de-oxygenation, biocides, and/or ultraviolet treatment are still in the developmental phase and will not be widely employed until at least 2016 (Tsolaki and Diamadopoulos, 2010; Lloyd's Register, 2011). The method of brine treatment presented above is an effective way to reduce the risk of ballast-mediated biological invasions that can be implemented immediately to treat non-compliant transoceanic vessels. This brine treatment could also serve as a 'back-up' strategy for cases where ballast water management systems, once approved and implemented, break down during ship operations.

Acknowledgments

We thank J. Bradie, F. Chan, B. Davey, M. Deneau, J. Gerlofsma and K. Wood for assistance in the field. We also thank Algoma Tankers, Canfornav Inc., Imperial Oil and the St. Lawrence Seaway Management Corporation for their participation in this study, with special thanks to E. Francis, K. Minkoff and ships' crews. Constructive comments from two anonymous reviewers are gratefully acknowledged. This research was supported by Fisheries and Oceans Canada, Transport Canada, NSERC's Canadian Aquatic Invasive Species Network, Ontario Ministry of Natural Resources, NSERC Discovery Grants to SAB and HJM, and by the U.S. National Oceanic and Atmospheric Administration Aquatic Invasive Species Program. GLERL Contribution #1599. The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of NOAA or the U.S. government.

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