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CONCENTRATED SODIUM CHLORIDE BRINE SOLUTIONS AS AN ADDITIONAL TREATMENT FOR PREVENTING THE INTRODUCTION OF NONINDIGENOUS SPECIES IN THE BALLAST TANKS OF SHIPS DECLARING NO BALLAST ON BOARD

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Abstract—Currently, seawater flushing is the only management strategy for reducing the number of viable organisms in residual sediments and water of ballast tanks of vessels declaring no ballast on board (NOBOB) that traffic ports of the eastern United States. Previously, we identified several species of freshwater and brackish-water peracarid crustaceans able to survive the osmotic shock that occurs during open-ocean ballast water exchange and, potentially, to disperse over long distances via ballasted ships and NOBOB vessels. We tested the efficacy of concentrated sodium chloride brine solutions as an additional treatment for eradicating the halotolerant taxa often present in the ballast tanks of NOBOB ships. The lowest brine treatments (30 ppt for 1 h) caused 100% mortality in several species of cladocerans and copepods collected from oligohaline habitats. Several brackish-water peracarid crustaceans, however, including some that can survive in freshwater as well, required higher brine concentrations and longer exposure durations (45–60 ppt for 3–24 h). The most resilient animals were widely introduced peracarid crustaceans that generally prefer mesohaline habitats but do not tolerate freshwater (required brine treatments of 60–110 ppt for 3–24 h). Brine treatments (30 ppt) also required less time to cause 100% mortality for eight taxa compared with treatments using 34 ppt seawater. Based on these experiments and published data, we present treatment strategies for the ballast tank biota often associated with NOBOB vessels entering the Great Lakes region. We estimate the lethal dosage of brine for 95% of the species in our experiments to be 110 ppt (95% confidence interval, 85–192 ppt) when the exposure time is 1 h and 60 ppt (95% confidence interval, 48–98 ppt) when the exposure duration is 6 h or longer.

Keywords—Introduced species   Ballast water   Zooplankton   Great Lakes   Salinity tolerance

INTRODUCTION

The main vector responsible for many of the nonindigenous species established in the estuaries and freshwater habitats of the eastern United States is ballast water of commercial ships originating from ports of the North, Baltic, Mediterranean, and Black–Azov seas [1–3]. Ports of the Baltic and North seas dominate the routes of U.S.-bound shipping traffic from Europe, and many of these ports are found in freshwater or mesohaline systems that provide an environmental match sufficient to allow the establishment of nonnative organisms [4] (http://www.glerl.noaa.gov/seagrant/GLERLUpdates/GLERLUpdates2007.html). Overall, ships carrying low-salinity water from source ports in northern Europe pose a significant risk to the freshwater and estuarine habitats of the eastern United States through the introduction of nonnative species.

A proportion of ships entering the Great Lakes and estuarine port systems of the eastern United States are not in a ballasted condition, reporting no ballast on board (NOBOB) [5,6]. Sediments and residual water in the ballast tanks of these ships often contain adults, larvae, and resting stages of many taxa [7]. As a consequence of off-loading and loading cargo, many commercial ships take on and discharge ballast water while in port, potentially releasing nonnative species [6,8]. To minimize the risk of species transfers from NOBOB vessels, ships entering the Great Lakes and St. Lawrence Seaway recently have been required to treat residuals with open-ocean seawater (seawater flushing [9]) before entering this region. Previously, we identified several freshwater and brackish-water taxa that can survive exposure to oceanic seawater (34 ppt) and are likely to be dispersed via the operations of commercial ships [10]. Furthermore, some ships entering the Great Lakes through the St. Lawrence Seaway are unable to follow the seawater flushing policy because of load-line restrictions and seaway draft constraints [11] (http://www.glerl.noaa.gov/res/projects/nobob/products/NOBOBFinalReport.pdf), and these ships may have low-salinity residual water in their tanks. Both of these factors create a potential risk of introducing nonindigenous species to the Great Lakes, especially if the ballast tank organisms originate from distant ports of similar environmental conditions [6,12]. Regulators responsible for the Great Lakes presently are faced with limited choices for dealing with such ships and may require them to return to an

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alternate exchange zone either at or beyond the mouth of the St. Lawrence River (a 2- to 4-d round trip) or to seal their tanks while in the Great Lakes. For these reasons, we conducted experiments to evaluate the efficacy of concentrated sodium chloride brine solutions as a treatment method to augment the current ballast water management strategy for NOBOB vessels based on seawater flushing. This treatment may provide a simple and effective method for eliminating halotolerant taxa within the sediments and residual water found in the ballast tanks of NOBOB vessels with minimal impacts on adjacent native flora and fauna of the Great Lakes.

MATERIALS AND METHODS

The goal of the present study was to build on our previous experiments investigating the salinity tolerance of native and introduced species [10]. In particular, we wanted to evaluate the use of concentrated sodium chloride brine solutions as a means of quickly eliminating freshwater and mesohaline taxa capable of surviving exposure to the average salinity of open-ocean seawater (34 ppt). We used our previously established experimental protocol based on seawater to identify seawater-intolerant and seawater-tolerant taxa and to compare their survivorship among several brine treatments.

Animal collection and sites

Initial experiments focused on native and introduced taxa of the Great Lakes (Lake Erie, Toledo, OH, USA; Lake Huron, Alpena, MI, USA; and Lake Michigan, Traverse City, MI, USA). The geographic scope was then expanded to source populations of species known to have been introduced to freshwater and brackish-water habitats of the United States (from the port of Rotterdam, The Netherlands; Curonian Lagoon, Lithuania; and the Vistula and Oder rivers, Poland). We also conducted experiments on native and introduced species of mesohaline habitats from two estuaries on the east and west coasts of the United States (Rhode River, MD, USA, and San Francisco and Suisun bays, CA, USA).

Most animals were collected directly from the field using a variety of sampling methods (plankton net, light trap, or seine net), sorted in the laboratory adjacent to the collection site, and then used within 12 h in the experiments outlined below. A small subset of species were reared in the laboratory from broods of adult animals (*Rhithropanopeus harrisii* and *Palaemonetes pugio*), which were held for a short time (days) in the laboratory until they developed into planktonic stages for experiments.

Seawater-tolerance protocols

Experiments were conducted in the laboratory with treatments simulating the two commonly used ballast water exchange (BWE) methods, flow-through (F-T) and empty-refill (E-R). Although for the present study we were not specifically interested in the osmotic effects of BWE, we used this protocol because it discriminated between seawater-tolerant and seawater-intolerant species. Animals were assigned randomly to one of three treatments: Control animals were kept at the salinity and temperature observed in their habitat at the time of collection, E-R animals experienced an abrupt shift to 34 ppt seawater, and F-T animals experienced a stepwise increase in salinity from ambient conditions to 14, 24, and 34 ppt seawater over a total period of 3 h (1 h per step). Each experiment included four replicates (bowls of animals) for each BWE method (F-T and E-R) and control treatment. Each replicate contained 10 individuals for each salinity treatment in a 100-ml glass bowl (120 total animals required for the control and experimental treatments). Each bowl was 4 cm in height and 8 cm in diameter, with a high surface to volume ratio to sustain high dissolved oxygen concentrations. Seawater solutions were made from filtered water (pore size, 0.2 μm) from the animal collection site and adjusted to the target salinity with artificial sea salt.

Each experiment had a time limit of 48 h that included plausible minimum exposure times before discharge for ships conducting BWE in open ocean [2,13]. All treatments were maintained in temperature-controlled incubators set at the ambient water temperatures measured during field collections. The water in the F-T treatment was changed at 1, 2, and 3 h, adjusting the salinity upward at each time point. Water in all treatments was changed at 24 h to limit any buildup of metabolites that might stress the organisms. No food was provided for these short-duration experiments.

Survivorship was recorded for all treatments after exposure times of 1, 2, 3, 24, and 48 h. If 100% mortality occurred in all replicates of a particular treatment (F-T or E-R), further observations at any subsequent time points usually were discontinued for that treatment. In all cases, survivorship was assessed visually using a stereomicroscope (×10–60) to detect any muscular contractions or respiratory currents produced by the animals. In the absence of any noticeable movement, even after contact with a probe, an animal was considered to be dead. At this point, the tissues of most animals were compromised and lost their natural pigment. When all individuals in a given treatment appeared to be dead or when 48 h had passed, animals were transferred back to their habitat water to assess whether any recovery occurred during the next 1 to 24 h. This latter step was particularly important for determining the survivorship of copepods and peracarid crustaceans.

Brine-tolerance protocol

We modified our seawater-tolerance protocol to track the survivorship over time of animals abruptly exposed to sodium chloride solutions of 30, 45, and 60 ppt. A group of seawater-tolerant species collected from the San Francisco and Suisun bays was exposed to sodium chloride solutions of 85 and 110 ppt to evaluate the efficacy of short-term brine exposures (1–3 h). Brine experiments had the same number of replicate bowls ($n = 4$) per control and treatment concentration and 10 individuals per bowl, as listed previously.

Cumulative survivorship was recorded at exposure times of 1, 2, 3, 6, 24, and 48 h. In a few experiments, when opportunity allowed, survivorship also was scored at 12 h. Brine solutions were composed of filtered water (pore size, 0.2 μm) from the collection site and adjusted to the target concentrations of sodium chloride (S-9888; Sigma-Aldrich, St. Louis, MO, USA) using a YSI 85 conductivity meter (YSI Incorporated, Yellow Springs, OH, USA) except for concentrations at 85 and 100 ppt, which exceeded the measuring range of the meter and were based on weight per volume. To ensure that any mortality was caused by changes in salt concentration and not by decreases in dissolved oxygen, the medium in all trials was replaced at 24 h. All treatments were conducted at the ambient temperature of the habitat from which the animals were collected. Survivorship was scored as discussed previously.

Data analyses

Previously, we assessed the proportion of species from low-salinity habitats that could withstand exposure to 34 ppt seawater [10]. In the present study, we summarized these experimental results as a function of mortality rate and treatment. For the purposes of the present study, if any individuals in either the F-T and E-R treatments survived after 48 h, this species was considered to be seawater tolerant. The effectiveness of the brine treatments was evaluated among seawater-intolerant and seawater-tolerant species by the relative exposure time necessary to cause 100% mortality in the experimental treatments as compared to the survivorship in the control treatments. The E-R and various brine treatments all abruptly exposed animals to elevated salt solutions, so mortality rate was only compared among these treatments and not to the F-T treatments. Using Probit (Ver 1.5; U.S. Environmental Protection Agency, Washington, DC), we estimated the sodium chloride concentrations capable of causing 100% mortality in 95% of the species in our experiments for several treatment durations. This analysis was preferred over other toxicological analyses, such as listing the time required to cause 95% mortality of individuals for each species, because we wished to estimate treatment levels that would eradicate groups of taxa.

Based on published data regarding the preferred habitat salinity ranges of species in our experiments [4], we used a logistic regression analysis to explore which factors (overall salinity range, habitat salinity and temperature at the time of collection) best predicted the ability of a species to survive in either the seawater-tolerance experiments (48 h) or intermediate brine exposures (45 ppt sodium chloride) for 3 h or more. In general, we categorized species into three salinity groups based on their habitat preferences: Freshwater and oligohaline animals were those confined to habitats of 0 to 5 ppt, brackish-water animals were those found in habitats that range from 0 to 20 ppt, and mesohaline species were those that usually occupy a salinity range of 5 to 25 ppt. Finally, the results from all our brine experiments were summarized as a function of the habitat salinity preferences of the species included, the cumulative proportion of the species eliminated, the effective brine concentration, and the exposure time. Additional supporting data for these regression models were gathered from the literature [7,14–26].

RESULTS

Seawater-tolerance experiments

Overall, 44% (F-T method) and 32% (E-R method) of the 54 taxa in our seawater-tolerance experiments had some survivorship at 48 h (Fig. 1). Among the species without 100% mortality, 42% (F-T method) and 53% (E-R method) of these taxa were peracarid crustaceans. These results support the need for additional treatments along with the current mandatory usage of BWE or seawater flushing, especially for eradicating seawater-tolerant taxa such as peracarid crustaceans. The following sections summarize our results for the sodium chloride treatments on a variety of seawater-intolerant and seawater-tolerant taxa.

Freshwater and oligohaline species

Adults of six freshwater cladoceran species, two copepod species (*Eucyclops* and *Eurytemora*), and one rotifer species experienced 100% mortality after a 1-h exposure, even at the lowest brine concentration (30 ppt). No differences were found in the time required to cause 100% mortality between the 30 ppt brine treatment and the 34 ppt seawater treatment for any of these species (Table 1). When placed back into their habitat water after the brine treatments, however, adults of two cladoceran species, *Eurycercus lamellatus* and *Bosmina longirostris*, burst and released completely developed, brooded juveniles. These juveniles survived in each of the three brine treatment concentrations (30, 45, and 60 ppt) when the exposure periods were limited to 1 h. Experiments repeated using reproductive adults of both species with late-stage broods, however, did not result with any survivorship when the exposure duration was at least 3 h (Table 1).

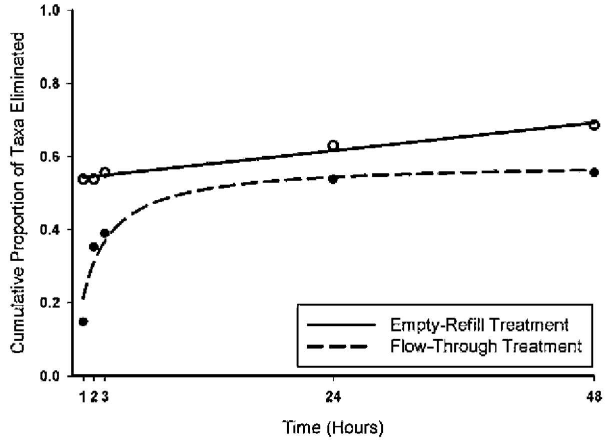

Fig. 1. The effect of osmotic shock using seawater on ballast water taxa. Both lines (dashed and solid) represent the cumulative number of taxa experiencing 100% mortality at specific dosages (ppt) and exposure durations (h). Dashed and solid lines indicate results from flow-through and empty-refill ballast water exchange protocols, respectively (see *Materials and Methods*). Model for flow-through experiments is based on single rectangular, two-parameter, hyperbolic nonlinear regression. Model for empty-refill experiments is a linear regression. Both adjusted r^2 values are greater than 0.98 (n = 54 taxa). Additional seawater-tolerance experiments were added to our previously published data [10] for this analysis.

Mussel veligers (mix of quagga and zebra mussels) retracted into their larval shells when exposed to either 34 ppt seawater or any of the brine solutions. Based on observations of velar lobe ciliary activity, mussel veligers survived for 48 h in 34 ppt seawater but only 3 h in 30 ppt sodium chloride. Mussel veligers were killed by both the 45 and 60 ppt brine treatments within 1 h. Two freshwater gammarid amphipods from Poland, *Gammarus fossarum* and *Gammarus roeseli*, were similar to each other in their tolerance to seawater (3 h) and levels of brine that caused 100% mortality (1–3 h). Individuals of *G. fossarum*, however, were eliminated with shorter exposure duration in the 30 ppt sodium chloride treatment as compared to individuals of *G. roeseli*.

Brackish-water and mesohaline species

Animals we classified as either brackish-water or mesohaline species included 12 peracarid crustacean species, one copepod species (*Acartia tonsa*), and the larvae of two decapod crustacean species. Newly released, stage-I zoeae from adult broods of the decapods, *R. harrisii* and *P. pugio*, generally were tolerant of 34 ppt seawater but differed in their tolerance to sodium chloride. *Rhithropanopeus harrisii* zoeae survived for 2 h at the lowest brine treatment (30 ppt), but *P. pugio* zoeae survived this treatment for at least 12 h (Table

Table 1. Species, sites, and results for the brine-tolerance experiments[a]

Collection site, date[b]	Species, seawater tolerance, and taxonomic group[c]	Habitat (°C, ppt)	C ± SD[h]	Exposure time (h) by concentration[i]		
				30 ppt	45 ppt	60 ppt
OH, 6/05/06	*Bosmina longirostris*[d] (C)	21, 0.1	1 ± 0	1	1	1
OH, 6/05/06	*Bosmina longirostris*[d] brooded juveniles (C)	21, 0.1	1 ± 0	3	3	3
OH, 6/01/06	*Daphnia retrocurva*[d] (C)	21, 0.1	1 ± 0	1	1	1
OH, 6/01/06	Quagga and zebra mussel larvae[e] (M)	21, 0.1	1 ± 0	3	1	1
TC, 8/04/06	*Asplanchna priodonta*[d] (R)	23, 0.1	1 ± 0	1	1	1
TC, 8/05/06	*Alona quadrangularis*[d] (C)	23, 0.1	1 ± 0	1	1	1
TC, 8/08/06	*Bythotrephes longimanus*[d] (C)	23, 0.1	0.95 ± 0.1	1	1	1
TC 8/06/06	*Cercopagis pengoi*[d] (C)	23, 0.1	1 ± 0	1	1	1
TC, 8/05/06	*Eurycercus lamellatus*[d] (C)	23, 0.1	0.98 ± 0.1	1	1	1
TC, 8/05/06	*Eurycercus lamellatus*[d] brooded juveniles (C)	23, 0.1	0.95 ± 0.1	3	3	3
LH, 5/03/07	*Chydorus sphaericus*[d] (C)	10, 0.1	0.98 ± 0.1	1	1	1
LH, 5/03/07	*Eucyclops speratus*[d] (CP)	10, 0.1	1 ± 0	1	1	1
LH, 4/30/07	*Eurytemora affinis*[d] (CP)	5, 0.1	1 ± 0	1	1	1
LH, 5/01/07	*Chaetogammarus ischnus*[f] (A)	5, 0.1	1 ± 0	24	3	2
VP, 7/18/07	*Gammarus fossarum*[d] (A)	15, 0.2	1 ± 0	2	2	2
VP, 7/28/07	*Dikerogammarus villosus*[g] (A)	18, 0.3	0.98 ± 0.1	24	6	6
VP, 7/18/07	*Gammarus roeseli*[d] (A)	17, 0.3	1 ± 0	3	2	1
VP, 7/17/07	*Pontogammarus robustoides*[f] (A)	18, 0.4	0.98 ± 0.1	24	6	3
RM, 7/25/06	*Neomysis integer*[f] (MY)	24, 1.8	1 ± 0	1	1	1
CL, 8/30/06	*Gammarus tigrinus*[f] (A)	17, 5	1 ± 0	24	24	2
CB, 7/29/06	*Acartia tonsa*[d] (CP)	20, 7.1	1 ± 0	1	1	1
CB, 7/24/06	*Gammarus tigrinus*[f] (A)	20, 7.1	0.90 ± 0.1	48	24	3
CB, 7/06/06	*Rhithropanopeus harrisii*[f] zoeae (D)	20, 9	0.90 ± 0.1	2	1	1
CB, 6/19/06	*Palaemonetes pugio*[f] zoeae (D)	20, 12.3	0.75 ± 0.1	12	2	1
SU, 10/22/07	*Grandidierella japonica*[f] (A)	16, 11	0.88 ± 0.1	*x*	*x*	48

[a] Exposure times (h) refer to the amount of time required to kill all individuals of a species within a particular sodium chloride concentration (30, 45, and 60 ppt). The details of the seawater-tolerance experiments have been previously reported [10], and some new data were added here.
[b] CB = Chesapeake Bay, Maryland, USA; CL = Curonian Lagoon, Lithuania; LH = Lake Huron (Alpena), Michigan, USA; OH = Lake Erie, Toledo, Ohio, USA; RM = Port of Rotterdam, The Netherlands; SU = Suisun Bay, California, USA; TC = Lake Michigan (Traverse City), Michigan, USA; VP = Oder and Vistula rivers, Poland.
[c] A = amphipods; C = cladocera; CP = copedpoda; D = decapoda; M = molluska; MY = Mysidae.
[d] All individuals killed within 1 to 3 h with exposure to 34 ppt seawater.
[e] Although zebra and quagga mussel larvae survived in 34 ppt seawater for 48 h, larvae died once they were put back into freshwater from their habitat.
[f] Survivorship greater than 50% after exposure to 34 ppt seawater for 48 h and placed back into habitat water.
[g] All individuals killed within 24 h with exposure to 34 ppt seawater.
[h] C ± SD = proportion of survivorship ± standard deviation in the control treatments for the brine experiments.
[i] *x* = treatment ineffective in killing all individuals of *Grandidierella japonica* (83 and 65% of individuals survived the 30 and 45 ppt sodium chloride treatments after 48 h, respectively).

1). Exposure durations causing 100% mortality also differed for these species in the 45 ppt brine treatments, but both species were killed within 1 h in the 60 ppt treatments.

Brackish-water peracarid crustaceans (except *Neomysis integer*) required much greater exposure times and sodium chloride concentrations to cause 100% mortality compared with other taxa. The introduced gammarid amphipod, *Chaetogammarus* (=*Echinogammarus*) *ischnus* was somewhat resilient in our brine experiments. Previous experiments showed that 5% of individuals of this species survived in 34 ppt seawater for at least 48 h [10]. In our brine experiments, specimens of *C. ischnus* were killed after 24-, 3-, and 2-h exposures in the 30, 45, and 60 ppt brine treatments, respectively. Although the amphipods *Dikerogammarus villosus* and *Pontogammarus robustoides* were both collected from freshwater rivers in Poland, they are found in brackish-water habitats as well. These two species survived for relatively long periods in the seawater treatments (24 and >48 h, respectively) but were killed within 24 h when treated with 30 ppt sodium chloride. Both species were eliminated within 3 to 6 h in 45 and 60 ppt sodium chloride treatments (Table 1). Two of the most resilient animals in our experiments were the gammarid amphipods, *Gammarus tigrinus* and *Grandidierella japonica*. Specimens of *Gammarus tigrinus* used in these experiments were collected from brackish-water habitats of both the Chesapeake Bay and Baltic Sea. Adults of this species tolerated the 30 and 45 ppt sodium chloride treatments for 12 to 24 h but were killed rather quickly in the 60 ppt treatments (2–3 h). Greater salinity tolerances were observed for adults of *Grandidierella japonica*, which that had high survivorships in the 30 and 45 ppt sodium chloride treatments after 48 h (83 and 65% survivorship, respectively) but experienced 100% mortality at 60 ppt after 48 h (Table 1).

The results with *Gammarus tigrinus* and *Grandidierella japonica* prompted us to focus more closely on short-term, high-salinity brine treatments capable of eliminating resistant brackish-water and mesohaline peracarid crustaceans. Populations of introduced and native peracarid species from the San Francisco and Suisun bays provided a useful source of test animals. Except for the amphipods *Hyalella* spp., all the species of peracarid crustaceans used in this portion of the present study were tolerant of 34 ppt seawater for 48 h. The amphipods *Hyalella* spp., *Americorophium spinicorne*, and *Gammarus daiberi* as well as the isopod *Synidotea laevidorsalis* and the

Table 2. Species, sites, and results for the concentrated brine-tolerance experiments[a]

Collection site, date[b]	Species and taxonomic group[c]	Habitat (°C, ppt)	C ± SD[d]	Exposure time (h) by concentration[e]	
				85 ppt	110 ppt
SF, 7/05/07	*Synidotea laevidorsalis* (I)	22, 25.6	1 ± 0	1	1
SU, 7/04/07	*Hyallela* spp. (A)	22, 1.8	1 ± 0	1	1
SU, 7/03/07	*Gammarus daiberi* (A)	22, 2.2	1 ± 0	1	1
SU, 7/04/07	*Americorophium spinicorne* (A)	22, 1.8	1 ± 0	1	1
SU, 7/15/07	*Neomysis kadiakensis* (MY)	22, 13	1 ± 0	1	1
SU, 7/06/07	*Grandidierella japonica* (A)	22, 11.8	1 ± 0	3	1
SU, 7/06/07	*Sinocorophium alienese* (A)	22, 11.8	0.95 ± 0.1	3	3
SU, 7/07/07	*Nippoleucon hinumensis* (CU)	22, 11.8	0.98 ± 0.1	>3[f]	3

[a] All species except *Hyalella* spp. had survivorships of greater than 50% after exposure to 34 ppt seawater for 48 h and a subsequent transfer to water from their collection habitat (all individuals of *Hyalella* spp. died at 24 h).
[b] SF = San Francisco Bay, CA, USA; SU = Suisun Bay, CA, USA.
[c] A = amphipoda; CU = cumacea; I = isopoda; MY = Mysidae.
[d] C ± SD = proportion of survivorship ± standard deviation in the control treatments for the brine experiments.
[e] Exposure times (h) refer to the amount of time required to kill all individuals of a species within a particular brine concentration (85 and 110 ppt).
[f] 30% of individuals of *Nippoleucon hinumensis* survived a 3-h exposure to 85-ppt brine.

mysid *Neomysis kadiakensis* were all killed by 1-h exposures to the 85 and 110 ppt brine treatments (Table 2). Two introduced amphipod species, *Corophium alienese* and *Grandidierella japonica*, and an introduced cumacean, *Nippoleucon hinumensis*, required exposure durations of at least 3 h or longer in the 85 ppt brine treatments and of 1 to 3 h exposures in the 110 ppt brine treatments to cause 100% mortality.

Data analyses

Comparing the results from the seawater-tolerance experiments to those of the 30 ppt sodium chloride treatments, the lowest sodium chloride concentration required less time to cause 100% mortality in eight freshwater and brackish-water taxa than did treatments with 34 ppt seawater. Sodium chloride concentrations causing 100% mortality in 95% of the species in our experiments are listed for exposure durations of 1, 3, and 6 h in Table 3.

The results of the logistic regression analyses of the three main species and habitat characteristics (salinity range, ambient salinity, and ambient temperature) were different between the treatment types. The best predictor of survivorship in our seawater-tolerance experiments was the overall salinity range documented for a particular species (t ratio = 2.43, p = 0.02). A species' salinity range, however, was not a good predictor of survivorship in the intermediate brine treatments. The remaining two factors, habitat salinity and temperature at the time of animal collection, were not significant predictors of survivorship for either treatment method.

Proposed brine treatment levels and their effectiveness are summarized in Figure 2. Cladocerans, copepods, and peracarid crustaceans limited to freshwater or oligohaline habitats (0–5 ppt) were killed within 1 to 3 h, even at the lowest brine concentration (30 ppt). Taxa that may occur in freshwater but that generally prefer brackish-water habitats (0–20 ppt) require higher brine concentrations (45–60 ppt) and longer treatment durations (3–24 h) to cause 100% mortality. The most resilient invertebrates in our experiments were peracarid crustaceans, such as *Grandidierella japonica* and *Nippoleucon hinumensis*, that prefer mesohaline habitats but do not usually survive in freshwater habitats [27] (http://www.southbayrestoration.org). These species required a minimum sodium chloride concentration of 60 ppt for at least 24 to 48 h to cause significant mortality. If the sodium chloride treatment was reduced to 3 h, then the brine concentrations needed to cause 100% mortality were 85 and 110 ppt for *Grandidierella japonica* and *Nippoleucon hinumensis*, respectively.

DISCUSSION

Despite the numerous proposals for ballast water treatments and systems [28–30], various protocols involving seawater flushing of commercial ship ballast tanks likely will remain an active, if not the primary, management strategy for preventing the spread of nonnative species during the next several years [31,32] (http://www.slc.ca.gov/Spec_Pub/MFD/Ballast_Water/Documents/Final_TechReport_121307_2.pdf and http://www.lr.org/NR/rdonlyres/55A3C43A-0A1B-43E0-9F336052F5BAE29D/80591/LRIMOMEPC56Report1.pdf). Based on this scenario, we previously demonstrated the dual effects of propagule reduction [33] (http://www.serc.si.edu/labs/marine_invasions/publications/PortOakfinalrep.pdf) and osmotic shock [10] occurring during BWE for coastal organisms within ballast tanks. From the present research, two overall conclusions can be drawn: First, the combined effects of propagule reduction and osmotic shock make ballast-water exchange and seawater flushing useful management tools for reducing the spread of nonindigenous species, especially among freshwater and oligohaline hab-

Table 3. Recommended minimum treatment concentrations for sodium chloride based on exposure time (n = 26 species)[a]

	Exposure duration		
	1 h	3 h	6 h
LC95 NaCl concentration (95% confidence interval)	110 (85–192) ppt	75 (59–137) ppt	60 (48–98) ppt

[a] A chi-square test of heterogeneity was not significant for any of the concentration–mortality distributions and supported the usage of a PROBIT analysis. LC95 = lethal concentration of sodium chloride causing 100% mortality for 95% of the species tested.

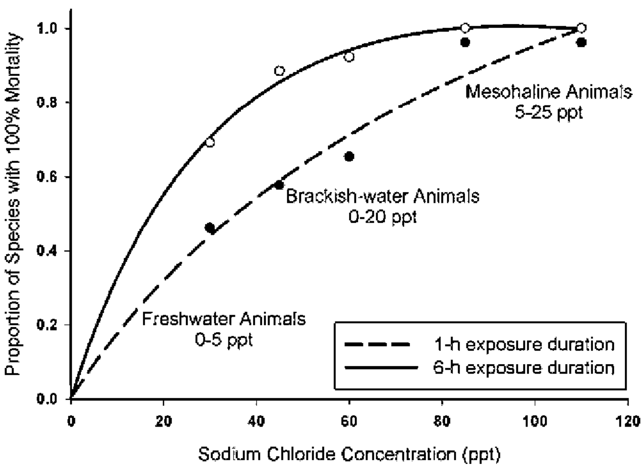

Fig. 2. Exposure time and dosage models for sodium chloride treatments. Each curve represents the total number of species experiencing 100% mortality for various brine concentrations at two exposure times. The 1-h exposure model is based on a four-parameter logistic curve. The 6-h exposure model is based on a single rectangular II, three-parameter curve. Both adjusted r^2 values are greater than 0.99. Freshwater and oligohaline organisms (those preferring salinities of 0–5 ppt) generally are intolerant of low-concentration brine exposures even at the shortest treatment duration. Brackish-water animals that also occur in freshwater (those preferring habitats of 0–20 ppt) require longer exposure durations and brine concentrations near 60 ppt. Animals that prefer mesohaline habitats (5–25 ppt) but generally are incapable of surviving and reproducing in freshwater require the greatest exposure times and brine concentrations (n = 26 species).

itats, and second, invertebrates more likely to overcome the osmotic effects of BWE and to disperse via ballast water discharges include peracarid crustaceans (amphipods, isopods, cumaceans, and mysids). In addition to the wide salinity tolerances exhibited by peracarid crustaceans, several other life-history characteristics contribute to their ability to expand their geographic range, including relatively high reproductive output [34] and, perhaps more importantly, their ability to establish populations in freshwater habitats. The latter trait is not unique to peracarid crustaceans; however, tolerance of both freshwater and hypersaline conditions may be more prevalent among introduced peracarid species than among other introduced crustacean species.

Toxicity experiments using solutions of equal concentration based on artificial sea salt or only sodium chloride against other freshwater macroinvertebrates have yielded results similar to those of the present study. The increased toxicity of sodium chloride solutions to that of seawater is linked to the elevated levels of sodium and chloride ions relative to those of other biologically important ions, such as calcium and potassium [20,21,26]. The differences in tolerance among various taxonomic groups in the present study also were observed for other species of freshwater macroinvertebrates in Australia [18,19]. In general, common freshwater invertebrates, such as fly larvae, were intolerant of increased salinity, but decapods, peracarids, and gastropods were the most resilient taxa, tolerating salinities ranging of 76 to 160 mS/cm (~50–100 ppt). Sodium chloride treatment also was evaluated for killing several species of fish, mollusks, and aquatic plants from the freshwater fishing nets of commercial vessels in New Zealand [35] (http: // yearofthemountains.org.nz/upload/documents/science-and-technical/drds261.pdf), and the recommended dosage was 70 ppt for 1 to 3 h. Although not included in the present study, we also have preliminary data that show sodium chloride treatments of 45 to 60 ppt kill the introduced round goby, *Apollonia melanostoma* (formerly *Neogobius melanostomus*, Lake Michigan population) within 1 h. The effectiveness of salt toxicity on microorganisms, such as bacteria and viruses, is more difficult to interpret. Some halotolerant bacteria from freshwater habitats can survive at 1 to 20 ppt—and in some species, up to saturation [24]. The physiological tolerances of viruses tend to coevolve along with their hosts and thus, in some cases, exhibit wide halotolerance [36].

Although many species were quickly killed by lower brine concentrations (45 ppt for 1–6 h) in our experiments, we conservatively propose, considering the additional salinity tolerance data cited here, that all sodium chloride treatments aim for a minimum exposure concentration of 80 ppt for at least 3 h. We list both the lower and upper 95% confidence limits for each treatment concentration, but we prefer to remain conservative regarding the effectiveness of sodium chloride treatments. Therefore, we suggest that the lower concentration limit for each of the three treatment durations be ignored. In some cases, it may be possible to treat the sediment and residual water in the ballast tanks of NOBOB vessels for longer periods (e.g., 12–24 h). Despite longer treatment durations, we do not recommend brine treatment concentrations less than 60 ppt for any exposure period.

The feasibility of this treatment strategy has been evaluated for use on commercial NOBOB ships in the Great Lakes region [37]. Several facilities can deliver highly concentrated sodium chloride solutions (up to 230 ppt) via trucks to many of the common ports of the region. Brine solutions could then be pumped into the ballast tanks of NOBOB vessels containing residual sediment and water. Residuals on most ships will be only a few metric tons per tank [11] and, thus, would require only a few metric tons of 230 ppt sodium chloride to produce a final salt concentration of at least 80 ppt. If the treatment is conducted at ports where cargo will be off-loaded, it should be possible to treat the tanks for 3 to 6 h before cargo off-loading would necessitate addition of ballast water to the tank.

One possible concern is that posttreatment discharge of highly saline water into freshwater habitats may negatively impact native species. After off-loading cargo and completion of the brine treatment, NOBOB vessels would need to fill their tanks with new freshwater ballast. In most cases, this would reduce the salinity in these tanks to much less than that of oceanic seawater and, along with the effects of dispersion, should not cause any greater environmental risk than the discharge of 30 ppt seawater already allowed under Great Lakes ballast regulations. Also, based on an analysis of ship traffic, the increased amount of sodium chloride introduced into the Great Lakes would not be significant compared to the levels already introduced via the deicing of roads using rock salt [37]. Furthermore, previous studies have found that the short-term toxic effects of sodium chloride as a result of road salt discharges do not threaten native freshwater taxa [14,15].

Another issue requiring further exploration is the effect of high-concentration sodium chloride on the metal structure of ballast tanks. This aspect has been addressed briefly, and in general, major tank-coating manufacturers have stated that their coatings would not be adversely affected by short exposures to sodium chloride solutions [37]. Tank coatings can become cracked over time, however, and such cracks would expose the underlying metal to the corrosion-causing brine solution. We acknowledge that this is a concern. On the other hand, the use of sodium chloride is not proposed as a regular treatment but, rather, as one to be used occasionally and for short durations. This may be a more acceptable alternative for ships that otherwise would have to return to an alternate exchange site (usually a 2- to 4-d round trip from Montreal, where most tank inspections are performed) or to seal their tanks.

CONCLUSION

If followed rigorously, current ballast water management strategies are a useful tool for reducing the spread of nonindigenous species, especially in freshwater systems. We have identified several species of peracarid crustaceans, however, with life-history characteristics that allow them to survive these management strategies and, thus, to exploit ballast tank transport as a means of long-distance dispersal that would otherwise be unavailable to them. In addition, some NOBOB ships are unable to conduct seawater flushing for safety reasons and may be carrying low-salinity, residual ballast water in some tanks. Our proposal to use concentrated sodium chloride solutions as an additional treatment for the residual organisms in the tanks of NOBOB ships provides a simple, economical, and effective method for eliminating numerous organisms, including halotolerant taxa. By timing the brine treatment to occur before ballasting, the resulting dilution will minimize both the corrosion potential in the treated ballast tanks and the potential for negative impacts on native flora and fauna during subsequent discharge (deballasting). Approval of brine as an alternative, limited-use treatment could be incorporated into existing ballast water management programs with minimal effort and cost to shipping operations, providing an additional tool for regulators to use against the spread of nonnative species.

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