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## **Brine-induced mortality of non-indigenous invertebrates in residual ballast water**

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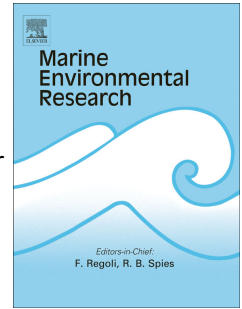
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# Accepted Manuscript



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2

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20 **Abstract**

21 All transoceanic vessels entering the Great Lakes are required to manage ballast  
22 water and ballast tank residuals with ballast water exchange and tank flushing,  
23 respectively. While these management procedures effectively reduce the density and  
24 richness of biota in ballast waters and thereby reduce the risk of transferring non-  
25 indigenous species, some ships are unable to uniformly manage all tanks. Laboratory  
26 experiments were conducted to evaluate sodium chloride brine as an emergency  
27 treatment for ballast tanks with non-compliant residuals. Invertebrate communities  
28 collected from i) Detroit River, ii) exchanged ballast tanks arriving in the Great Lakes,  
29 and iii) North Sea ports, were exposed to a range of brine concentrations (15‰-115‰)  
30 until complete mortality was reached. Results indicate that a one-hour exposure to  
31 115‰ brine is a broadly effective treatment (>99.9% mortality) regardless of treatment  
32 temperature, taxonomic group, or species' source habitat salinity. A median of 0.00%  
33 (range 0.00-5.33) of individuals are expected to survive treatment and the expected  
34 number of viable individuals released after treatment is within Canadian and proposed  
35 international discharge standards. Before implementation, validation with ship-scale  
36 trials is recommended.

37  
38 Keywords: ballast water treatment; Great Lakes; non-indigenous species; sodium  
39 chloride brine; acute toxicity; zooplankton; salinity; introduced species

40  
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## 42 1. Introduction

43 Ballast water is utilized to control the trim, stability and stresses on operational ships  
44 that lack cargo. Worldwide, shipping operations move 10 billion m<sup>3</sup> of ballast water and  
45 the biota contained therein annually (Rigby *et al.*, 1999). Ballast water transfer provides  
46 a potent mechanism for dispersal of aquatic biota to locations far more distant than  
47 natural mechanisms alone could effect (Locke *et al.*, 1993; Minton *et al.*, 2005) and  
48 contributes significantly to non-indigenous species (NIS) introductions to aquatic  
49 systems globally (Carlton, 1985; MacIsaac *et al.*, 2002).

50 Ships entering the Laurentian Great Lakes are effectively required to replace ballast  
51 water with mid-ocean seawater by exchanging full tanks or flushing residuals. Ballast  
52 water exchange and flushing reduce the risk of spreading NIS, particularly between  
53 freshwater regions, through a combination of purging and osmotic stress (see Gray *et*  
54 *al.*, 2007; Santagata *et al.*, 2008). However, at least 4% of ballast tanks in transoceanic  
55 vessels arriving to the Great Lakes in 2007 were non-compliant with ballast  
56 management regulations (120 tanks of 2867 inspected; M.G. Deneau, Fisheries and  
57 Oceans Canada, unpubl. results). Although ships with non-compliant ballast water have  
58 the choice to retain non-compliant ballast water on board for the duration of their Great  
59 Lakes' operations, or to return to sea to complete ballast exchange and/or tank flushing,  
60 a rapid and effective back-up treatment is highly desirable to minimize ship delays. As  
61 the majority of ships' ballast tanks contain only residual ballast water at entry to the  
62 Great Lakes, adequate treatment of tank residuals is a high priority.

63 The addition of sodium chloride (NaCl) brine, herein referred to as brine, has been  
64 proposed as a cost-effective, readily-available treatment for management of non-

65 compliant residual ballast water (Jenkins, 2007). A feasibility study indicated that brine  
66 could be easily applied to ballast tanks at port through the ship's sounding tube and that  
67 short-term exposure of ballast tanks to high salinity brine should not cause undue  
68 corrosion (Jenkins, 2007). In practice, because most ships will load ballast water into  
69 tanks at their first port-of-call in the Great Lakes, brine treatment of residual ballast  
70 could only be applied for a short time interval in some cases. As a result, an  
71 examination of the acute toxicity of brine exposure to aquatic invertebrates that are, or  
72 may be, transported by ship's ballast water is warranted.

73         As seawater ( $\geq 30\text{‰}$  salinity) used in current ballast management practices is  
74 effective in reducing the viability of fresh- and brackish-water taxa through osmotic  
75 stress, brine ( $> 230\text{‰}$ ) is expected to be at least as effective as current ballast  
76 management if the final salinity concentration is optimized for the duration of exposure.  
77 Altering the salinity of the surrounding environment can induce changes in the activity  
78 rate, internal volume, volume regulation, internal osmotic concentration, internal ionic  
79 content, ionic regulation, respiration rate, and oxygen requirements of aquatic  
80 organisms (Schlieper, 1971, Hart *et al.*, 1991). A large and rapid increase in salinity is  
81 expected to disrupt the aforementioned processes resulting in mortality of ballast-  
82 dwelling invertebrates. Furthermore, brine, which is manufactured from rock salt, differs  
83 substantially in ionic composition from natural ocean water, having 250% more sodium,  
84 chloride, calcium and strontium and  $< 20\%$  of the potassium and magnesium found in  
85 natural ocean water (Turekian, 1968; Hovanec and Coshland, 2004; J.N. Bradie,  
86 unpubl. results). A high concentration of salts in an "unnatural" balance should cause

87 mortality in aquatic taxa, and in fact, studies have shown that acute tolerance to NaCl is  
88 usually lower than acute tolerance to natural or artificial seawater (Kefford *et al.*, 2004).

89 Here the acute toxicity of NaCl brine exposure to aquatic invertebrates is  
90 examined *in vitro*. Addition of brine at high concentrations, for short time periods (i.e.,  
91 hours), is expected to be the most feasible treatment application under typical  
92 operational schedules (Jenkins, 2007). As the regulatory standard for treatment using  
93 ballast water exchange is at least 95% volumetric exchange of ballast with a final  
94 salinity of 30‰, we aim to determine the brine concentration required to exterminate at  
95 least 95% of aquatic invertebrates contained in ballast water, given a short duration of  
96 exposure. While Santagata *et al.* (2009) determined that a one hour exposure to 110‰  
97 brine was sufficient to cause 95% mortality, they conducted species-specific trials with  
98 only 25 species. In order to understand the efficacy of brine treatment more  
99 comprehensively, trials are conducted with diverse invertebrate communities collected  
100 from marine, freshwater and brackish-water habitats. We examine the effect of brine  
101 concentration, exposure time, treatment temperature, habitat salinity, and taxonomic  
102 affiliation on treatment efficacy to ensure that results are sufficiently robust to be  
103 applicable to all incoming vessels to the Great Lakes.

## 104 **2. Materials and methods**

### 105 *2.1 Collection of samples*

106 Invertebrates were collected in the field using vertical plankton net tows (53µm) and  
107 rinsed from the plankton net cod end into 25L unfiltered site water for transport to the  
108 laboratory. Ambient salinity and temperature of site water were measured at the time of  
109 collection using a YSI 556 multi-parameter instrument (YSI Incorporated, Yellow

110 Springs, OH, USA) or digital thermometer and digital refractometer. Ambient site  
111 salinities ranged from 0-39‰ and temperatures ranged from 5.0-24.2°C. An extra 25L of  
112 ambient site water was collected for each trial and filtered (GF/F Whatman filter, 0.7 µm  
113 pore size) to remove organisms and other organic matter for later dilution of brine to  
114 pre-determined test concentrations.

115 Collection sites were: i) exchanged ballast tanks of five ships arriving in the Great  
116 Lakes between July and November 2007; ii) the Detroit River, collected between August  
117 2007 and May 2008; and iii) the North Sea ports of Rotterdam, Antwerp and Bremen,  
118 with collections made between July and August 2008. One additional sample collected  
119 from the Waal River, the main distributary in the Rhine delta, at Nijmegen, The  
120 Netherlands, is treated herein as a North Sea sample. North Sea taxa were of  
121 significant interest because there is high shipping traffic between this region and the  
122 Great Lakes (Ricciardi and MacIsaac, 2000; Colautti *et al.*, 2003) and because similar  
123 climatic conditions make it probable that individuals from North Sea ports will tolerate  
124 the abiotic conditions of Great Lakes' ports (Reid and Orlova, 2002). In addition, tidal  
125 salinity fluctuations in North Sea ports result in invertebrate assemblages that are at  
126 least moderately tolerant of salinity changes (Barnes, 1994), so they should be a  
127 conservative indicator of brine treatment efficacy. Slight variations in methodology  
128 occurred during trials for each site, and are described below as ballast tank, Detroit  
129 River, and North Sea experiments, respectively.

130

131 *2.2 NaCl brine exposure experiments*



132 Since most aquatic invertebrates are essentially thermo-conformers, temperature  
133 changes directly alter their metabolic rate, thereby affecting their ability to osmo-  
134 regulate in hyperosmotic salinities (Kinne, 1963, Schlieper, 1971). Brine treatment  
135 efficacy was examined at exposure temperatures of 11°C and 22°C to investigate  
136 efficacy throughout the shipping season. These temperatures were chosen based on  
137 ballast tank temperatures measured during sample collection in August and December  
138 for vessels entering the Great Lakes. North Sea experiments were conducted only at  
139 22°C since there was no significant difference attributed to temperature after analyzing  
140 results from ballast tank and Detroit River trials (see 3.3). Brine treatment  
141 concentrations were chosen based on preliminary trials and findings of a brine  
142 treatment feasibility study (Jenkins, 2007). Ballast tank taxa were exposed to brine  
143 concentrations of 60‰, 77‰, and 115‰ as recommended by Jenkins (2007). In  
144 preliminary trials, Detroit River invertebrates displayed high mortality after exposure to  
145 60‰ brine, so Detroit River samples were subsequently tested at 15‰, 30‰, and 60‰.  
146 In contrast, North Sea taxa were exposed to brine concentrations of 77‰ and 115‰,  
147 since high variability in efficacy was observed after one hour exposure to 60‰ brine  
148 during ballast tank trials (see 3.2). The number of trials for each brine concentration and  
149 temperature is summarized in Table 1. A total of 17 replicated experiments were  
150 conducted.

151 Samples collected from sites with water temperatures of 17.3 to 24.2°C were stored  
152 at room temperature (22±1°C) until trials began, whereas samples collected from cold  
153 water sites (5.0 to 15.0°C) were placed in an environmental chamber at 11°C.  
154 Experiments began no more than 24 hours after sample collection and invertebrates

155 were not fed during this interval. Treatment brine concentrations were produced by  
156 diluting stock brine (300‰; Pollard Highway Products, Harrow, ON, Canada) with  
157 filtered site water; final concentrations were checked using an optical or digital  
158 refractometer. Experiments were initiated by filtering a randomly drawn subsample  
159 through a 40µm sieve and rinsing retained invertebrates into a counting tray with ~80mL  
160 of diluted brine at a pre-determined treatment concentration or filtered site water  
161 (control). The volume of filtrate was dependent on animal density, with a target of ≥50  
162 individuals per replicate for ballast tank and Detroit River experiments and ≥100  
163 individuals per replicate for North Sea experiments. The number of individuals and  
164 taxonomic composition of each replicate was subject to random variation. Four  
165 replicates were set up for each treatment and control, with the exception of ballast tank  
166 trials and the Detroit River trial at 22°C, for which five replicates were used.

167 Invertebrate survival was assessed hourly in each replicate by viewing individuals  
168 under a Leica dissecting microscope at 10-80x magnification. Invertebrates that did not  
169 exhibit any movement, even in reaction to stimulation with a dissection probe, were  
170 considered dead. When all individuals in all replicates of a treatment appeared dead,  
171 brine exposure was ended. Individuals were then transferred to filtered site water for  
172 one hour to allow possible recovery, and then reassessed. If living individuals were  
173 found after the recovery period, survival rates for earlier time periods were adjusted to  
174 correct for later, higher, survival rates. Due to time constraints, at each observation time  
175 point, control groups were counted to determine the number of dead individuals in each  
176 tray whereas treatment groups were counted to determine the number of live individuals  
177 in each tray; at the termination of each trial, samples were preserved in 95% ethanol

178 and counted in entirety to enable the calculation of percent mortality. Exposure times  
179 varied between one hour and six days due to variation in brine tolerance of taxa. Water  
180 samples from each replicate were tested to ensure that treatment concentration and  
181 temperature were maintained until the experiment was ended. Taxonomic identification  
182 was conducted for all individuals from ballast water experiments. Additionally, fixed-  
183 count sampling techniques were employed to subsample 100 individuals from each  
184 North Sea and Detroit River trial to identify to genus level (Barbour and Gerritsen,  
185 1996). For North Sea trials, individuals surviving brine treatment were preserved  
186 separately, allowing for identification of resistant taxa. Invertebrates were identified  
187 using Koste (1978), Balcer *et al.* (1984), Barnes (1994), Hayward and Ryland (1995),  
188 Johnson and Allen (2005), Bartsch (2006), and Newell and Newell (2006); taxa from at  
189 least 37 genera were included in trials.

### 190 2.3 Data Analysis

191 Survival rates from brine exposure experiments were calculated as the proportion of  
192 individuals alive when brine exposure was ended. The number of dead individuals found  
193 in treatment groups may be attributed to individuals dead at the beginning of testing,  
194 individuals that died naturally during the test, or individuals that died as a result of brine  
195 exposure. To accurately report the mortality caused by brine treatment alone, it was  
196 necessary to exclude from analysis individuals that died from the former two sources.  
197 Survival rate to brine treatment was calculated as:

$$198 \quad \text{Survival rate (\%)} = TS / CS \times 100\% \quad \text{Equation 1}$$

199 where *TS* is the number of viable individuals / total number of individuals in the  
200 treatment (15‰, 30‰, 60‰, 77‰, 115‰) and *CS* is the number of viable individuals /

201 total number of individuals in the control (filtered site water) at a given point in time  
202 (Abbott, 1925). In cases where this equation yielded a survival rate greater than 100,  
203 this value was reduced to 100 for further analysis.

204 For statistical analysis, any replicate, or taxonomic group within a replicate, that had  
205 less than 10 individuals was excluded. Kruskal-Wallis tests were performed to  
206 determine if survival rates for different brine treatments or different treatment  
207 temperatures varied significantly (Zar, 1999). Kruskal-Wallis tests were also used to  
208 determine if there was a difference in survival to brine treatment based on an  
209 individual's life history (collection salinity, taxonomic affiliation). Wilcoxon rank sum tests  
210 were used to perform pair-wise comparisons of variables found to be significantly  
211 different using a Kruskal-Wallis test. A significance level of 95% was used for all  
212 analyses, except in cases where multiple tests were conducted on the same dataset, in  
213 which case a Bonferroni correction was applied. All tests, with the exception of one,  
214 compared experiments with an equal number of replicates; for the exception, one  
215 replicate was randomly excluded from analysis. It was not possible to test for a  
216 difference in survival due to temperature after exposure to 60‰ brine, because there  
217 were differences amongst experiments within treatments (see 3.4).

218

### 219 **3 Results**

#### 220 *3.1 Taxonomic Affiliation*

221 Invertebrates tested were grouped as copepods, copepod nauplii, rotifers, and  
222 "other" taxa. Taxa in the "other" group included Cladocera (including, but not limited to,  
223 *Bosmina* spp., *Leptodora* spp., and *Diaphanosoma* spp.), Gastropoda and their larvae,

224 Halacaridae, Insecta, Mysida, Nanorchestidae, Noctilucaceae, Polychaeta, and Protista.  
225 Nauplii of Cirripedia were present in three trials at high abundance, and were  
226 considered a separate group in these trials. There was a significant difference in  
227 survival amongst taxonomic groups at 77‰ and 115‰ brine treatments (Fig. 1; Kruskal-  
228 Wallis,  $p < 0.001$ ), but not at the 60‰ concentration ( $p > 0.05$ ). For both 77‰ and 115‰  
229 treatments, significantly more Cirripedia larvae survived than copepods, copepod  
230 nauplii, or rotifers (Wilcoxon rank sum test with Bonferroni correction,  $p < 0.015$ ), and  
231 survival of “other” species did not differ from that of other groups (Wilcoxon rank sum  
232 test with Bonferroni correction,  $p > 0.04$ ).

### 233 3.2 NaCl brine toxicity

234 Survival decreased with increasing brine concentration. The median survival rate  
235 for Detroit River invertebrates exposed to three hours of 15‰ brine treatment was  
236 0.00% (range 0.00-29.82) (Fig. 2), whereas all Detroit River invertebrates died after one  
237 hour of exposure to 30‰ or 60‰ brine treatment (322 individuals) (Fig. 2a). For all  
238 invertebrates tested, the median survival rate after one hour of treatment with 60‰  
239 brine, 77‰ brine, or 115‰ brine was 0.00%. However, there were important differences  
240 in the range of survival rates observed, which reflects variability in the consistency of  
241 treatment efficacy. One hour of 60‰ brine treatment resulted in survival rates between  
242 0% and 100% (Fig. 2), whereas after two hours of exposure, survival rates were  
243 between 0.00% and 4.36% (Fig. 2). Survival rates between 0.00% and 12.09% were  
244 seen for 77‰ brine treatment, and survival rates between 0.00% and 5.33% were seen  
245 for 115‰ brine treatment. Survival rates for 60‰ are not directly comparable to results  
246 from 77‰ and 115‰ treatments, since they were not generated from the same

247 experiments (see Table 1). A total of 126 of 13188 individuals tested (<0.01%) survived  
248 exposure to 77‰ brine, whereas only five of 13183 individuals tested (3.79%) survived  
249 exposure to 115‰ brine. Significantly greater mortality occurred with exposure to 115‰  
250 than in 77‰ brine in four experiments (Fig. 3; Kruskal-Wallis,  $p < 0.05$ ). There was no  
251 significant difference in survival for ballast tank invertebrates exposed to 60‰ or 77‰  
252 brine (Fig. 2c; Kruskal-Wallis,  $p > 0.15$ ).

### 253 3.3 Temperature

254 The effect of brine exposure was examined at 22°C and 11°C for all ballast tank  
255 and Detroit River trials. Although two copepod nauplii survived 115‰ brine treatment at  
256 11°C and zero individuals survived the treatment at 22°C, the difference was not  
257 statistically significant. In fact, there was no significant difference in survival rates  
258 between the two temperatures for any of the treatment concentrations tested (Fig. 4;  
259 Kruskal-Wallis,  $p \geq 0.05$ ).

### 260 3.4 Source Habitat Salinity

261 Taxa collected from freshwater habitats were much more susceptible to brine  
262 treatment than those from either brackish or marine habitats. No freshwater taxa  
263 survived one hour of 30‰ brine treatment (Fig. 2a), whereas some individuals of  
264 brackish and marine taxa survived treatment with 60‰, 77‰ and 115‰ brine (Figs. 2b,  
265 2c). Furthermore, survival after one hour of 77‰ brine treatment was significantly  
266 greater for individuals from 20 to 22‰ habitats than for those from 1 to 9‰ habitats  
267 (Fig. 3; Kruskal-Wallis,  $p < 0.001$ ; Wilcoxon rank sum test with Bonferroni correction,  
268  $p < 0.005$ ). There was no difference in survival of taxa from different habitats after one  
269 hour of 115‰ brine treatment (Kruskal-Wallis,  $p > 0.05$ ).

### 270 3.5 Identification of Survivors

271 98 individuals in North Sea trials and 17 individuals in ballast tank trials survived  
272 one hour of 77‰ brine treatment. These survivors were 93 Cirripedia nauplii, four  
273 copepod nauplii, one *Nanorchestes* sp., one *Rhombognathides* sp., one *Nereis* sp., and  
274 15 unidentified individuals. The median survival rate for Cirripedia nauplii was 2.06%  
275 (range 0.00-12.21) for this treatment. Five individuals, including two Cirripedia nauplii,  
276 two copepod nauplii, and one *Nanorchestes* sp., survived one hour of 115‰ brine  
277 treatment. The median survival rate for Cirripedia nauplii was 0.00% (range 0.00-0.09)  
278 in this treatment.

279

### 280 Discussion

281 This study indicates that treatment with 115‰ NaCl brine for one hour can be a  
282 rapid and effective strategy for emergency management of residual ballast water to  
283 prevent the introduction of nonindigenous invertebrate species to the Great Lakes.  
284 While one hour treatment with 77‰ and 115‰ brine were both very effective (>99%  
285 mortality) against all taxa examined, 115‰ treatment was statistically more effective in  
286 several trials and yielded complete mortality more frequently, making this management  
287 option more preferable.

288 The effect of temperature on treatment efficacy was explored to ensure  
289 meaningful results irrespective of season, because the effects of salinity changes on  
290 aquatic taxa can be modulated by temperature (Kinne, 1963; Browne and  
291 Wanigasekera, 2000) and surface water temperature may vary between 0° and 27°C  
292 when international ships are active on the Great Lakes (Reid and Orlova, 2002). At the

293 brine concentrations examined here, survival was not significantly affected by  
294 temperature and thus brine treatment is expected to be effective throughout the  
295 shipping season. Since a species' salinity tolerance is influenced by the salinity of its  
296 natural habitat (Costlow *et al.*, 1966; Laughlin and Neff, 1981; Fockedeey *et al.*, 2005),  
297 our experiments included invertebrates collected from salinities between 0‰ and 34‰  
298 to determine if all taxa arriving to the Great Lakes via ballast water would be susceptible  
299 to brine treatment. Mortality was not influenced by habitat salinity when taxa were  
300 treated with 115‰ brine, providing further evidence for the broad efficacy of this  
301 treatment. 77‰ brine treatment is not as broadly effective since taxa collected from 20  
302 to 22‰ habitats survived this treatment significantly better than did those collected from  
303 1 to 9‰ environments. However, these results show that taxa in ballast tanks that  
304 originate from habitats with low salinity - which would pose the greatest establishment  
305 threat to the Great Lakes - are the least likely to survive exposure to brine treatment.

306         The most resistant taxon to brine treatment was *Cirripedia nauplii*. *Cirripedia*  
307 *nauplii* are only infrequently observed and at very low density in residual ballast  
308 samples and therefore pose a relatively low risk for introduction to the Great Lakes  
309 (Duggan *et al.*, 2005). Additionally, even if *Cirripedia* was introduced to the Great Lakes,  
310 it is a marine taxon that is not expected to survive in freshwater habitats. In fact, a  
311 comprehensive study on hull fouling found that *Cirripedia* were always dead or in poor  
312 condition when attached to ship hulls in the Great Lakes, presumably due to exposure  
313 to freshwater (Sylvester and Maclsaac, 2010).

314         We estimate that treatment using 115‰ brine for one hour of exposure will be at  
315 least as effective as ballast water exchange, exterminating >99% of marine, brackish



316 and freshwater organisms from residual ballast water. However, Canadian regulations  
317 require that any ballast water treatment other than ballast water exchange or tank  
318 flushing must reduce concentrations of viable organisms and indicator microbes below a  
319 specified discharge standard. For aquatic invertebrates with a minimum dimension  
320 greater than or equal to 50 $\mu\text{m}$  (i.e., the invertebrates examined in this study), the  
321 relevant discharge standard is less than 10 viable individuals  $\text{m}^{-3}$  (Government of  
322 Canada, 2006; see also USCG (2009) and IMO (2004) for proposed equivalent  
323 American and International standards). Given a median survival rate of 0.00% (range  
324 0.00-5.33) and assuming a median density of 280 individuals  $\text{m}^{-3}$  in untreated residual  
325 ballast water (Duggan *et al.*, 2005), treatment with 115‰ brine for one hour is expected  
326 to result in a median density of 0 (range 0-15) individuals  $\text{m}^{-3}$ . Therefore, our  
327 recommended treatment application is expected to achieve results that are largely  
328 compliant with the relevant discharge standard. Furthermore, taxa arriving in ballast  
329 water are likely in poor condition from transit, and may be more susceptible to  
330 unfavourable conditions than would the healthy port taxa tested here (Wonham *et al.*,  
331 2001).

332 While brine treatment at our recommended dosage appears highly effective, we  
333 acknowledge two limitations to our study. First, although ballast water may contain  
334 many different taxa, mainly zooplankton were tested in these experiments. Zooplankton  
335 were used as model organisms because they are abundant in ballast tanks, because  
336 their viability can be assessed easily using light microscopy, and because the Great  
337 Lakes have sustained many invasions by this group (e.g. *Bythotrephes longimanus*,  
338 *Cercopagis pengoi*, *Daphnia lumholtzi*). However, discharge standards for ballast water

339 regulate all aquatic taxa greater than 10  $\mu\text{m}$  in minimum dimension, as well as indicator  
340 microbes. Thus, it is necessary to consider a broader range of taxa when assessing  
341 brine treatment as results from zooplankton alone may not reflect efficacy against all  
342 biotic groups. It is known that NaCl concentrations  $>10\text{‰}$  will eliminate most bacteria  
343 and NaCl concentrations  $>30\text{‰}$  will be toxic to many fungi (Dr. Carol Litchfield, George  
344 Mason University, personal communication). Additionally, preliminary tests have shown  
345 that the round goby (*Neogobius melanostomus*), a previously introduced fish known to  
346 be susceptible to ballast water exchange (Ellis and MacIsaac, 2009), is killed by brine  
347 exposure of  $45\text{‰}$  to  $60\text{‰}$  (Santagata *et al.*, 2008). Therefore, although it requires  
348 empirical examination, we expect that non-halophilic taxa that are transported in ballast  
349 water will be negatively affected by brine treatment.

350         Second, our recommendations are based on laboratory, rather than ship-scale,  
351 trials. Laboratory studies were used to establish a 'proof of principle' and because they  
352 allowed us to manipulate variables that would not have been feasible in shipboard  
353 studies. Our recommendation of  $115\text{‰}$  treatment for one hour assumes complete  
354 mixing in tanks to achieve a uniform salinity. However, ballast tank structure is complex,  
355 with multiple longitudinal and transverse members that could restrict uniform application  
356 of brine to ballast residuals; higher survival rates would be expected if brine application  
357 is spatially heterogeneous. Ship-scale studies are required to determine if brine  
358 treatment will be equally effective under operational conditions.

359         Before implementation of this treatment, the environmental impact of releasing  
360 brine into the Great Lakes must be considered. Concern exists about the environmental  
361 consequences of road salt run-off entering waterways (d'Itri, 1992; Jones *et al.*, 1992;

362 Forman and Alexander, 1998) and because brine would be released into the Great  
363 Lakes post-treatment, it could contribute to the problem. Residual ballast typically  
364 amounts to less than 0.5% of tank capacity (~10 tonnes), so only small volumes of brine  
365 (~10 tonnes) would be required to conduct 115‰ treatment. As a result, the treated tank  
366 could (and should) be filled with Great Lakes water before discharge to dilute the brine  
367 to concentrations  $\leq 10\text{‰}$ . The brine is expected to be further diluted by at least a factor  
368 of 10 with discharge, so the impact of brine treatment should be minimal and spatially  
369 localized.

#### 370 **4 Conclusions**

371 One-hour treatment of 115‰ brine exterminated nearly all invertebrate ballast  
372 water taxa (>99.99%) in laboratory trials. Survival is not affected by temperature,  
373 species' habitat salinity, or by taxonomic affiliation. Post-treatment densities of viable  
374 invertebrates comply with relevant Canadian and proposed international discharge  
375 standards and should be at least as effective as current ballast water management  
376 practices of vessels entering the Great Lakes.

377

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386

## 387 **References**

388 Abbott, W.S., 1925. A method for computing the effectiveness of an insecticide. *J. Econ.*

389 *Entomol.* 18, 265-267.

390 Balcer, M.D., Korda, N.L., Dodson, S.I., 1984. *Zooplankton of the Great Lakes: A guide*

391 *to the identification and ecology of the common crustacean species.* University of

392 *Wisconsin Press, Madison.*

393 Barbour, M.T., Gerritsen, J., 1996. *Subsampling of benthic samples: A defense of the*

394 *fixed-count method.* *J. N. Am. Benth. Soc.* 15, 386-391.

395 Barnes, R.S.K., 1994. *The brackish-water fauna of northwestern Europe: An*

396 *identification guide to brackish-water habitats, ecology and macrofauna for field*

397 *workers, naturalists and students.* Cambridge University Press, Cambridge.

398 Bartsch, I., 2006. *Halacaroidea Acari.: a guide to marine genera.* *Org. Divers. Evol.* 6,

399 103-108.

400 Browne, R.A., Wanigasekera, G., 2000. *Combined effects of salinity and temperature*

401 *on survival and reproduction of five species of Artemia.* *J. Exp. Mar. Biol. Ecol.* 244,

402 29-44.

403 Carlton, J.T., 1985. *Trans-oceanic and interoceanic dispersal of coastal marine*

404 *organisms – the biology of ballast water.* *Oceanogr. Mar. Biol.* 23, 313-371.

405 Colautti, R.I., Niimi A.J., van Overdijk, C.D.A., Mills E.L., Holeck, K., MacIsaac, H.J.,

406 2003. *Spatial and temporal analysis of shipping vectors to the Great Lakes.* In: Ruiz,

- 407 G.M., Carlton, J.T., Mack, R.N. (Eds.), Invasion pathways: Analysis of invasion  
408 patterns and pathway management, pp. 227-246, Island Press, Washington, D.C.
- 409 Costlow, J.D., Bookhout, C.G., Monroe, R., 1966. Studies on the larval development of  
410 the crab, *Rhithropanopeus harrisii* Gould. I. The effect of salinity and temperature on  
411 larval development. *Physiol. Zool.* 61, 81-100.
- 412 d'Itri, F.M. (Ed.), 1992. Chemical Deicers and the Environment. Lewis Publishers,  
413 Chelsea, MI, USA.
- 414 Duggan, I.C., van Overdijk, C.D.A., Bailey, S.A., Jenkins, P.T., Limén, H., Maclsaac,  
415 H.J., 2005. Invertebrates associated with residual ballast water and sediments of  
416 cargo-carrying ships entering the Great Lakes. *Can. J. Fish. Aquat. Sci.* 62, 2463-  
417 2474.
- 418 Ellis, S., Maclsaac, H.J. 2009. Salinity tolerance of Great Lakes invaders.  
419 *Freshwater Biol.* 54, 77-89.
- 420 Fockedey, N., Mees, J., Vangheluwe, M., Verslycke, T., Janssen, C.R., Vincx, M., 2005.  
421 Temperature and salinity effects on post-marsupial growth of *Neomysis integer*  
422 Crustacea: Mysidacea.. *J. Exp. Mar. Biol. Ecol.* 326, 27-47.
- 423 Forman, R.T.T., Alexander, L.E., 1998. Roads and their major ecological effects. *Ann.*  
424 *Rev. Ecol. Syst.* 29, 207-231.
- 425 Government of Canada, 2006. Ballast water control and management regulations.  
426 *Canada Gazette* 140 (13), SOR/2006-129.
- 427 Gray, D.K., Johengen, T.H., Reid, D.F, Maclsaac, H.J., 2007. Efficacy of open-ocean  
428 ballast water exchange as a means of preventing invertebrate invasions between  
429 freshwater ports. *Limnol. Oceanogr.* 52, 2386-2397.

- 430 Hart, B.T., Bailey, P., Edwards, R., Hortle, K., James, K., McMahon, A., Meredith, C.,  
431 Swadling, K., 1991. A review of the salt sensitivity of the Australian freshwater biota.  
432 *Hydrobiologia* 210, 105-144.
- 433 Hayward, P.J., Ryland, J.S. (Eds.), 1995. Handbook of the Marine Fauna of North-West  
434 Europe, Oxford University Press, Oxford, Great Britain.
- 435 Hovanec, T.A., Coshland, J.L., 2004. A chemical analysis of select trace elements in  
436 synthetic sea salts and natural seawater. *Advanced Aquarist Online*  
437 (<http://advancedaquarist.com/issues/sept2004/feature.htm>).
- 438 International Maritime Organization, 2004. International convention for the control and  
439 management of ships' ballast water and sediments. Adopted 13 February 2004.
- 440 Jenkins, P.T., 2007. Brine as a treatment solution for the control of aquatic nuisance  
441 species introductions into the great lakes by NOBOB vessels. Transport Canada  
442 T8275-05-0249. Technical Report. Transport Canada-Marine Safety, Ottawa, ON.
- 443 Johnson, W.S., Allen, D.M., 2005. Zooplankton of the Atlantic and Gulf Coasts: A guide  
444 to their identification and ecology. Johns Hopkins University Press, Baltimore.
- 445 Jones, P.H., Jeffrey, B.A., Watler, P.K., Hutchon, H., 1992. Environmental impact of  
446 road salting. In: d'Itri, F. M. (ed.) *Chemical Deicers and the Environment*. Lewis  
447 Publishing, Chelsea, MI, pp. 1-116.
- 448 Kefford, B.J., Palmer, C.G., Pakhomova, L., Nuggeoda, D., 2004. Comparing test  
449 systems to measure the salinity tolerance of freshwater invertebrates. *Water SA*, 30,  
450 499-506.

- 451 Kinne, O., 1963. The effects of temperature and salinity on marine and brackish water  
452 animals. In: Barnes, H. (ed.) Oceanography and marine biology: An annual review.  
453 Unwin Brothers Limited, London, pp 301-340.
- 454 Koste, W. [ed.] 1978. Rotatoria. Gebrüder Borntraeger, Berlin.
- 455 Laughlin, R.B.Jr., Neff, J.M., 1981. Ontogeny of respiratory and growth responses of  
456 larval mud crabs *Rhithropanopeus harrisi* exposed to different temperatures,  
457 salinities and naphthalene concentrations. Mar Ecol-Prog. Ser. 5, 319-332.
- 458 Locke, A., Reid, D.M., van Leeuwen, H.C., Sprules, W.G., Carlton, J.T., 1993. Ballast  
459 water exchange as a means of controlling dispersal of freshwater organisms by  
460 ships. Can. J. Fish. Aquat. Sci. 50, 2086-2093.
- 461 Maclsaac, H.J., Robbins, T.C., Lewis, M.A., 2002. Modelling ships' ballast water as  
462 invasion threats to the Great Lakes. Can. J. Fish. Aquat. Sci. 59, 1245-1256.
- 463 Minton, M.S., Verling, E., Miller, A.W., Ruiz G.M., 2005. Reducing propagule supply and  
464 coastal invasions via ships: Effects of emerging strategies. Front. Ecol. Environ. 3,  
465 304-308.
- 466 Newell, G.E., Newell, R.C., 2006. Marine plankton. Hutchinson Educational, London.
- 467 Reid, D.F., Orlova, M., 2002. Geological and evolutionary underpinnings for the success  
468 of Ponto-Caspian species invasions in the Baltic Sea and North American Great  
469 Lakes. Can. J. Fish. Aquat. Sci. 59, 1144-1158.
- 470 Ricciardi, A., Maclsaac, H.J., 2000. Recent mass invasion of the North American Great  
471 Lakes by Ponto-Caspian species. Trends Ecol. Evol. 15, 62–65.

- 472 Rigby G.R., Hallegraef G.M., Sutton C., 1999. Novel ballast water heating technique  
473 offers cost-effective treatment to reduce the risk of global transport of harmful marine  
474 organisms. *Mar. Ecol-Prog. Ser.* 191, 289–293.
- 475 Santagata, S. Gasiunaite, Z.R., Verling, E., Cordell, J.R., Eason, K., Cohen, J.S.,  
476 Bacela, K., Quilez-Badia, G., Johengen, T.H., Reid, D.F., Ruiz, G.M., 2008. Effect of  
477 osmotic shock as a management strategy to reduce transfers of non-indigenous  
478 species among ports by ships. *Aquatic Invasions*, 3, 61-76.
- 479 Santagata S., Bacela, K., Reid, D., McLean, K.A., Cohen, J.S., Cordell, J.R., Brown,  
480 C.W., Johengen, T.H., Ruiz, G.M., 2009. Concentrated sodium chloride brine  
481 solutions as an additional treatment for preventing the introduction of nonindigenous  
482 species in the ballast tanks of ships declaring no ballast on board. *Environ. Toxicol.*  
483 *Chem.* 28, 346-353.
- 484 Schlieper, C., 1971. Part II: Physiology of brackish water, in: Remane A., Schlieper, C,  
485 *Biology of brackish water.* Wiley Interscience, Stuttgart, pp. 211-321.
- 486 Sylvester, F. and Maclsaac, H. 2010. Is vessel hull fouling an invasion threat to the  
487 Great Lakes?. *Divers. Distrib.* 16, 132-143.
- 488 Turekian, K.K., 1968. *Oceans.* Prentice-Hall, New Jersey.
- 489 United States Coast Guard, 2009. Proposed ballast water discharge standard  
490 rulemaking. USCG-2001-10486. (<http://www.regulations.gov>)
- 491 Wonham, M.J., Walton, W.C., Ruiz, G.M., Frese, A.M, Galil, B.S., 2001. Going to the  
492 source: role of the invasion pathway in determining potential invaders. *Mar. Ecol-*  
493 *Prog. Ser.* 215, 1-12.
- 494 Zar, J.H. 1999. *Biostatistical Analysis,* Prentice-Hall, New Jersey.



495 **Table 1.** Number of trials conducted for each temperature x brine concentration  
 496 combination, by sample source location. Sample source locations: T- ballast tank  
 497 that has undergone ballast water exchange; D- Detroit River; A= Port of Antwerp;  
 498 R- Port of Rotterdam; B=Port of Bremen; N- Waal River, Nijmegen.

	11°C					22°C				
Sample Source	15	30	60	77	115	15	30	60	77	115
T			3	3	3			2	2	2
D	1	1	1			1	1	1		
A									3	3
R									5	5
B									1	1
N									1	1

499

**500 Figure Legends**

501 Figure 1. Mean (+SD) survival rate for copepods (black bars), copepod nauplii  
502 (open bars), rotifers (vertically striped bars), “other” taxa (grey bars), and  
503 Cirripedia nauplii (diagonally striped bars) exposed to one hour of NaCl brine.  
504 Median values greater than 0 are indicated by horizontal lines. (\*) indicates  
505 statistically significant difference in survival between groups. Survival rates have  
506 been corrected to account for mortality in controls (see 2.3).

507

508 Figure 2. Mean (+SD) survival rate for (A) freshwater, (B) North Sea, and (C)  
509 ballast water organisms exposed to NaCl brine. Median value is zero for all bars.  
510 Survival rates have been corrected to account for mortality in controls (see 2.3).

511

512 Figure 3. Mean (+SD) survival rate for organisms exposed to one hour of 77‰  
513 (solid bar) or 115‰ (open bar) NaCl brine treatment. Each pair of bars  
514 represents a separate trial conducted at a given habitat salinity. Median values  
515 greater than 0 are indicated by horizontal white lines (77‰) and black lines  
516 (115‰). (\*) indicates statistically significant lower survival in 115‰ treatment  
517 than in 77‰ treatment. Survival rates have been corrected to account for  
518 mortality in controls (see 2.3).

519

520 Figure 4. Mean (+SD) survival rate for (A) freshwater, and (B) ballast water  
521 organisms exposed to NaCl brine treatment at 22°C (solid bar) and 11°C (open  
522 bar). Median values greater than 0 are indicated by white lines (22°C) and black

523 lines (11°C). Exposure time is one hour unless concentration is marked with an <sup>§</sup>  
524 (3 hours). Survival rates have been corrected to account for mortality in controls  
525 (see 2.3). The effect of temperature on survival rate was found to be statistically  
526 insignificant.

