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

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Abstract

Many transoceanic vessels enter the Great Lakes carrying residual ballast water and sediment that harbours live animals and diapausing eggs. In this study, we examine the potential for sodium hypochlorite (NaOCl) to reduce the risk of species introductions from diapausing invertebrate eggs in residual ballast sediment. We collected sediment from three transoceanic vessels and from Lake Erie and exposed them to NaOCl concentrations between 0 and 10,000 mg/L for 24 h. Hatching success was reduced by >89% in all four experiments at 1000 mg/L relative to unexposed controls. Fewer species hatched at high than at low NaOCl concentrations. Based on an average residual ballast of 46.8 tonnes, the volume of NaOCl required to treat inbound vessels is 374 L. Impacts of NaOCl use could be minimized by neutralization of treated residuals with sodium bisulfite. Further research is needed, however, to evaluate the effect of NaOCl on ballast tank corrosion.

Keywords: Diapausing eggs; Sodium hypochlorite; Ballast; NOBOB; Nonindigenous species

1. Introduction

Greater than 90% of inbound shipping traffic to the Great Lakes is comprised by vessels carrying cargo (Colautti et al., 2003). These vessels declare no-ballast-on-board (NOBOB) status when entering the Great Lakes, and are exempt from existing ballast exchange regulations. Such regulations in ballasted ships are intended to purge nonindigenous species (NIS) from ballast tanks, and kill those remaining in the tanks with high salinity water (United States Coastguard, 1993). Owing to design constraints, ballast tanks on NOBOB vessels typically contain residual water and sediment that support an abundance and variety of live invertebrate species, and hundreds of thousands of viable invertebrate diapausing eggs, including those of NIS (Bailey et al., 2003, 2005a; Duggan et al., 2005). Such species can be introduced to the Great Lakes when a NOBOB vessel loads and then subsequently discharges ballast during multi-port operations within the system. Diapausing eggs may enter the system either directly by disturbance of the residual sediment during deballasting, or, more likely, as live individuals after hatching within the ballast tanks (Bailey et al., 2005b).

Designing treatment strategies to reduce the risk posed by diapausing invertebrate eggs could prove difficult because they are contained within the ballast sediments and are not easily flushed from the tanks (Bailey et al., 2005a), and because the eggs are resistant to a wide array of adverse conditions including freezing, desiccation, disinfection, and anoxia (Gilbert, 1974; Hairston, 1996; Williams, 1998). Laboratory experiments indicate that salt water exposure, as might be achieved with open ocean ballast tank flushing, is an ineffective treatment method for this life stage but effective for water-borne active stages (Gray et al., 2005; Bailey et al., 2006). Here we examine

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whether exposure to sodium hypochlorite (NaOCl) can reduce the risk of introductions from diapausing eggs contained in NOBOB vessels entering the Great Lakes. This chemical oxidizes live tissue, and is also effective on cysts of parasitic tapeworms (Zehnder et al., 2002; Karaoglanoglu et al., 2004). At low concentrations (e.g., 1.0 mg/L), NaOCl increases hatching success of diapausing eggs or cysts, owing to its antibacterial activity and scarification effect on egg coverings (Balompapueng et al., 1997; Douillet, 1998). Biocidal effects of NaOCl when used at high concentrations on diapausing eggs have been investigated, though studies have been conducted for a limited number of species and only on eggs isolated from sediment (Pati and Belmonte, 2003; Sano et al., 2004). Consequently, it is difficult to determine the effectiveness of sodium hypochlorite as a biocide for ships' ballast. To evaluate this potential, we performed acute toxicity experiments by exposing whole sediments containing diapausing eggs of invertebrate species to concentrations of NaOCl ranging from 0 to 10,000 mg/L (active ingredient) for 24 h.

2. Methods

2.1. Sample collection

Ballast sediment for experimentation was obtained from three transoceanic vessels inbound to the Great Lakes on November 2001, July 2003, and June 2005 (Sediments 1, 2, and 3, respectively). Sediment was collected from at least five areas of the ballast tanks using sterile scoops and spatulas. An additional, natural sediment sample was collected in March 2005 from Lake Erie at the marina in Amherstburg, Ontario, Canada using a 15 cm × 15 cm ponar grab (Sediment 4). Sediment samples were stored in the dark at 4 °C for at least three weeks prior to their use in experiments in order to allow diapausing eggs to experience a refractory period (Marcus and Lutz, 1998; Jo and Marcus, 2004). To evaluate the density of diapausing eggs present in the sediments, three 40 g replicates were washed through a 45-μm sieve to remove fine sediment. The eggs were then separated from the sediment using a Ludox® HS-40 protocol (Burgess, 2001) and were enumerated under a dissecting microscope at ~32× magnification. Organic carbon content was measured using loss-on-ignition methods (K. Drouillard, Great Lakes Institute for Environmental Research, Windsor, Ontario).

2.2. NaOCl exposure experiments

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

After removal from storage, sediment was thoroughly mixed and 40 g was distributed into 500 mL glass vessels. NaOCl solution (150 mL) ranging in concentration from 0 to 10,000 mg/L was added to each vessel, which were then agitated by hand for approximately five seconds. Each of five treatment levels of NaOCl concentration was replicated three times. NaOCl solutions were prepared by mixing quantities of commercial Javex® bleach (5.25% NaOCl by volume) with synthetic pond water (Hebert and Crease, 1980). NaOCl concentrations used for the first experiment (Sediment 1) were 0, 50, 100, 1000 and 10,000 mg/L. Based on results from this experiment, which suggested lower concentrations (50, 100 mg/L) were ineffective, we altered the treatment concentrations for the subsequent experiment (Sediment 4) to 0, 500, 1000, 5000, and 10,000 mg/L. NaOCl treatment seemed most effective between 1000 and 5000 mg/L, thus the final two experiments (Sediments 2 and 3) included a 2500 mg/L treatment.

After adding NaOCl solution to the vessels, they were placed in an environmental chamber at 20 °C with a 16:8 light : dark cycle. Sediment was exposed to NaOCl solution for 24 h, after which time water from each vessel was carefully decanted and replaced with fresh synthetic pond water. This exchange procedure was performed twice in succession with each vessel to ensure that any residual NaOCl solution left after the first exchange was adequately diluted. Each exchange left $\leqslant$10 mL of the media within the sediment, resulting in an NaOCl concentration of approximately 62.5 and 666 mg/L after the first exchange for vessels treated at 1000 and 10,000 mg/L, respectively. After the second exchange this solution would be diluted to 3.9 mg/L for 1000 mg/L treatments and to 44 mg/L for 10,000 mg/L treatments. This is likely an overestimate of the residual chlorine levels since the sediment chlorine demand would have lowered the available chlorine levels after the 24 h exposure period. However, even at concentrations of 3.9 and 44 mg/L we would not expect a significant effect on hatching success as it was not significantly altered at NaOCl levels $\leqslant$100 mg/L in this study.

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

2.3. Data analysis

We calculated the reduction in hatching success in treatments relative to controls as

$$\% \text{ reduction} = 100\left(\frac{\text{HC} - \text{HT}}{\text{HC}}\right),$$

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

3. Results and discussion

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

A total of 20 rotifer and cladoceran species hatched from diapausing eggs during this study (Table 2). Nineteen species hatched from sediment collected from Lake Erie (Sediment 4), six species hatched from Sediments 2 and 3, and two hatched from Sediment 1. Copepod nauplii hatched from Sediments 2 and 4, but could not be cultured to an identifiable stage.

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

Table 1
Mean diapause egg density per 40 g by taxon

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

NS, natural sediment; BT, ballast tank.

The amount of NaOCl solution (usually 500 or 1000 mg/L) required to reduce hatching success in this study was much higher than that observed in previous trials that used diapausing eggs or cysts isolated from sediment. For example, exposure to 53 mg/L NaOCl solution for 24 h killed 90% of isolated *Artemia* cysts (Sano et al., 2004), while ~77 mg/L killed 99% of *Daphnia magna* ephippia (BMT Fleet Technology Ltd., 2002). The presence of sediment is likely responsible for the high concentrations required in this study, as it serves as a physical barrier preventing or limiting exposure of the eggs to the biocide. As well, sediment generates a high chlorine demand, reducing the effective concentration of NaOCl to which the diapausing eggs are exposed (Sano et al., 2004; BMT Fleet Technology Ltd., 2002).

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

The high NaOCl concentration required to prevent hatching of diapausing eggs in this study indicates that NaOCl treatment would not be feasible for vessels with filled ballast tanks, which can contain between ~4000 and 14,000 tonnes of ballast (Niimi and Reid, 2003). However, NOBOB cargo vessels typically contain relatively small volumes of residual water (average 46.8, range 0.0–153.0 metric tonnes per vessel; Duggan et al., 2005). To achieve a 90% or more reduction in hatching, water in ballast tanks would need to be exposed to a solution of NaOCl of at least 1000 mg/L for 24 h before being deballasted. Assuming

Table 2
List of species that emerged during hatching experiments after 24 h exposure to various NaOCl concentrations

Sediment	Group	Species	Treatment (mg/L NaOCl)							
			0	50	100	500	1000	2500	5000	10,000
1 BT	Rotifera	*Brachionus angularis*	×	×	×	–	○	–	–	○
		Brachionus calyciflorus	×	×	×	–	×	–	–	○
2 BT	Rotifera	*Brachionus angularis*	×	–	–	○	○	○	○	–
		Brachionus bidentata	×	–	–	○	○	○	○	–
		Brachionus calyciflorus	×	–	–	○	○	○	○	–
		Keratella quadrata	×	–	–	○	○	○	○	–
		Proales sp.	×	–	–	○	○	○	○	–
		Trichocerca pusilla	×	–	–	○	○	○	○	–
	Copepoda	Copepod nauplii	×	–	–	×	○	○	○	–
3 BT	Rotifera	*Brachionus calyciflorus*	×	–	–	×	×	○	○	–
		Brachionus urceolaris	○	–	–	○	×	○	○	–
		Keratella cochlearis	○	–	–	×	○	○	○	–
		Pompholyx sulcata	×	–	–	○	○	○	○	–
		Synchaeta kitina	×	–	–	○	○	○	○	–
		Trichocerca pusilla	×	–	–	×	○	○	○	–
4 NS	Rotifera	*Asplanchna brightwelli*	×	–	–	×	○	–	○	○
		Brachionus angularis	×	–	–	×	×	–	○	○
		Brachionus bidentata	○	–	–	×	○	–	○	○
		Brachionus budapestinensis	×	–	–	○	○	–	○	○
		Brachionus calyciflorus	×	–	–	×	○	–	○	○
		Brachionus caudatus	×	–	–	○	○	–	○	○
		Brachionus urceolaris	○	–	–	×	○	–	○	○
		Filinia longiseta	×	–	–	×	×	–	○	○
		Hexarthra mira	×	–	–	×	○	–	○	○
		Keratella cochlearis	×	–	–	○	○	–	○	○
		Keratella quadrata	×	–	–	×	×	–	○	○
		Polyarthra dolichoptera	×	–	–	×	×	–	○	○
		Proales sp.	×	–	–	○	○	–	○	○
		Synchaeta grandis	×	–	–	×	×	–	○	○
		Synchaeta kitina	×	–	–	×	×	–	○	○
		Synchaeta lakowitziana	×	–	–	×	○	–	○	○
		Trichocerca pusilla	×	–	–	×	×	–	○	○
	Cladocera	*Bosmina longirostris*	○	–	–	×	○	–	○	○
		Diaphanosoma birgei	×	–	–	×	×	–	○	○
	Copepoda	Copepod nauplii	×	–	–	×	○	–	○	○

NS, natural sediment (L. Erie); BT, ballast sediment; ×, species present; ○, species absent; –, treatment not run at this concentration.

the use of industrial grade NaOCl (12.5% NaOCl by volume), between 0 and 1224 L (average 374 L) would be required to treat the full range of NOBOB ships surveyed recently on the Great Lakes (Duggan et al., 2005). Considerably less volume would be required for vessels that did not intend to discharge water from all of their tanks while operating within the system, or for ships that contain design features, such as a dedicated stripping system, intended to limit sediment and water accumulation in tanks.

Use of NaOCl for treating diapausing invertebrate eggs in NOBOB vessels could have several advantages. First, the NaOCl concentration needed to eliminate many live invertebrates and algae is far lower than the 1000 mg/L treatment level suggested in this study (BMT Fleet Technology Ltd., 2002; Sano et al., 2004). This suggests that the use of NaOCl would not only reduce the invasion risk from diapausing invertebrate eggs, but it would kill live individuals present in ballast residuals. Second, the chlorine in treated ballast water could be treated with sodium bisulfite before discharge into the Great Lakes, thereby minimizing its environmental impacts (BMT Fleet Technology Ltd., 2002). The dose of sodium bisulfite required to neutralize residual chlorine could itself act as a biocide before reacting with the residual chlorine since this compound has been shown to be toxic to *D. magna*, resulting in immobility and mortality at solutions greater than 80 mg/L (Freeman and Fowler, 1953; Dowden and Bennett, 1965). Third, the cost of using NaOCl would be relatively low. Using an average contract price of ~US\$0.13 L^{-1} industrial grade NaOCl (Kirschner, 2003) costs of purchase for the average vessel would only be ~US\$50. Additional hardware or administrative costs would be necessary for the acquisition of NaOCl, its safe storage, and for safe dosing of ballast tanks. As well, regulatory hurdles pertaining to discharge of chlorinated residuals would have to be addressed.

Despite the encouraging results from this study, further research is required before the use of NaOCl in NOBOB

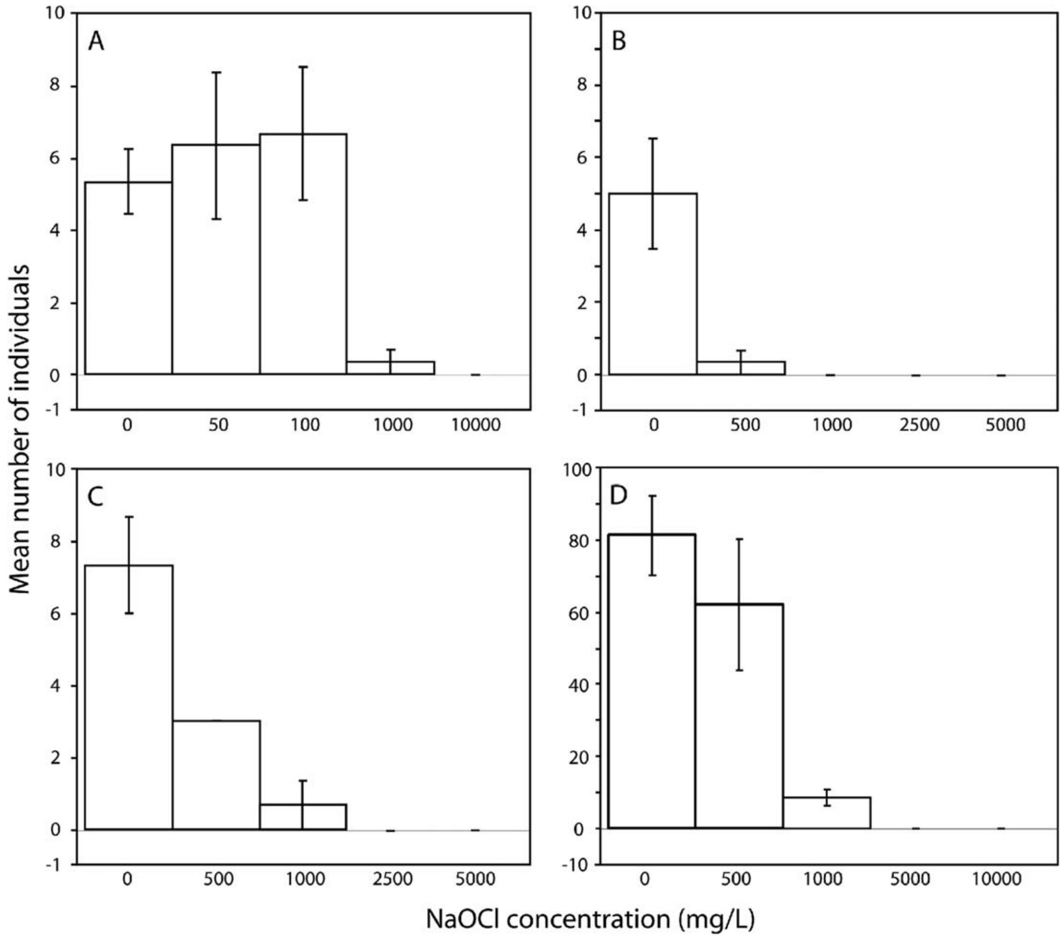

Fig. 1. Mean number of individuals (±SE) hatched from sediments after exposure to various NaOCl concentrations. (A) Sediment 1, (B) Sediment 2, (C) Sediment 3, (D) Sediment 4 (Lake Erie). Sediments 1–3 were obtained from ballast tanks of NOBOB vessels. Note differences in values on the *x* and *y* axes.

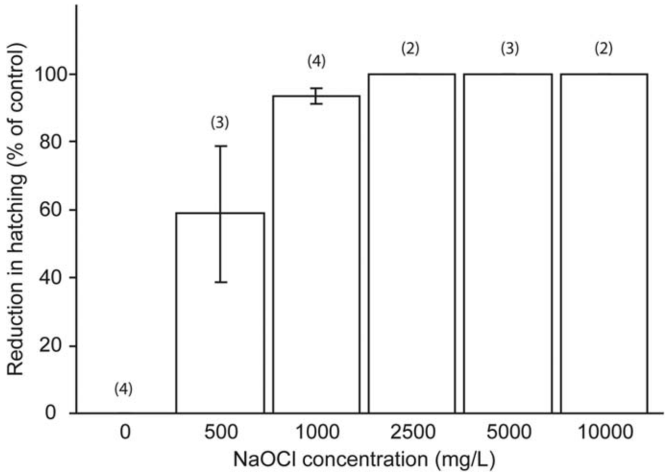

Fig. 2. Mean reduction in hatching (±SE) observed at various NaOCl concentrations compared to controls. Percent reduction compared to controls was calculated as 100((HC − HT)/HC), where HC and HT are the mean number of individuals hatched in control and experimental treatments, respectively. Values shown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

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

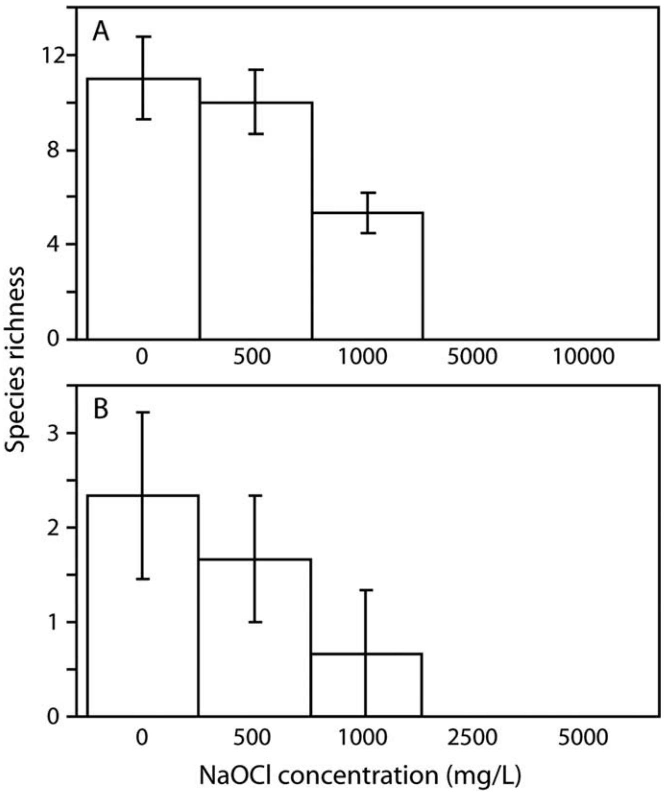

Fig. 3. Mean number of species hatched from sediments (±SE) after exposure to various NaOCl concentrations. (A) Sediment 4, (B) Sediment 3. The data of Sediments 1 and 2 were omitted from the figure since very few individuals hatched at concentrations above zero to meaningfully compare species richness among treatments. Note differences in values on the *y* axes.

efficient method of extracting water from the tanks would be required. Second, for NaOCl treatment to work effectively it would have to be well mixed within the tanks. This might be facilitated by the movement of the vessel during normal operations, but would probably require that at least 8–10 cm of residual water be present in the tanks. Even if the NaOCl is well mixed, it may not penetrate through deep residual sediment. Residual sediment is often very compacted (D. Gray, personal observation), and may provide a refuge for invertebrates, such as nematodes, which were occasionally found alive in this study after treatment with 1000 mg/L NaOCl for 24 h. However, the effect of these refugia on treatment success is probably minimal since diapausing eggs and invertebrates deep within the sediments would probably not be available for discharge during ballasting operations. Organisms close to the sediment surface that could be resuspended during ballasting operations would have presumably been exposed to the NaOCl treatment and perished as a result. Third, studies on the impact of NaOCl on ballast tank and piping corrosion at 1000 mg/L or higher is imperative since hypochlorous acid can increase corrosion of steel in water under some conditions (Gracki et al., 2002). BMT Fleet Technology Ltd. (2002) noted accelerated corrosion at 10 mg/L NaOCl, suggesting that dosing with 1000 mg/L could be a major concern. We contacted individuals familiar with ballast treatment technology and ballast coatings. All suggested that dosing with 1000 mg/L bleach could have a negative impact on ballast coatings and tank corrosion (T. Wilkins, INTERTANKO; D. Stocks, BMT Fleet Technology Ltd.; Capt. P.T. Jenkins, Philip T. Jenkins & Associates Ltd.; J.A. van Marle, Valvoline EMEA, personal communications). However, the typical vessel would only enter the Great Lakes a maximum of five times each shipping season (P.T. Jenkins, Philip T. Jenkins and Associates Ltd., personal communication), requiring a maximum of five 24 h NaOCl treatments. It should be noted that quantitative data on the impact of this treatment regime are lacking. Therefore, we propose that additional work is needed on this topic before reaching any conclusions. If damage is significant, NaOCl treatment may not be a viable option unless residual sediment loads are decreased, which would allow for lower NaOCl dosages. Currently, chlorine treatment is required for vessels entering Buenos Aires, Argentina from areas in which cholera is endemic, and Chile provides vessels with the option of using powdered NaOCl as a substitute for ballast exchange (100 mg/L; INTERTANKO, http://www.intertanko.com/tankerfacts/environmental/ballast/ballastreq.htm). Thus, a precedent exists with respect to use of chlorine for ballast water treatment. Fifth, the impact of the chlorine neutralizing agent sodium bisulfite on organisms within the tank should also be studied as it has been shown to be toxic to certain species (Freeman and Fowler, 1953; Dowden and Bennett, 1965).

In conclusion, our results suggest that the use of NaOCl in vessels could represent an effective method for reducing the risk of future species introductions via invertebrate resting stages contained in NOBOB ships' residual sediments. Although NOBOB ballast residuals have been studied most intensively in the Great Lakes, the risk of introductions from these vessels is probably widespread. NaOCl has the potential to be a cheap, effective, and environmentally benign treatment for all areas with regular shipping traffic and therefore warrants consideration by researchers and policy-makers.

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