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AN EVALUATION OF THE RESIDUAL TOXICITY AND CHEMISTRY OF A SODIUM HYDROXIDE-BASED BALLAST WATER TREATMENT SYSTEM FOR FRESHWATER SHIPS

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Abstract: Nonnative organisms in the ballast water of freshwater ships must be killed to prevent the spread of invasive species. The ideal ballast water treatment system (BWTS) would kill 100% of ballast water organisms with minimal residual toxicity to organisms in receiving waters. In the present study, the residual toxicity and chemistry of a BWTS was evaluated. Sodium hydroxide was added to elevate pH to >11.5 to kill ballast water organisms, then reduced to pH <9 by sparging with wet-scrubbed diesel exhaust (the source of CO_2). Cladocerans (*Ceriodaphnia dubia*), amphipods (*Hyalella azteca*), and fathead minnows (*Pimephales promelas*) were exposed for 2 d to BWTS water under an air atmosphere (pH drifted to ≥9) or a 2.5% CO_2 atmosphere (pH 7.5–8.2), then transferred to control water for 5 d to assess potential delayed toxicity. Chemical concentrations in the BWTS water met vessel discharge guidelines with the exception of concentrations of copper. There was little to no residual toxicity to cladocerans or fish, but the BWTS water was toxic to amphipods. Maintaining a neutral pH and diluting BWTS water by 50% eliminated toxicity to the amphipods. The toxicity of BWTS water would likely be minimal because of rapid dilution in the receiving water, with subsurface release likely preventing pH rise. This BWTS has the potential to become a viable method for treating ballast water released into freshwater systems. *Environ Toxicol Chem* 2015;34:1405–1416. © 2015 SETAC

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INTRODUCTION

The release of nonnative species in ballast water can have catastrophic effects on coastal and freshwater ecosystems and economies [1,2]. Recent studies have identified domestic ballast water transfer by ships that exclusively use the Great Lakes (referred to as "Lakers" [3]) as an important pathway of secondary spread of nonnative species in the Great Lakes [3,4]. This is, in part, the result of the large volume of ballast water transported by these vessels annually, which is 20 times greater than the volume of local ballast water transferred by coastal and seagoing vessels combined [3]. Current regulations for ballast water management differ for seagoing and nonseagoing vessels. For seagoing vessels, undiluted ballast water must meet biological standards (density of organisms within specified size classes must be under a given threshold) and biocide standards (residues of biocides or their derivatives must meet US Environmental Protection Agency [USEPA] national recommended water-quality criteria) [5]. In contrast, nonseagoing vessels, such as Lakers, are currently exempt from these standards [6] because there are no ballast water treatment systems (BWTSs) available for use on these ships. The purpose of the present study was to develop a viable BWTS for use on Great Lakes ships to prevent or reduce the spread of invasive species among large freshwater systems.

Shipboard methods for ballast water must be practical, economically feasible, and environmentally safe. Ballast tank volumes per ship can exceed tens of thousands of cubic meters [7], and the water is pumped at high rates (e.g., ∼40 m^3/min for each pump for the Great Lakes bulk carrier *M/V Indiana Harbor* [8]). A formidable challenge is to devise a method that kills 100% of the organisms in large volumes of ballast water within a ship's trip length (e.g., 18–48 h) yet retains minimal subsequent toxicity (residual toxicity) to organisms in waters receiving the treated ballast water.

An economical approach to killing ballast-residing organisms is to elevate the pH of the ballast water to ≥11.5 with sodium hydroxide (NaOH), hydrated lime $Ca(OH)_2$, or other strong bases [9,10]. One benefit of NaOH is that, unlike oxidant-based BWTSs [1], NaOH addition to water does not result in the production of additional, potentially toxic by-products. Tests of NaOH as a biocide treatment for freshwater organisms found that a 4-h exposure to pH 11.5 resulted in 100% mortality of zooplankton (adult rotifer *Branchionus calyciflorus*, cladoceran *Daphnia magna* and its resting eggs [ephippia], and copepod *Eucyclops* spp.), and a 2-d exposure to pH 12.5 reduced survival of the green alga *Selenastrum* sp. and rotifer cysts to 0% [9]. Survival of microbes (*Escherichia coli*, *Enterococci*, and heterotrophic bacteria) was reduced by >92% within 2 h in pH 11.5 treatments [9]. Exposure to a NaOH solution at pH 12 reduced survival of quagga mussel (*Dreissena rostriformis bugensis*) veligers to 0 within 30 min (C. Moffitt et al., University of Idaho, US Geological Survey, Idaho Cooperative Fish and Wildlife Research Unit, Moscow, ID, unpublished manuscript) and killed 100% of gram-negative and gram-positive bacterial cultures within 72 h, with the majority of the

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species killed within 4 h [11]. Tests on the residual toxicity of this high-pH water indicated no residual toxicity in 2-d to 4-d exposures to commonly tested organisms (amphipods [*Hyalella azteca*], fathead minnows [*Pimephales promelas*], or cladocerans [*Ceriodaphnia dubia*]) if the treated water of pH 11.5 to 12 was diluted by 100- or 1000-fold [9]. However, such high dilution rates prerelease are not feasible for shipboard treatment of ballast water.

An alternative to prerelease dilution to reduce pH is to lower the pH of NaOH-treated water to <9 by sparging with carbon dioxide (CO_2). When dissolved in water, CO_2 forms carbonic acid, which drives down the pH. Tests conducted aboard the *M/V Indiana Harbor* demonstrated that sparging NaOH-treated ballast water with compressed CO_2 lowered the pH of BWTS water (approximately 3400 m^3/tank) from approximately 12 to approximately 7.8 to 8.0 in less than 2 h [12]. Although sparging with CO_2 proved to be an effective method for reducing pH, vessel operators would prefer not to carry compressed gas onboard for safety reasons.

An alternative source of CO_2 is diesel exhaust. Diesel exhaust contains approximately 3% to 8% CO_2; it also contains a broad range of other pollutants, including metals and organic compounds in both vapor and particulate forms [13,14]. Because components in diesel exhaust have the potential to be present at concentrations toxic to freshwater organisms, it is essential to reduce the soot and particulate load of diesel exhaust by using exhaust gas–cleaning systems, known as "scrubbers" [15]. Unlike scrubbers designed to reduce sulfur dioxides in diesel exhaust, the wet-scrubber used in the present study reduces soot and particulates but maintains concentrations of CO_2 in the exhaust used to neutralize the pH of the treated water (Supplemental Data, Figure S1).

There is a need to establish whether there is residual toxicity of NaOH addition and scrubbed diesel exhaust treatment of ballast water under the proposed BWTS before this procedure can be used by the Great Lakes shipping industry. In the present study, we chemically characterized a NaOH-based BWTS and investigated whether there was residual toxicity associated with this system: addition of NaOH to bring pH to 11.5, sparging with wet-scrubbed diesel exhaust to bring the pH to <9, and aeration to bring dissolved oxygen to >8 mg/L. The BWTS water was prepared using laboratory control water because ballast water has a complex composition that varies with the ship, the season, and the port where the water is acquired, introducing complexities to data interpretation. We examined the lethal and sublethal effects of BWTS-related changes in water chemistry (pH drift ≥9, alkalinity, components associated with wet-scrubbed diesel exhaust, and combinations of these factors) on cladocerans, fathead minnows, and amphipods.

MATERIALS AND METHODS

Test organisms

The Supplemental Data provide detailed information on the testing conditions for the cladoceran, amphipod, and fathead minnow test organisms (Supplemental Data, Tables S1–S3). Neonate *Ceriodaphnia dubia* (less than 1 d old and all within 8 h of the same age) were cultured at the Columbia Environmental Research Center laboratory in Columbia, Missouri, USA, in well water diluted to 100 mg/L hardness (as $CaCO_3$), hereafter referred to as "control water." Newly hatched fathead minnow (*Pimephales promelas*, 1–2 d posthatch) were obtained from Columbia Environmental Research Center cultures or from a commercial source. *Hyalella azteca* were obtained from Columbia Environmental Research Center cultures. *Pimephales promelas* and *H. azteca* were acclimated to the 100-mg/L hardness control water for at least 24 h before the start of the toxicity tests. *Pimephales promelas* and *C. dubia* are commonly used in standard USEPA toxicity testing [16], and the amphipod *H. azteca* is commonly used to conduct toxicity tests with contaminants in water or in sediment (e.g., Morrison et al. [17] and Harper et al. [18]).

pH control: Headspace conditions

Preliminary tests indicated that when BWTS water is exposed to air, the pH of BWTS water drifts from approximately 7 to 8 to approximately 9, resulting from a decline in the partial pressure of CO_2 in the BWTS water. To distinguish toxicity attributable to elevated pH from that resulting from other water-quality changes associated with the proposed BWTS, we assessed toxicity under conditions in which pH was allowed to drift above pH 9 in an air atmosphere or was maintained below pH 9 using a CO_2 atmosphere [19]. Details on the use of CO_2 in the headspace are provided in the Supplemental Data.

Preparation of BWTS water

The Supplemental Data provide detailed information on BWTS water preparation aboard the freshwater tanker *M/V Indiana Harbor*. Briefly, NaOH was added to control water to bring the pH to approximately 11.5, and the water was sparged with diesel exhaust from the ship's engines, which had been wet-scrubbed to remove diesel components and aerated to achieve a concentration of dissolved oxygen greater than 8 mg/L. To evaluate whether there was any loss in the wet-scrubber's efficiency in removing toxicants from the exhaust, the scrubber was operated continuously for 6 h, and gas components in the scrubbed diesel exhaust were measured when the scrubber was clean and unused (0 h of use) and again after exhaust had been continuously pumped into the scrubber for the next 6 h (6 h of scrubber use). The 6-h operating period represents the longest possible duration needed to depress treated ballast water from a pH of approximately 11.5 to approximately 8 with scrubbed diesel exhaust on board the *M/V Indiana Harbor*. The NaOH-treated laboratory water was sparged onboard the ship with diesel exhaust that had been scrubbed after 0 h (BWTS-0) and after 6 h (BWTS-6) of scrubber use. The scrubbed BWTS water was then aerated with oxygen.

Study 1: BWTS toxicity test experimental design

Test organisms (*C. dubia*, *H. azteca*, *P. promelas*) were exposed to BWTS-0 and BWTS-6, undiluted (100%) or diluted with control water (50%), under an air atmosphere and under a 2.5% CO_2 atmosphere for 2 d at 25 °C. To gain knowledge about possible long-term effects of an initial 2-d exposure to BWTS water, with and without elevated pH, we transferred surviving test organisms to control water, where they were maintained under an air atmosphere for an additional 5 d with water renewal and feeding as described (Supplemental Data, Tables S1–S3). This exposure sequence likely represents a worst-case scenario because organisms in the receiving water of a lake would be exposed to undiluted ballast water for a very short time, based on recent studies demonstrating that the concentration of ballast water in a harbor (measured in transects along the ship) falls to 1% to 5% of concentrations in the ballast tank within hours of ballast water discharge, reaching a dilution factor of 10^3 in 1 d [20]. In the BWTS tests, toxicity greater than that expected based on alkalinity (with pH ~8) likely would be attributable to

components from the scrubbed diesel or from alterations in water chemistry associated with NaOH addition. Survival (*C. dubia*, *H. azteca*, *P. promelas*) was determined after the initial 2-d exposure and after the 5-d postexposure, and the weight of *P. promelas* and the reproduction of *C. dubia* were determined at the end of the 5-d postexposure. Sublethal effects on *H. azteca* are not typically measured in short-term exposures given the limited growth for this species expected in 7-d exposures without feeding for the first 2 d.

Study 2: Sodium bicarbonate toxicity test experimental design

Toxicity tests with $NaHCO_3$ were conducted to determine if alkalinity levels observed in the BWTS tests were potential sources of toxicity and to calculate median lethal concentrations (LC50s) for alkalinity for each of the 3 species. Test organisms were exposed to 6 alkalinities (100 mg $CaCO_3$/L, 200 mg $CaCO_3$/L, 400 mg $CaCO_3$/L, 800 mg $CaCO_3$/L, 1600 mg $CaCO_3$/L, and 3200 mg $CaCO_3$/L) under 2 atmospheres (air and 2.5% CO_2) for 2 d at 25 °C. To achieve these alkalinities, $NaHCO_3$ was added to control water (hardness 100 mg alkalinity 100 mg $CaCO_3$/L, pH 8.2) [21]. Alkalinities were chosen to bracket the alkalinity of 100% BWTS water (~750 mg $CaCO_3$/L) and the published LC50 values for alkalinity toxicity for our test organisms (500–1139 mg $CaCO_3$/L) [18,22–24]. Exposure times and end points were as described for the BWTS test.

Toxicity testing

Toxicity testing protocols followed ASTM International and USEPA effluent toxicity testing methods [19,25,26] except where noted (Supplemental Data, Tables S1–S3). The test acceptability criteria were set at ≥90% control survival at the end of the initial 2-d exposure period and ≥80% overall control survival at the end of the subsequent 5-d postexposure period. In accordance with standard protocols for 2-d toxicity tests, organisms were not fed during the 2-d exposure period as the addition of food can compromise water quality during the exposure. This is not the standard protocol for 7-d testing, in which organisms are fed throughout the exposure period. The lack of food during the first 2 d in the present studies may have contributed to the lower reproduction of cladocerans by the end of the 5-d postexposure period.

Water quality and chemical analysis

The pH, conductivity, hardness, alkalinity, dissolved oxygen, and total ammonia nitrogen were measured at the beginning of the exposure (day 0) and at the end of the exposure (day 2) on single composite samples pooled from replicate beakers for each treatment and time point. To determine if the BWTS water met the vessel permit guidelines for water quality as defined by the USEPA [15] and the International Marine Organization [27], polynuclear aromatic hydrocarbons (PAHs), metals, and major anions were analyzed in BWTS-0, BWTS-6, and control water, and PAHs were analyzed in water from Lake Superior (inlet water 0). To assess the efficiency of the wet-scrubber to remove pollutants from the diesel exhaust, levels of these chemicals in scrubber water were compared with levels in BWTS water. Scrubber water is water that is recirculated within the closed-loop scrubbing system. The charge water for the scrubber system is from Lake Superior (inlet water). To determine if the efficiency of the scrubber decreased over time, chemical levels in BWTS water were compared after 0 h and 6 h of scrubber use (BWTS-0, BWTS-6). Thirty-four individual PAHs were analyzed, including the priority pollutant PAHs and alkylated PAHs (Supplemental Data, Table S4), as described [28,29]. Detection limits for the PAHs differed for sample sets analyzed on different days, a reflection of higher method and instrument detection limits for the second set of samples. Detection limits were 0.002 μg/L for the initial set of shipboard samples (inlet water, scrubber water, BWTS water) and 0.010 μg/L for the laboratory water (control water) analyzed a few weeks later. The PAH concentrations were corrected for percent recovery using deuterated surrogates spiked into the samples. Dissolved and total metals were analyzed according to USEPA inductively coupled plasma–mass spectrometry (ICP-MS) methods 200.8 and 6020A [30], with specific quality-assurance and sample-handling procedures for ICP-MS analyses following USEPA method 1638 [31]. Because arsenic and selenium levels in BWTS water were found to be at or below method detection limits of the ICP-MS method, the more accurate hydride generation atomic absorption methods (USEPA 7062 and 7742, respectively) were not used for confirmatory analyses for these 2 metalloids. Digestion of unfiltered subsamples for total metals was conducted using nitric acid addition and microwave heating according to USEPA method 3015a [32]. Recovery of elements spiked into the BWTS water ranged from 86% to 100% (unfiltered) and from 81% to 100% (filtered). Metal concentrations were corrected for blank values, when appropriate, but were not corrected for recovery. Analysis of major anions was conducted on 5-μm filtered subsamples following USEPA method 9056a [33].

Statistical analyses

Tests of the biological effects of treatment (untreated vs treated) on dependent variables (survival, reproduction, weight) were made using analysis of variance (ANOVA) or the Kruskal-Wallis test on ranks if the data were not normally distributed, followed by post hoc tests. Survival percentage data were transformed using arcsine-square root (arcsine [sqrt {percent survival/100}] × 2) and tested for normality (Shapiro-Wilk test) and equal variance (F test) before analysis. For *C. dubia* reproduction (number of offspring per female), count data were removed for females that died without producing offspring ("structural 0s"), and count data were log-transformed prior to statistical analysis. Statistical significance was determined at $\alpha = 0.05$ using SigmaPlot™ (Ver 12). The 2-d and 5-d postexposure LC50s for each treatment in the sodium bicarbonate study were estimated where applicable using the Toxicity Relationship Analysis Program [34]. In general, a normal probability distribution model was used for survival data. The exposure concentrations were log-transformed, and the response of each replicate was used for the calculation. Lowest-observed-effect concentrations (LOECs) were calculated using ANOVA. Because only those *C. dubia* surviving the initial 2-d exposure were used for the 5-d postexposure reproduction tests, the cumulative survival for *C. dubia* was determined by multiplying the proportion surviving to 2 d by the proportion surviving to 5 d postexposure. This allowed calculation of LC50s, but not LOECs, for the 5-d postexposure *C. dubia* in the sodium bicarbonate study. Two-way ANOVAs were conducted to determine if there was any effect of dose or atmosphere (air vs CO_2) or an interaction of the 2 on the toxicity of alkalinity (sodium bicarbonate study) or on the toxicity of the BWTS water. Three-way ANOVAs indicated that there was no significant interaction among atmosphere, dilution (50% vs

100% BWTS water), and hours of scrubber use (0 vs 6) on the toxicity of BWTS water.

RESULTS

Chemical concentrations

The wet-scrubber efficiently removed PAHs, metals, and major anions from the diesel exhaust of the *M/V Indiana Harbor* as indicated by the small amounts of these pollutants in BWTS water compared with scrubber water. For total PAHs (tPAH), concentrations were 2 to 3 times lower in 100% BWTS water than in scrubber water (Supplemental Data, Table S4). Concentrations of tPAH in BWTS water were 0.11 μg/L (100% BWTS-0) and 0.13 μg/L (100% BWTS-6), of which approximately one-half (0.05 μg/L) was derived from 2 compounds (phenanthrene and fluoranthene) in the control water. Background concentrations in the lake water used to fill the scrubber unit (0.04 μg/L inlet water) account for 12% to 15% of scrubber water tPAH (0.27–0.36 μg/L scrubber water).

Metal concentrations were up to 15 times lower in BWTS-0 compared with scrubber water 0 (Supplemental Data, Table S5). Metal concentrations in BWTS water ranged from <0.01 μg/L (titanium, 100% BWTS-0) to 62 μg/L (zinc, 100% BWTS-6), levels that were only slightly higher than levels of metals in control water (Supplemental Data, Table S5), with 2 exceptions: Zn and Cu concentrations were 6 to 28 times higher, respectively, in 100% BWTS-6 water relative to levels in control water.

The sum of nitrates and nitrites was 17 times lower in BWTS-0 than in scrubber water 0 (Supplemental Data, Table S6). Concentrations of all major anions in BWTS waters were similar to those in control water, with 1 exception: the sum of nitrates + nitrites was 39 to 47 times higher in 100% BWTS-0 and 100% BWTS-6 water (3.9–4.7 mg/L) than in control water (<0.1 mg/L; Supplemental Data, Table S6).

Water quality in the BWTS water toxicity test

Water quality (alkalinity, hardness, pH) for the BWTS toxicity test was measured on composite samples pooled from replicate beakers collected on day 0 (initial composite) and after the 2-d exposure (2-d composite) and was similar for all 3 species (Supplemental Data, Figure S2). Representative water-quality data for *H. azteca* are shown in Figure 1. Following the addition of NaOH, sparging with wet-scrubbed diesel (which contained 6.2%–6.3% CO_2), and overnight shipment at approximately 5 °C for toxicity testing, the alkalinity at the start of the exposures increased from approximately 100 mg/L in control water to 412 mg/L to 420 mg/L (50% BWTS) and to 714 mg/L to 752 mg/L (100% BWTS; Figure 1A). The alkalinity of BWTS water was relatively unaffected after the 2-d exposure under an air atmosphere (black bars) or after the 2-d exposure under a 2.5% CO_2 atmosphere (striped bars) compared with initial alkalinity (white bars). The initial hardness was 100 mg/L in control water, 90 mg/L to 92 mg/L in the 50% BWTS water, and 60 mg/L to 70 mg/L in the 100% BWTS water (white bars; Figure 1B). After the 2-d exposure, there was a small drop in hardness levels in the BWTS water under an air atmosphere (black bars), likely a result of the precipitation of calcium and magnesium out of solution as CO_2 degassed from the beakers. Hardness remained similar to initial levels during the 2-d exposures in all waters under a 2.5% CO_2 atmosphere (striped bars). The pH of control water, 50% BWTS water, and 100% BWTS water at the beginning of the exposures was 8.3 to 8.4 (white bars; Figure 1C). After the 2-d exposures,

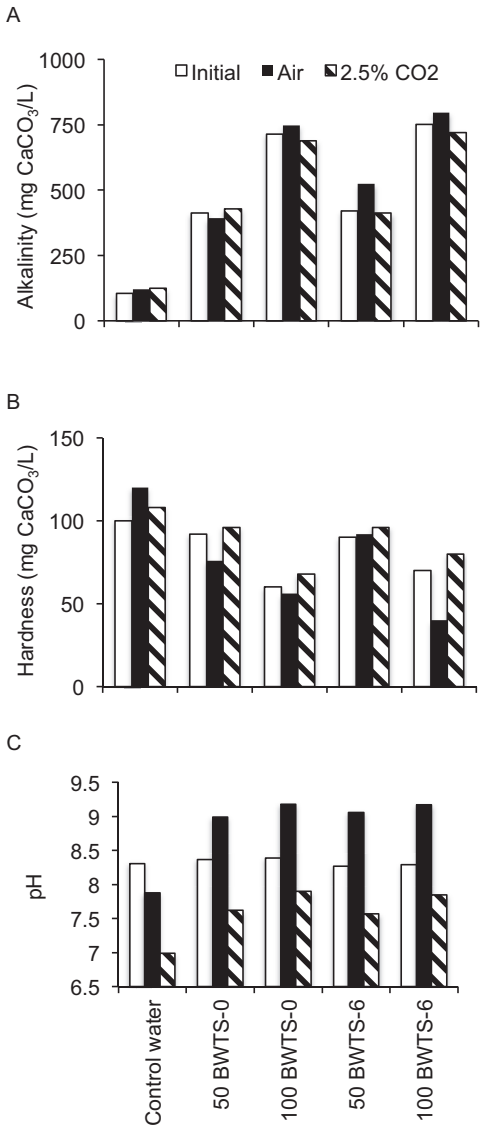

Figure 1. Water-quality characteristics of the control water or water treated with NaOH, sparged with wet-scrubbed diesel exhaust, and aerated (BWTS water) in beakers containing *Hyalella azteca* at the start of the 2-d exposures (white bars) and at the end of the 2-d exposures under an air atmosphere (black bars) or under a 2.5% CO_2 atmosphere (striped bars): (**A**) alkalinity, (**B**) hardness, (**C**) pH. Values are for composite samples pooled from 4 replicate beakers. BWTS-0 = NaOH-treated laboratory water sparged with diesel exhaust that had been scrubbed after 0 h of scrubber use; BWTS-6 = NaOH-treated laboratory water sparged with diesel exhaust that had been scrubbed after 0 h of scrubber use; 100 BTWS = undiluted ballast water treatment system water; 50 BWTS = ballast water treatment system water diluted to 50% with control water.

the pH of the 100% BWTS water increased to ≥9 (~9–9.2) under an air atmosphere (black bars), whereas the pH of the 100% BWTS water under a 2.5% CO_2 atmosphere was maintained at 7.5 to 7.9 (striped bars). The pH of the control water after the 2-d exposure was approximately 7.9 under an air atmosphere and approximately 7.0 under a CO_2 atmosphere. Ammonia levels in the test chambers at 2 d (0.17–2.8 mg NO_3-N/L) were well below mean acute toxicity values for the test species (144–193 mg/L) [35].

Residual toxicity of BWTS water to test organisms

For the BWTS toxicity tests, the 2-d control survival was >90% for all 3 species (Table 1). There were no effects on the

Table 1. Mean survival percentages of *Ceriodaphnia dubia*, *Pimephales promelas*, and *Hyalella azteca* in the ballast water treatment system (BWTS) toxicity test[a]

		Air[b]		2.5% CO_2[b]	
Species	Treatment	2-d exposure	5-d postexposure	2-d exposure	5-d postexposure
C. dubia					
	Control water	100 (0)	100[c]	100 (0)	80
	50 BWTS-0	98 (5)	98	100 (0)	100
	100 BWTS-0	98 (5)	98	95 (6)	76
	50 BWTS-6	95 (6)	86	100 (0)	100
	100 BWTS-6	95 (6)	95	100 (0)	100
P. promelas					
	Control water	100 (0)	98 (5)	100 (0)	100 (0)
	50 BWTS-0	100 (0)	98 (5)	100 (0)	98 (5)
	100 BWTS-0	100 (0)	100 (0)	98 (5)	98 (5)
	50 BWTS-6	100 (0)	100 (0)	100 (0)	100 (0)
	100 BWTS-6	100 (0)	100 (0)	98 (5)	98 (5)
H. azteca					
	Control water	100 (0)	81 (17)	100 (0)	95 (6)
	50 BWTS-0	95 (6)	50 (26)	100 (0)	90 (12)
	100 BWTS-0	65 (13)*	25 (19)*	100 (0)	68 (5)*
	50 BWTS-6	80 (14)*	45 (10)	100 (0)	88 (18)
	100 BWTS-6	68 (22)*	45 (25)	98 (5)	75 (6)*

[a]Organisms were exposed to dilution water control or to treated water after 0 h and 6 h of scrubber use (BWTS-0, BWTS-6) either diluted 50% (50 BWTS) or undiluted (100 BWTS) and held under an air atmosphere or under a 2.5% CO_2 atmosphere during the 2-d exposure. After the 2-d exposure, surviving test organisms were transferred to dilution control water under an air atmosphere for 5 d.
[b]Values are mean (standard deviation in parentheses). For the 2-d end point, $n = 4$ replicates of 10 individuals. For the 5-d end point, $n = 4$ replicates of 10 individuals/replicate (*P. promelas*, *H. azteca*) or $n = 10$ replicates of 1 individual/replicate (*C. dubia*).
[c]See text for calculation of 5-d postexposure survival for *C. dubia*.
*Significantly different from respective control water at $p < 0.05$.

survival of *C. dubia* exposed to 100% or 50% BWTS-0 or BWTS-6 water at the end of the initial 2-d exposure under both atmospheres (i.e., with or without pH control). Reproduction of *C. dubia* in the control water (~5–6 young per female under both atmospheres; Figure 2A) was below the recommended rate of 15 young per female, likely a result of not feeding the organisms during the first 2 d of the exposure. Effects of BWTS water on *C. dubia* reproduction were inconsistent; the average number of offspring produced per female being significantly higher for *C. dubia* exposed for 2 d to 50% BWTS under both atmospheres (~9–14 offspring per female) relative to controls, whereas no significant effects of exposure to 100% BWTS water were observed (~2–9 offspring per female; Figure 2A).

There was no effect on the survival of *P. promelas* exposed to either 100% or 50% BWTS-0 or BWTS-6 water, either at the end of the initial 2-d exposure period or at the end of the 5-d postexposure period in control water, with or without pH control during the initial 2-d exposures (Table 1). There was also no effect of the initial 2-d exposures to any BWTS treatment on the weight of *P. promelas* after the 5-d postexposure (average range, 0.28–0.51 g dry wt/fish across treatments), regardless of the atmosphere tested (Figure 2B).

The atmosphere under which amphipods were exposed and the dilution of BWTS water to which they were exposed significantly affected *H. azteca* survival. A 2-d exposure under an air atmosphere with increased pH significantly reduced survival of *H. azteca*, with survival ranging from 65% to 80% in the 100% BWTS-0, 100% BWTS-6, and 50% BWTS-6 treatments (Table 1). Additional mortality was observed at 5 d postexposure after the initial 2-d exposure in all BWTS treatments under an air atmosphere, though survival was only significantly different from controls in the 100% BWTS-0 treatment. In contrast, when pH was controlled under a CO_2 atmosphere during the initial 2-d exposures, BWTS treatments were not lethal to *H. azteca* at the end of the 2-d exposures (100% survival in all BWTS treatments). After the 5-d postexposure, however, mortality was observed in the undiluted 100% BWTS treatments, even with pH control (68% and 75% survival at the end of the 5-d postexposure period). For the 2-d exposure, the effect of atmosphere on the toxicity of BWTS water was statistically significant (atmosphere × dilution, $p < 0.001$).

Water quality in the sodium bicarbonate toxicity test

Water quality (alkalinity, hardness, pH) for the sodium bicarbonate toxicity test was measured on day 0 and after the 2-d exposure (separate composite samples for each species) and was similar for all 3 species (Supplemental Data, Figure S3). Representative water-quality data for 1 species, *H. azteca,* are shown in Figure 3. Alkalinity was unaffected by the 2-d exposure to either atmosphere for target alkalinities <1600 mg $CaCO_3$/L (Figure 3A). At alkalinity ≥1600 mg/L, however, measured alkalinity tended to be higher than target alkalinity under an air atmosphere (black bars) for *H. azteca* (measured vs target: 2150 vs 1600, 4150 vs 3200; Figure 3A) and *P. promelas* (1770 vs 1600, 3690 vs 3200; Supplemental Data, Figure S3). It is unclear why some measured alkalinities were higher than target alkalinities, particularly at the 3200 mg/L level, under an air atmosphere (and not a CO_2 atmosphere), and why higher alkalinity occurred in the exposure beakers of only 2 of the 3 species (atmosphere had little effect on alkalinity in the *C. dubia* exposure chambers; Supplemental Data, Figure S3). Because these measurements were conducted on a single composite sample from replicate exposure beakers, statistical analyses could not be conducted.

The addition of sodium bicarbonate, as well as the atmosphere in the headspace, altered hardness. At the start of the exposures, the addition of sodium bicarbonate to achieve an alkalinity ≥1600 mg/L had the effect of lowering hardness from

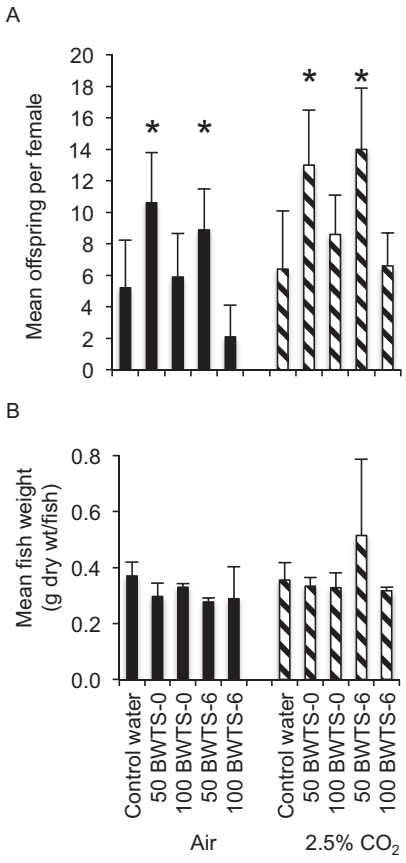

Figure 2. Effects of 2-d exposure to ballast water treatment system (BWTS) water under an air atmosphere (black bars) and under a 2.5% CO_2 atmosphere (striped bars) on (**A**) reproduction of *Ceriodaphnia dubia* and (**B**) the weight of *Pimephales promelas* at 5 d postexposure. Mean ± standard deviation ($n = 10$, *C. dubia*; $n = 4$, *P. promelas*). * Significantly different from respective control at $p < 0.05$. See Figure 1 caption for definitions of abbreviations.

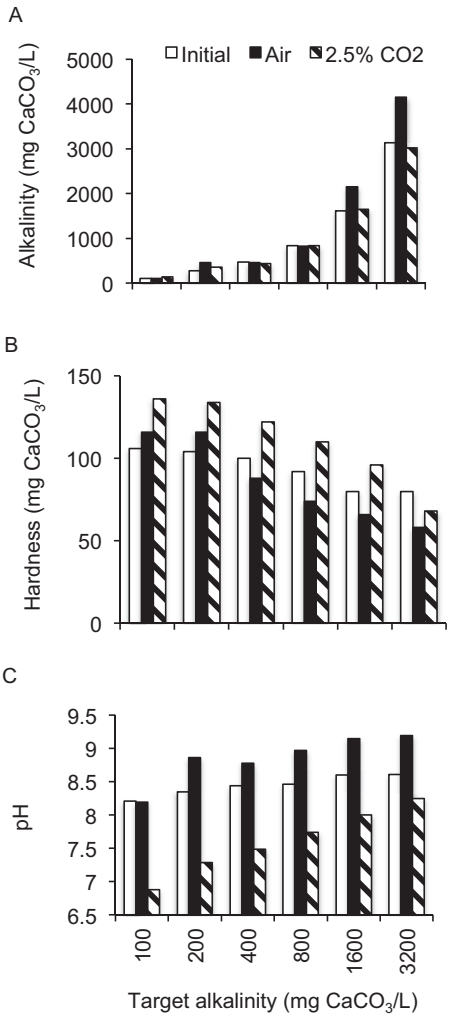

Figure 3. Water-quality characteristics of the exposure water in the sodium bicarbonate toxicity test in beakers containing *Hyalella azteca* at the start of the 2-d exposures (white bars) and at the end of the 2-d exposures under an air atmosphere (black bars) or under a 2.5% CO_2 atmosphere (striped bars): (**A**) alkalinity, (**B**) hardness, (**C**) pH. Values are for composite samples pooled from 4 replicate beakers.

100 mg $CaCO_3$/L in the control to 80 mg $CaCO_3$/L in the 1600-mg/L and 3200-mg/L alkalinity treatments (Figure 3B, white bars). After the 2-d exposures, hardness in exposure beakers at an alkalinity ≥400 mg/L to 1600 mg/L held under an air atmosphere (black bars) decreased from an initial range of 100 mg/L to 80 mg/L to a range of 88 mg/L to 50 mg/L for all 3 species. In contrast, hardness in exposure beakers at an alkalinity of ≥400 mg/L to 1600 mg/L held under a 2.5% CO_2 atmosphere (striped bars) remained in the initial range (108–80 mg/L for *C. dubia* and *P. promelas*; Supplemental Data, Figure S3B) or increased (136–96 mg/L for *H. azteca* at target alkalinity ≥100 mg/L; Figure 3B). After the 2-d exposures, hardness dropped in the 3200–mg/L exposure beakers, regardless of atmosphere, from 80 mg/L to a range of 50 mg/L to 58 mg/L (air atmosphere) and 62 mg/L to 68 mg/L (2.5% CO_2 atmosphere; Figure 3A; Supplemental Data, Figure S3B). As expected, pH increased over the 2-d exposure period in beakers held under an air atmosphere (black bars) from an initial value (8.2–8.6, white bars) to a value of ≥9 (9–9.2) at an alkalinity of >800 mg $CaCO_3$/L (Figure 3C). In contrast, pH was maintained between 6.7 and 8.3 under the 2.5% CO_2 atmosphere (striped bars), even at the 3200 mg/L alkalinity. Ammonia levels in the test chambers (0.22–1.3 mg NO_3-N/L) were well below mean acute toxicity values for the test species (e.g., 144–193 mg/L) [35].

Alkalinity toxicity

The 2-d control survival for all 3 species in the sodium bicarbonate toxicity test was ≥90%, and the 5-d postexposure control survival was >80% (Table 2). Differences in alkalinity toxicity were observed among species, with pH significantly affecting toxicity (Table 2). The 2-d LC50 and LOECs were estimated based on the average of the initial alkalinity and the alkalinity measured in the beakers at the end of the 2-d exposures. The mortality curves for alkalinity were steep for all 3 species. This resulted in LC50 values bracketed by the no-observed-effect concentration and the LOEC and produced LOEC values that, in some instances, were higher than the LC50s. The 2-d LC50s (LOECs in parentheses) under air with pH drift ≥9 were 1809 mg/L (1679 mg/L) for *C. dubia*, 3393 mg/L (3412 mg/L) for *P. promelas*, and 791 mg/L (1879 mg/L) for *H. azteca*. When pH was neutral under a CO_2 atmosphere, alkalinity was less toxic: 2-d LC50s (LOECs in parentheses) under a CO_2 atmosphere were 2033 mg/L (2992 mg/L) for *C. dubia*, >3092 mg/L (>3092 mg/L) for *P. promelas*, and 1689 mg/L (1629 mg/L)

Table 2. Mean survival percentages of *Ceriodaphnia dubia*, *Pimephales promelas*, and *Hyalella azteca* in the sodium bicarbonate toxicity test[a]

Species	Target alkalinity (mg $CaCO_3$/L)	Air		2.5% CO_2	
		2 d exposure	5-d postexposure	2 d exposure	5-d postexposure
C. dubia					
	100[b]	100 (0.0)	90	100 (0)	90
	200	98 (5.0)	78	100 (0)	30
	400	95 (5.8)	95	100 (0)	70
	800	100 (0)	80	95 (5.8)	86
	1600	68 (9.6)*	68	98 (5)	98
	3200	0*	0	0*	0
	LC50[c]	1809 (−6371 to 9989)	1942 (1674 to 2209)	2033 (ND[d])	2511
	LOEC[c]	1679	ND	2992	ND
P. promelas					
	100[b]	100 (0)	93 (5)	100 (0)	95 (10)
	200	98 (5)	100 (0)	98 (5)	100 (0)
	400	98 (8.2)	95 (10)	100 (0)	95 (5.8)
	800	100 (0)	98 (5)	100 (0)	90 (8.2)
	1600	100 (0)	98 (5)	95 (10)	95 (10)
	3200	48 (26.3)*	45 (26.5)*	90 (14.1)	85 (17.3)
	LC50[c]	3393 (−7391 to 14 178)	3371 (−47 908 to 54 649)	>3092	>3092
	LOEC[c]	3412	3412	>3092	>3092
H. azteca					
	100[b]	98 (5.0)	93 (9.6)	100 (0)	98 (5)
	200	100 (0)	93 (9.6)	98(5)	93 (9.6)
	400	98 (5)	93 (9.6)	95 (10)	93 (9.6)
	800	40 (31.6)	15 (12.9)	98 (5)	98 (5.0)
	1600	0*	0*	58 (17.1)*	38 (29.9)*
	3200	0*	0*	0*	0*
	LC50[c]	791 (719 to 863)	889 (705 to 1072)	1689 (1084 to 2293)	1565 (1318 to 1811)
	LOEC[c]	1879	1879	1629	1629

[a]Organisms were exposed to sodium bicarbonate–produced alkalinities and held under an air atmosphere or under a 2.5% CO_2 atmosphere during the 2-d exposure. After the 2-d exposure, surviving test organisms were transferred to dilution control water under an air atmosphere for 5 d. See Table 1 footnotes a, b, and c for details.
[b]The 100-mg/L treatment is untreated laboratory water and served as the control.
[c]The 2-d and 5-d postexposure median lethal concentrations (with upper and lower 95% confidence limits in parentheses) and lowest-observed-effect concentrations are based on measured alkalinity (Figure 3).
[d]Confidence limits could not be calculated because there was no partial kill for this species at this time point.
*Significantly different from respective control (100 mg/L) at $p < 0.05$.
LC50 = 50% lethal concentration; LOEC = lowest-observed-effect concentration.

for *H. azteca*. The 5-d postexposure LC50s and LOECs under air atmosphere with pH drift or under a CO_2 atmosphere were similar to the 2-d LC50s and LOECs, indicating that there was not substantial additional toxicity observed to the 3 test organisms following the initial 2-d exposure. Together, the initial 2-d exposure data and the 5-d postexposure data indicate a relative species sensitivity to alkalinity based on survival of *H. azteca* >*C. dubia* > *P. promelas*. Water quality (alkalinity, hardness, pH) was similar for all 3 species (Supplemental Data, Figure S3), indicating that differences in species sensitivity were not the result of differences in water quality among species-specific exposures.

Similar to the BWTS exposures, reproduction of *C. dubia* in the 100 mg/L control water was below the recommended rate of 15 young per female (∼4 young/female under an air atmosphere and ∼11 young/female under a 2.5% CO_2 atmosphere; Figure 4A), likely the result of not feeding the organisms during the first 2 d of the exposure. Reproductive output increased with increasing alkalinity up to 800 mg/L (∼14 offspring/female) under an air atmosphere with pH ≥9 but was significantly lower when pH was controlled under a 2.5% CO_2 atmosphere (∼4 offspring/female at pH 7.8 and an alkalinity of 800 mg/L and at pH 8.12 and an alkalinity of 1600 mg/L; Figure 4A). However, because effects of alkalinity and/or pH may be confounded by effects of lack of food, these data may not be representative of the effect of alkalinity on reproductive output by *C. dubia*. The weight of *P. promelas* was relatively unaffected by alkalinity, with all larvae reaching approximately the same weight at the end of 5 d postexposure (0.18–0.25 g dry wt/fish; Figure 4B). Atmosphere significantly affected the toxicity of alkalinity to the reproductive output of *C. dubia* (atmosphere × alkalinity, $p < 0.001$).

DISCUSSION

Laboratory water treated with the BWTS protocol met vessel discharge permit limits for water quality, organic contaminants, and toxic metals, with the exception of copper. Although exposure to BWTS water did not affect the survival of fish (*P. promelas*) or cladocerans (*C. dubia*), undiluted BWTS, even with neutral pH, was toxic to the amphipod *H. azteca*, indicating that changes in the water chemistry of BWTS water, independent of pH drift, were likely responsible for the observed residual toxicity to *H. azteca*.

Vessel permit guidelines

The BWTS water met vessel general permit limits for tPAH in discharge water [15], being 0.04% and 0.08% of permit levels for 100% BWTS-0 and 100% BWTS-6, respectively (Table 3). Total PAH concentrations in the BWTS water fell within

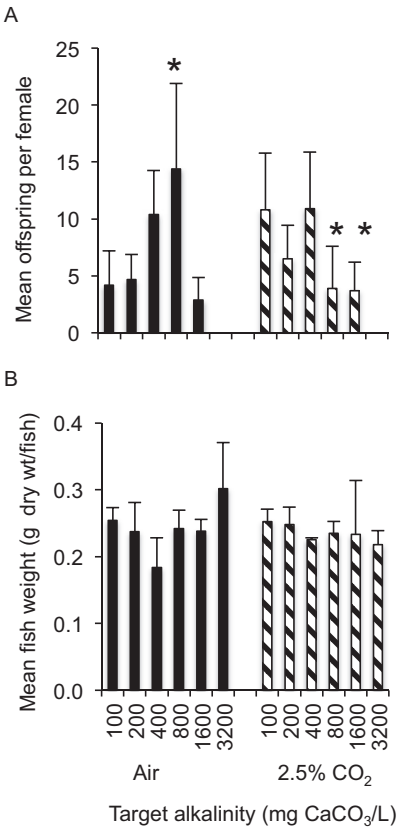

Figure 4. Effects of 2-d exposure to a range of alkalinities under an air atmosphere (black bars) and under a 2.5% CO_2 atmosphere (striped bars) on (**A**) the reproduction of *Ceriodaphnia dubia* and (**B**) the weight of *Pimephales promelas* at 5 d postexposure. Mean ± standard deviation ($n = 10$, *C. dubia*; $n = 4$, *P. promelas*). * Significantly different from respective control (100 mg/L) at $p < 0.05$.

ranges considered low for US surface waters: 0.0014 μg/L to 0.077 μg/L for York River estuary (VA, USA), 0.09 μg/L for lakes in Grand Teton National Park (WY, USA), and 18 μg/L for Occoquan Reservoir (VA, USA) [36].

As expected, PAH profiles in inlet water (lake water), in treated water (BWTS), and in scrubber water were dominated by parent PAHs, with few alkylated compounds (Supplemental Data, Table S4), reflecting the combustion source of these compounds [37]. Phenanthrene is often found in surface waters [38] and comprised 65% of the tPAH detected in the lake water (inlet water) in the present study.

The 100% BWTS-0 and 100% BWTS-6 waters met permit limits for nitrates and nitrites (Table 3) as well as for pH. The sum of nitrates + nitrites in 100% BWTS-0 (3.9 mg/L) and 100% BWTS-6 water (4.7 mg/L) was 6.6% and 7.8%, respectively, of the permit limit of 60 mg/L, based on a wash water flow rate of 45 ton/MWh [15]. The pH levels of 100% BWTS-0 (7.4) and 100% BWTS-6 (7.6) were well within the vessel general permit limit requiring that discharge pH not be less than 6.5.

Metal concentrations in BWTS water (Table 4) were similar to or below the USEPA national recommended water-quality criteria acute thresholds for the protection of freshwater organisms [39], except for copper. Copper concentrations ranged from 0.6 to 2.9 times higher than the aquatic life criteria based on hardness values for Lake Superior and Lake Michigan and on the biotic ligand model criteria for copper.

BWTS water toxicity

The residual toxicity of the BWTS water to *H. azteca* may have been influenced by nitrite or copper but was not likely directly influenced by hardness, alkalinity, or sodium. The mean alkalinity of 100% BWTS treatments under pH control ranged from 701 mg/L to 737 mg/L, alkalinities that are below 836 mg/L, an alkalinity that, with pH control, was not toxic to *H. azteca* in the sodium bicarbonate toxicity test (98% survival at 5 d postexposure; Table 2). The concentration of Na resulting from the addition of NaOH to the control water was also not likely a factor because the reagent-grade NaOH used in the present study was approximately 14 g NaOH/24 L (~0.58 g NaOH/L) in control water, which would result in a concentration of approximately 350 mg Na/L relative to a concentration of 10 mg Na/L in the control water. A concentration of approximately 350 mg Na/L is well below the 2-d LC50 for *H. azteca* of 6000 mg NaCl/L (which would be ~2400 mg Na/L in control water at 100 mg $CaCO_3$/L hardness; C. Ingersoll, unpublished data).

Although concentrations of nitrates + nitrites in undiluted BWTS water (3.9–4.7 mg nitrates+nitrites/L) were well below vessel permit discharge limits (60 mg/L) [15], they may have been high enough to deleteriously affect *H. azteca*. Amphipods can be very sensitive to nitrite (2-d LC50, 3.8 mg nitrite/L for adult *Echinogammarus echinosetosus* [40]; 96-h LC50, 12.5 mg nitrite/L for juvenile *H. azteca* [41]). Another consideration is chloride (Cl), which reduces the toxicity of

Table 3. Concentrations of polynuclear aromatic hydrocarbons (PAHs) and the sum of nitrates and nitrites in treated laboratory water relative to vessel permit discharge guidelines[a]

	Concentration in sample		Concentration in sample as percentage of permit limit	
	Total PAHs (μg/L)	NO_2+NO_3 (mg/L)	Total PAHs (%)[b]	NO_2+NO_3 (%)[c]
100% BWTS-0	0.11	4.0	0.04	6.6
100% BWTS-6	0.13	4.7		7.8
Inlet water	0.04	—[d]		
Control water	0.05	—		

[a]At a wash water flow rate of 45 ton/MWh, the permit limit for PAHs is ≤50 μg/L above inlet water PAH concentration, and the permit limit for the sum of nitrate and nitrite is 60 mg/L [15].
[b]Percent of permit limit = [(PAH concentration in sample − PAH concentration in control water − PAH concentration in inlet water)/50 μg L^{-1}] × 100.
[c]Percent of permit limit = [(NO_2 + NO_3 concentration in sample)/60 mg L^{-1}] × 100.
[d]Not measured.
PAH = polynuclear aromatic hydrocarbon; NO_2 = nitrite; NO_3 = nitrate; BWTS = laboratory water treated with NaOH, sparged with scrubbed diesel exhaust after 0 h (100% BWTS-0) and 6 h (100% BWTS-6) of scrubber use, and aerated; 100% BWTS = undiluted BWTS water.

Table 4. Metal concentrations in treated laboratory water relative to vessel permit discharge limits[a]

	Cr	Ni	Cu	Zn	As	Cd	Pb
Concentrations in samples (μg/L)[b]							
100% BWTS-0	< 1.0	4.4	15.2	22	<0.5	0.04	0.64
100% BWTS-6	< 1.0	1.7	19.1	62	<0.5	0.03	0.30
Water-quality criteria (μg/L)[c]							
CMC, Lake Michigan[d]	16	600	18	150	340	2.7	89
CMC, Lake Superior[e]	16	247	6.6	62	340	0.92	23
BLM, Lake Ontario[f]			24				
BLM, Lake Superior[g]			8				
Concentration in sample/water-quality criteria at measured hardness							
100% BWTS-0							
CMC, Lake Michigan	– [h,i]	0.01	0.8	0.15	–	0.01	0.01
CMC, Lake Superior	–	0.02	2.3	0.36	–	0.04	0.03
BLM, Lake Ontario			0.6				
BLM, Lake Superior			1.9				
100% BWTS-6							
CMC, Lake Michigan	–	0.003	1.1	0.41	–	0.01	0.003
CMC, Lake Superior	–	0.01	2.9	1.0	–	0.04	0.01
BLM, Lake Ontario			0.8				
BLM, Lake Superior			2.4				

[a]Vessel permit discharge limits for metals are from US Environmental Protection Agency (USEPA) [15].
[b]Where concentrations differed between filtered and unfiltered (total) subsamples (Supplemental Data, Table S5), the higher concentration is shown here.
[c]Water-quality criteria for copper are from the USEPA aquatic life ambient freshwater quality criteria [48]; water-quality critera for all other metals are from the USEPA national recommended water quality criteria [39].
[d]Metal criteria maximum concentration (CMC; acute) national recommended water-quality criteria normalized to 134 mg/L hardness (Lake Michigan) using hardness data from the US Environmental Protection Agency [51].
[e]Metal CMC (acute) national recommended water quality criteria normalized to 47 mg/L hardness (Lake Superior) using hardness data from the US Environmental Protection Agency [51].
[f]Biotic ligand model criteria for copper in Lake Ontario water. Criteria calculated by C. Mebane, US Geological Survey, Boise, ID, using data from the US Environmental Protection Agency [48], Alsop and Wood [52], and unpublished data.
[g]Biotic ligand model criteria for copper in Lake Superior water. Criteria calculated by C. Mebane, US Geological Survey, Boise, ID, using data from the US Environmental Protection Agency [48], Alsop and Wood [52], and unpublished data.
[h]There are separate CMC criteria for chromium III and chromium VI [39]. We measured total chromium and used the lower of the 2 CMC values (Cr VI) for these calculations.
[i]Concentration of analyte was below detection.
BWTS = laboratory water treated with NaOH, sparged with scrubbed diesel exhaust after 0 h (100% BWTS-0) and 6 h (100% BWTS-6) of scrubber use, and aerated.

nitrite to amphipods [42]. The nitrite LC50s of 3.8 mg nitrite/L and 12.5 mg nitrite/L were determined in exposure water containing concentrations of 56 mg Cl/L [40] and 72 mg Cl/L [41], respectively. The 100% BWTS water contained only 10.2 mg Cl/L (Supplemental Data, Table S6), making it possible that in this low-Cl water even nitrite levels of <3.8 mg/L may be toxic to amphipods. In our method, interconversion of nitrate and nitrite is possible prior to analysis. Hence, we cannot determine the specific concentration of nitrite in the BWTS water or if nitrite contributed to the observed toxicity to *H. azteca*.

The addition of NaOH to control water may have led to the precipitation of $CaCO_3$ out of solution, resulting in decreased Ca levels [43] and decreased water hardness [44]. Water hardness affects the toxicity of many contaminants to freshwater organisms [45,46], with several metals having higher toxicity to *H. azteca* in softer water [47]. However, the observed drop in water hardness in the present study was not likely sufficient to contribute to the observed toxicity to *H. azteca* exposed to BWTS water. Under pH control with a CO_2 atmosphere, water hardness in *H. azteca* exposure beakers remained relatively high, ranging from 68 mg/L in the 100% BWTS-0 water to 80 mg/L in the 100% BWTS-6 water compared with 96 mg/L in the 50% diluted BWTS water and 108 mg/L in control water. In contrast, in a study of the residual toxicity of BWTS water conducted in 2011, water hardness was substantially lower and may have contributed to the observed toxicity to *C. dubia* (20–50% survival, 1–2 offspring/female) [12]. In that study, *C. dubia* were exposed for 7 d to 8 d, with daily renewal, to ballast water treated with NaOH and sparged with compressed CO_2 (rather than scrubbed diesel exhaust) under an air atmosphere with pH drift >9. Hardness levels in that water were low (25–27 mg $CaCO_3$/L) compared with hardness levels of the 100% BWTS water in the *C. dubia* exposure chambers in the present study (58–63 mg/L), which did not affect *C. dubia* survival even with pH drift ≥9. Future studies should examine alterations in hardness and in the concentrations and activities of major ions, including analyses specific for nitrite, in treated ballast water.

The concentrations of copper in 100% BWTS water were similar to the USEPA national recommended water-quality acute criteria (Table 4) and may have contributed to the toxicity of BWTS water to *H. azteca*. Copper concentrations in 100% BWTS water were 15.2 μg/L and 19.1 μg/L, which are similar to acute criteria based on water hardness (18–6.6 μg/L for Lake Michigan and Lake Superior, respectively; Table 4), within criteria based on the biotic ligand model for Lake Ontario (24 μg/L) and Lake Superior (8 μg/L), and similar to biotic ligand model LC50 values for copper for *H. azteca* (8–19 μg/L) [48]. It is possible that copper toxicity may have occurred under conditions of reduced calcium; the effects of calcium on copper toxicity to *H. azteca* are complex [49]. Experiments examining the interplay of copper and calcium in

mediating toxicity to *H. azteca*, in addition to toxicity identification evaluation tests, may be useful in identifying factors contributing to the observed toxicity.

The inconsistent reproductive output of *C. dubia* in the present study may reflect a physiological compensation response to multiple stressors. Nutritional quality and food quantity significantly affect the net reproductive rate of *C. dubia* [50], and in the present study the lack of food during the first few days of life may have altered reproductive output over subsequent days. Exposure to BWTS water, elevated alkalinity, and/or elevated pH may have provoked additional physiological changes, including altering feeding rates, which could affect fecundity. Given the possible complexity of the response, it is perhaps not surprising that there was no consistent relationship between the number of offspring produced and exposure to either BWTS water, alkalinity, or pH (Supplemental Data, Figure S4). Future studies could include treatments where *C. dubia* are fed during the initial 2-d exposure period to further evaluate potential effects of limited food.

Minimal residual toxicity of diluted, pH-neutral BWTS water

The 50% dilution of BWTS water was not toxic to *H. azteca* under a CO_2 atmosphere, indicating that prevention of pH rise, coupled with dilution of ballast water during discharge, is likely sufficient to minimize residual toxicity of the BWTS to *H. azteca*. Discharge of ballast water at 6 m below the ship's waterline would likely prevent the BWTS water from coming into contact with air and thus prevent a pH rise, although this has not been determined empirically. The pH of the receiving water should be monitored during and after discharge to confirm that it does not rise in the receiving water. Dilution and dispersion rates of ballast water vary, depending on density differences between ballast water and harbor water, wind-driven currents, thermal gradients, the presence of other boats in the harbor, and port structural features (semi-enclosed, open). In the Great Lakes, ports generally are sheltered with limited current speeds. A study of ballast water release by Wells et al. [20] into Goderich Harbor, an extremely small port (200 m × 400 m) on Lake Huron, under calm conditions with little to no boat traffic over a study period of 3 d, might represent a worst-case situation. Wells et al. reported that peak concentrations of dyed ballast water inside the harbor (measured along transects) were diluted to 1% to 5% of the initial concentrations in the ballast tank immediately after discharge, reaching a dilution factor of 10^3 after 1 d [20]. This dilution rate is likely sufficient to eliminate residual toxicity, even without pH control. A control water treated with NaOH to achieve pH 11.5 to 12 produced no residual toxicity in 2-d to 4-d exposures to *H. azteca*, *P. promelas*, or *C. dubia* if the treated water was diluted by 100-fold or 1000-fold [9].

Alkalinity toxicity and pH

The toxicity of alkalinity in the sodium bicarbonate test was influenced by pH. Acute alkalinity LC50s for *C. dubia*, *H. azteca*, and *P. promelas* are typically evaluated under conditions where pH is not controlled at hardness ranging from low (36 mg $CaCO_3$/L) to moderate (80–180 mg $CaCO_3$/L). The acute LC50 for alkalinity (tested as $NaHCO_3$) ranged from 500 mg/L to 1075 mg/L for *C. dubia* (2-d LC50) [23,24] and was 662 mg/L (4-d LC50) for *H. azteca* [22]. Although not strictly comparable to tests conducted in laboratory water, the alkalinity LC50 for *P. promelas* in reconstituted river water was 1139 mg /L (4-d LC50) [18]. These are similar to the 2-d LC50s for alkalinity in the present study under pH drift (pH ~9.2, hardness of 65–110 mg/L): 1809 mg/L (*C. dubia*), 791 mg/L (*H. azteca*), and 3393 mg/L (*P. promelas*) (Table 2). However, when pH was controlled in the present study (pH range ~8–8.3), the 2-d LC50s increased to 2033 mg/L for *C. dubia*, 1689 mg/L for *H. azteca*, and >3092 mg/L for *P. promelas* (the highest exposure concentration). Given the potential effect of pH on the toxicity of alkalinity, it is important that pH be reported with alkalinity LC50s. These data demonstrate the importance of controlling pH to control the toxicity of water with elevated alkalinities.

Efficacy of NaOH-BWTS to reduce organism density

Laboratory and shipboard studies conducted by the Great Ships Initiative demonstrate the efficacy of the NaOH-BWTS to reduce the density of organisms in ballast tank water. Laboratory tests indicated that a 4-h exposure to pH 11.5 resulted in 0% survival of zooplankton (adult rotifer *B. calyciflorus*, cladoceran *D. magna* and its resting eggs [ephippia], copepod *Eucyclops* spp.) and that a 2-d exposure to pH 12.5 reduced survival of the green alga *Selenastrum* sp. and rotifer cysts to 0 [9]. In the Great Ships Initiative shipboard trials [12], NaOH solutions were added to ballast tanks to achieve a pH of approximately 12; pH remained elevated until in-tank carbonation systems were activated, approximately 18 h prior to discharging the tanks dockside 3 d later. This 3-d exposure significantly reduced the density of organisms in the ≥50 μm size range (mainly dreissenid bivalve veligers [zebra and/or quagga mussels]; loricate rotifers in the genus *Keratella*; illoricate rotifers, including *Polyarthra*, *Synchaeta*, and *Conochilus*; and copepod nauplii), but densities were still above the <10 organisms/m^3 ballast water performance standard requirement of the International Maritime Organization convention [12]. However, densities of organisms of ≥10 μm and <50 μm (mainly blue-green algae and diatoms) were below the standard (<10/mL). The authors of that study concluded that the NaOH-BWTS significantly reduces live densities of zooplankton and phytoplankton relative to control discharge densities [12]. It is important to note that the data reported by the Great Ships Initiative in its shipboard study are from a single trial in which the authors note that some samples may have been compromised by technical issues [12]. Thus, although promising, the study conducted by the Great Ships Initiative is not definitive, and further testing is necessary, particularly with respect to the ability of the BWTS to reduce the density of ≥50-μm organisms, a size range which includes the veligers of invasive mussels of concern.

Ideally, BWTSs would produce treated ballast water that has no residual toxicity to organisms in the receiving water. The toxicity tests performed in the present study indicate that undiluted BWTS water is likely to have minimal residual toxicity to organisms in receiving waters. Ballast water is released at high rates (e.g., ~40 m^3/min for each pump on the *M/V Indiana Harbor* [8]) during the cargo loading process to maintain proper trim of the ship. It is typically released >6 m below the waterline of a cargo ship and even in restricted ports can be rapidly and significantly diluted [20]. Under these conditions, pH in the receiving water would not likely rise, ballast water would likely be rapidly diluted, and toxicity would not be likely to occur based on the findings of the present study. The present experiments were conducted using laboratory water treated with the proposed BWTS. Additional studies are needed on ballast water treated with this BWTS to ensure full compliance with vessel discharge permit limits for residual toxicity, turbidity, PAHs, metals, and nitrates, and

nitrites of discharged water. Moreover, monitoring of receiving water during and after deballasting should be done to confirm that the release of treated ballast water does not alter the quality (e.g., pH, hardness) of the receiving water. This approach has the potential to become a viable method for treating large volumes of ballast water released into freshwater systems.

SUPPLEMENTAL DATA

Tables S1–S6.
Figures S1–S4. (1.6 MB PDF).

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Data Availability—Data, associated metadata, and calculation tools are available on request from the first author (aelskus@usgs.gov).

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