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Research Article

# Hydroxide stabilization as a new tool for ballast disinfection: efficacy of treatment on zooplankton

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## Abstract

Effective and economical tools are needed for treating ship ballast to meet new regulatory requirements designed to reduce the introduction of invasive aquatic species from ship traffic. We tested the efficacy of hydroxide stabilization as a ballast disinfection tool in replicated, sequential field trials on board the M/V Ranger III in waters of Lake Superior. Ballast water was introduced into each of four identical 1,320 L stainless steel tanks during a simulated ballasting operation. Two tanks were treated with NaOH to elevate the pH to 11.7 and the remaining two tanks were held as controls without pH alteration. After retention on board for 14–18 h, $CO_2$-rich gas recovered from one of two diesel propulsion engines was sparged into tanks treated with NaOH for 2 h to force conversion of NaOH ultimately to sodium bicarbonate, thereby lowering pH to about 7.1. Prior to gas sparging, the engine exhaust was treated by a unique catalytic converter/wet scrubber process train to remove unwanted combustion byproducts and to provide cooling. The contents of each tank were then drained and filtered through 35-µm mesh plankton nets to collect all zooplankton. The composition and relative survival of zooplankton in each tank were evaluated by microscopy. Zooplankton populations were dominated by rotifers, but copepods and cladocerans were also observed. Hydroxide stabilization was 100% effective in killing all zooplankton present at the start of the tests. Our results suggest hydroxide stabilization has potential to be an effective and practical tool to disinfect ship ballast. Further, using $CO_2$ released from the ship engine reduces emissions and the neutralized by product, sodium bicarbonate, can have beneficial impacts on the aquatic environment.

**Key words:** Great Lakes, NaOH, ballast water management, freshwater

## Introduction

The unintended introduction of non-indigenous aquatic species has been identified as a major threat to freshwater and marine ecosystems, with undesirable biodiversity, economic and social consequences (Pimentel et al. 2005; Colautti et al. 2006; Horan and Lupi 2010; Hyytiäinen et al. 2013). An important vector responsible for introduction is the process of ballast water uptake, transport and release related to ship traffic (Molnar et al. 2008; Gallardo and Aldridge 2013). The quantity of ballast water moved in ships worldwide, often from one continent to another, is greater than 10 million tons annually (Nanayakkara et al. 2011).

Control of ballast discharge from ships has been addressed with regulations at the international level by the International Maritime Organization (IMO) and its subsidiary body, the Marine Environment Protection Committee (MEPC) (Goncalves and Gagnon 2012; Albert et al. 2013). In addition, various authorities within individual countries, states and provinces have developed ballast water management plans with the goal of preventing the spread of non-native organisms.

Methods for disinfection of ballast water have received considerable attention by regulators and treatment developers and the options include de-oxygenation, ultraviolet irradiation, use of sodium chloride brine and an array of chemical biocides (Gregg and Hallegraeff 2007; Gregg et al. 2009;

Tsolaki and Diamadopoulos 2010; Bradie et al. 2010; Maranda et al. 2013; de Lafontaine and Despatie 2014). These methods have had varying degrees of success at killing the many taxonomic groups including bacteria, phytoplankton, invertebrates and vertebrates found in ballast water.

Ballast water discharge from commercial cargo ships into the Laurentian Great Lakes has been linked to the recent introduction of the zebra mussel *Dreissena polymorpha* (Pallas, 1771), spiny water flea *Bythotrephes longimanus* (Leydig, 1869), bloody red shrimp *Hemimysis anomala* (G.O. Sars, 1907), and round goby *Neogobius melanostomus* (Pallas, 1814) (as reviewed by Briski et al. 2012). Many of these species have spread further into other North American lakes and river systems through fishing and other recreational activities. Disinfecting ballast linked to the freshwater fleet operating in the Great Lakes is problematic given unusually high ballasting/deballasting rates (up to 230 $m^3$/min), relatively large ballast volumes (up to 64,000 $m^3$), limited shipboard space to install treatment systems, and the lack of coatings inside the ballast tanks that are commonly used in marine vessels to retard corrosion. The absence of the corrosion coatings can prevent the application of brine or common oxidizing biocides.

There is a pressing need for new treatment technologies that are both cost-efficient and environmentally safe (Gregg and Hallegraeff 2007; Delacroix et al. 2013; Maranda et al. 2013). A treatment system that meets the unique operational demands of ships traveling in the Great Lakes was developed by the U.S. Geological Survey and has been tested at the bench scale, at a land-based testing facility (Great Ships Initiative, Duluth, MN) and onboard the American Steamship Company's M/V Indiana Harbor (TenEyck et al. 2009; 2013; Cangelosi et al. 2011, 2013; Starliper and Watten 2013; Moffitt et al. 2015; Starliper et al. 2015). With these methods, stabilization of biologically active water is achieved through elevation of pH to targets of 11–12 by applying lye (NaOH) or hydrated lime [$Ca(OH)_2$] based on success in early trials with treatment of municipal wastewater and sludge (Grabow et al. 1969; Sattar et al. 1976; Grabow et al. 1978). The process is attractive in shipboard applications given the ability of elevated pH to retard steel corrosion and the stability of the pH observed during transit. The pH is readily returned to neutral levels, just prior to release, with carbonation. Carbonation results in desirable alkalinity products - sodium bicarbonate in the case of NaOH addition or calcium bicarbonate in the case of hydrated lime addition.

In the study presented herein we evaluate treatment of ship ballast water with NaOH followed by introduction of carbon dioxide ($CO_2$) recovered from cleaned ship engine exhaust. Most commercially available $CO_2$ is recovered from industrial waste streams, but in our case, engine exhaust from the ship's propulsion system provided the gas required. This step eliminated the need for gas liquefaction, storage under pressure and forced vaporization of the liquid prior to use as is required for application of commercial $CO_2$ in this process. Previous shipboard trials of elevated pH occurred aboard a Great Lakes freight vessel with a transit time of over 48 h (Cangelosi et al. 2013) with treatment holding times of over 24 h. However, many freshwater ships such as barges and ferries have significantly shorter hold times, thus this testing aims to confirm practicality for such vessels.

The objectives of our study were to test the efficacy of the refined NaOH treatment process on micro and macro-zooplankton in a smaller-scale vessel with holding times of less than a day. Additionally, our study was focused to confirm the applicability of the neutralization system in shallow tanks, such as those found on small vessels or in double bottom areas of tanks on larger vessels.

## Methods

### *Location and test design*

Two trials were conducted sequentially from 20 to 22 September 2013, on board the National Park Service M/V Ranger III (Table 1). This ship carries passengers and cargo between Houghton, MI and the Isle Royale National Park located on Lake Superior. The M/V Ranger III measures 50 m in length, 4.6 m in height and 10 m in width providing a deadweight cargo capacity of about 758 metric tons. Twelve ballast tanks onboard provide a total ballast capacity of 140 $m^3$. The ballast transfer system utilizes a single stage self-priming centrifugal pump capable of delivering 0.68 $m^3$/h at 9.1 m total dynamic head.

We installed and secured four stainless steel rectangular mock ballast tanks measuring 107 cm in width, 122 cm in length and 109 cm in height (Model 115678-C; American Machining Inc. Somonauk, IL) at a point just forward of the ship wheelhouse. The top of each tank was sealed, except for a raised 10-cm diameter vent, which allowed for in situ water chemistry monitoring

**Table 1.** Summary of steps in sequential trials conducted on the M/V Ranger III in September 2013. Times for the start and duration of fill neutralization, and final plankton sampling are provided. Plankton filling was at ship home port in Portage Lake, Houghton, MI (latitude 47.123199 and longitude -88.563936).

| Trial and dates | Filling | | NaOH addition | Neutralization | | Plankton sampling |
|---|---|---|---|---|---|---|
| | Start | Duration | | Start | Duration | |
| 1 (20 - 21 Sept 2013) | 13:01 | 0:50 | 15:30 | 10:20 | 2:15 | 14:31 |
| 2 (21 - 22 Sept 2013) | 18:50 | 1:20 | 20:00 | 9:53 | 2:07 | 13:30 |

with submersible probes and off-gassing release during required pH depression steps. NaOH tests were conducted in the port aft and starboard forward tanks. The remaining two tanks were used for untreated control tanks (Figure 1). All tanks were filled with the ship ballast pump through a manifold system that provided distribution of infill simultaneously to the four tanks via 1.9-cm diameter rubber hose. In addition, the manifold had a port with hose for concurrent zooplankton sampling of infill and a pressure relief valve. Prior to fill, the tanks were disinfected with chlorine then rinsed. During the test, tanks were filled to 93% of their capacity (1,320 L) to leave room for NaOH dosing and expansion of the ballast water during the addition of the $CO_2$.

NaOH treatment of test tanks was achieved by introducing 700 mL of a 50% solution of NaOH (caustic soda, UN1824, Hawkins, Inc, Minneapolis, Minnesota) with manual stirring. This addition provided a NaOH dose of 0.0108 N that reached a target pH of about 11.7. After retention for 14–18 h, $CO_2$-rich gas recovered from one of two diesel propulsion engines was sparged into both tanks treated with NaOH for a period of 2 h to force conversion of NaOH ultimately to sodium bicarbonate, thereby lowering pH to a target of about 7.1. The engine exhaust was treated, prior to use, by a unique catalytic converter/wet scrubber method. The engine exhaust gas scrubber was designed primarily to convert carbon monoxide to carbon dioxide, to reduce particulate carbon based material and to reduce gas temperature for compression and flow measurement. Major components of the system (Figure 2) included (1) a DCL International Mine-X Catalytic converter Model DCP-DP (Concord, ON, Canada) attached directly to the test engines turbine exhaust; (2) a 2.54-cm stainless steel Type 484 water jet exhauster (Schutte and Koerting, Trevose, PA) coupled to a stainless steel pressurized gas – water separator measuring 30 cm in diameter and 45 cm in height, (3) a stainless steel pressure tank measuring 18 cm in diameter and 23 cm in height was packed with plastic 3.81-cm diameter packing rings (Flexiring™, Koch-Glitsch, Wichita, KS), (4) two variable area gas flow meters (Dwyer Instruments, Michigan City, IN), (5) a Dayton type multistage booster pump, (6) a Signet type paddlewheel water flow meter, and (7) a Maxichanger type counter current flow (plate type) heat exchanger. In operation, hot engine exhaust was pulled through the catalytic converter for reduction of carbon monoxide/ particulates by action of the water jet exhauster. The exhauster mixed a re-circulated scrubbing solution (water) with the gas, and then delivered the gas-water mixture below the surface of the water maintained within the gas/water separator shown as the reaction tank (Figure 2). The cleaned and cooled gas that accumulated in the headspace of the tank was then directed through the packed bed pressure tank for mist and condensate removal. Gas was then forced through the variable area flow meters for delivery to both stainless steel test ballast tanks. Gas was sparged via a fine bubble 34 cm diameter by 4-cm high diffuser (Flexair™ Model D350) positioned at the floor of the tanks. Water required for operation of the jet exhauster was re-circulated in a closed loop (liquid volume 28 L) that included the booster pump, water flow meter and the heat exchanger. Cooling flow to the heat exchanger was provided by an isolated stream of ship service water. Ship service water was also used to charge the scrubbing system just prior to the start of the scrubbing tests.

Conditions maintained throughout operation of the scrubbing system were: water jet exhauster water flow rate, 37 L/min; scrubber liquid temperature, 21.1–26.7°C; gas flow rate to each test tank sparger, 63.7 L/min. During tests, gas was pulled from the starboard engine of the M/V Ranger III. The selected engine was one of two Caterpillar 3508B diesel, 4 cycle 8 cylinder engines that provide propulsion for the ship via two 1.93 m-diameter variable pitch propellers. Each engine was capable of developing 850 hp (2,745 kW) at about 1,200 rpm. Both ship engines operated on an ultra low sulfur fuel (<15ppm) with a specific fuel consumption per engine of about 280 L/h.

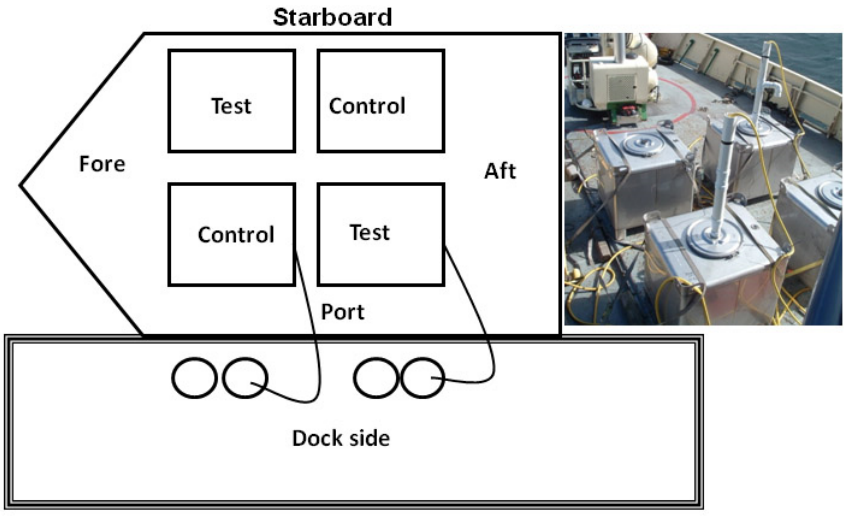


**Figure 1.** Schematic of the arrangement of the four mock ballast tanks, and the directional scheme for gravity filtration from tanks on board the M/V Ranger III. Photo insert shows placement of tanks on deck.

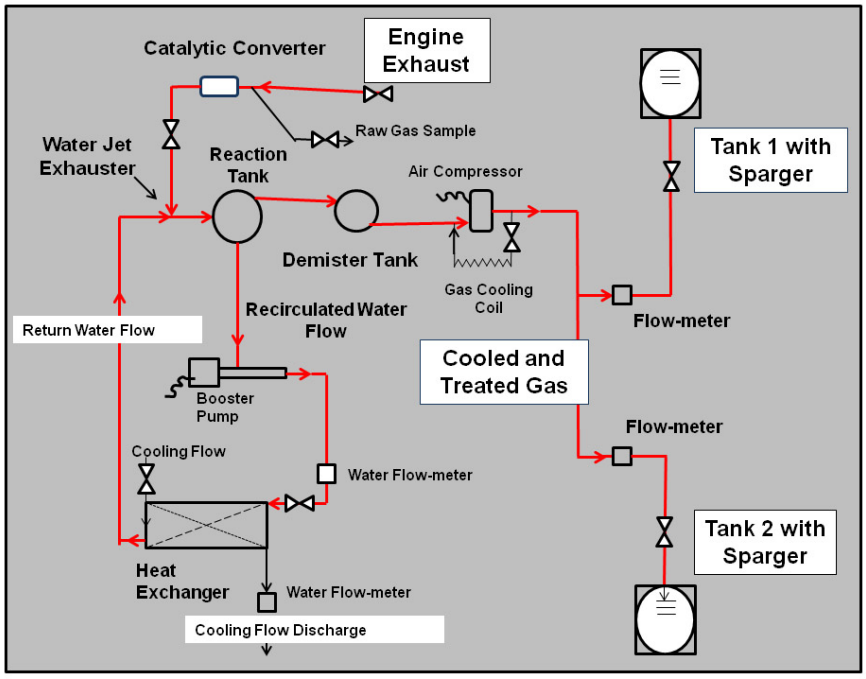


**Figure 2.** Schematic of the engine exhaust treatment and delivery system used to provide cleaned and cooled gas to spargers in the two tanks to neutralize the elevated hydroxide.

Engine shaft speed during tests was fixed to minimize changes in exhaust gas composition/temperature, and was 1100 rpm. The resultant propeller shaft speed, and propeller pitch were maintained similarly for each trial. During the carbonation process, we monitored the composition of gas entering the catalytic converter (oxygen, carbon dioxide, carbon monoxide, nitrogen oxide and sulfur dioxide) using a portable combustion analyzer (PCA 3, Bacharach, New Kensington, PA). The ship was motored out from Houghton, MI into Portage Lake, through Keweenaw Waterway into Keweenaw Bay of Lake Superior (Latitude 46°57'30"; Longitude 088°20'56"). The distance traveled during each trial was approximately 130 km. Just prior to tank dosing with NaOH and again following the carbonation step, 500-mL grab samples were removed and analyzed for pH

(Ecosense 100, YSI, Yellow Springs, OH), dissolved oxygen/temperature (HQ40D, Hach, Loveland, CO), conductivity (Model 30, YSI, Yellow Springs, OH) and turbidity (Model 2100P, Hach, Loveland, CO). The pH decline of NaOH treated tank water during carbonation was monitored, in situ, with WPH-310-NN pH controllers coupled with WEL-PHF-NN 38/10 electrodes (Walchem, Holliston, MA).

*Sampling of plankton*

At the beginning of each trial, a sample of inflow ballast water equal to the volume of a tank (1,320 L) was filtered to characterize the plankton community. At the end of each trial, after the treatment and neutralization process were completed, the water from each tank was filtered (Table 1). Sampling of inflow at tank filling was accomplished using a port from the manifold used to deliver ballast water to the tanks. Sampling at the completion of trials was accomplished using a port at the bottom of each tank. In both procedures, all water was diverted through a rubber hose and then filtered through a 35-µm mesh plankton net that was 30-cm in diameter, 90-cm long and terminated with a 1-L cod jar (Sea-Gear, Melbourne, FL). Filtration was conducted dockside using gravity flow. To prevent cross contamination, individual plankton nets were assigned to sampling of fill, control or test tanks. Each net was supported by a steel frame and the hose gently placed inside each net. During filtration, nets were contained inside 132-L barrels that served to collect filtrate and support the net. We recorded the total volume filtered using a calibration mark on each barrel, rotating and counting the emptied barrels to record the total volume filtered.

In Trial 1, samples of zooplankton at the filling were obtained with a constrictor nozzle attached to the hose. However, to limit any potential mortality from this pressure, the nozzle was removed for all subsequent sampling. We retained plankton from the 1,320 L filtered from the manifold at filling (pre-fill sample) in two cod jars (750 mL and 350 mL) to reduce loading density of plankton, but after observing plankton, all subsequent samples were filtered and concentrated into one cod jar (1-L). The contents of each cod jar were then stored in individually labeled acid washed 1-L containers on ice until analysis.

Filtration of water from the tanks in Trial 1 was conducted sequentially with control tanks sampled first, and treated tanks second. For Trial 2, two tanks (one control and one test) were drained simultaneously (though sampled separately) to reduce the sampling time. Between trials, all equipment (nets, cod ends, funnels, 132 L barrels) was disinfected with chlorinated municipal water. To obtain water for diluting the samples during counting we prepared approximately 20 L of ultra-filtered water from the same source of water that was used for the tests. Ultra-filtration was achieved by filtering the already filtered water (35-µm mesh) through a 10-µm mesh.

*Enumeration of plankton*

Plankton samples were transported in coolers to the Lake Superior Research Center, Michigan Technological University. In the laboratory, the contents of each container of filtrate were split into equal aliquots with a Folsom plankton splitter (Wildco®, Yulee, FL). Split samples were returned to the chilled cooler in clean acid washed plastic beakers with lids until processing. Analysis of zooplankton collected at the filling of the tanks in Trial 1 was accomplished using 4 subsamples, split from one container (750 L) only. To characterize zooplankton at filling of tanks in Trial 2, all plankton from the 1,320 L volume filtered were combined and enumerated using 2 subsamples. To assess each test and control tank in both trials, zooplankton were assessed from 4 subsamples, using a Ward counting wheel for a total of 4 mL per tank evaluated.

To process each subsample we inverted the jar gently several times, removed the lid and mixed the sample with a clean disposable pipette in a figure-8 motion. We used a Henson-Stempel pipette to remove a 1 mL sample that was placed carefully into a Ward counting wheel. The sample was diluted in the wheel as necessary using the ultra-filtered lake water. With a dissecting microscope we carefully evaluated the composition. Dead zooplankton were counted and separated into rotifers, cladocerans, or copepods. An organism was recorded as dead if no motion was observed, no cilia were in motion, or no heart beat was evident. We then added 2–5 drops of 50% acetic acid to each counting wheel to kill all live zooplankton. Then all zooplankton were counted again. The number of live zooplankton was determined by subtracting the number of dead plankton counted after adding the acetic acid from the number of dead plankton before it was added. For samples taken after treatment with NaOH, no live zooplankton were observed, and the killing step was omitted. To extrapolate the count data to tank loading and estimate the total density of live and dead zooplankton, the counts

from each sample analyzed were averaged to provide an average count per mL. These counts were extrapolated to equivalent counts per L of filtrate. To estimate the density of organisms introduced into the test systems, the extrapolated count per L was divided by the volume filtered in each tank (1,320 L) and then multiplied by 1,000 to convert to density of organisms per $m^3$.

After field analysis was completed, each of the subsamples was combined, and the volume was concentrated to ~ 40 mL by filtering the samples through the 10µm mesh. The concentrated samples were preserved with 10% formalin and transported back to the University of Idaho for a more complete analysis of the composition of plankton. To perform this analysis, we diluted each sample to a constant volume of 150 mL and removed triplicate 1 mL samples with a Henson-Stempel pipette, evaluated the contents in a gridded Sedgwick-Rafter slide, counting 100 squares, or 10% of the total area with a compound microscope (Leitz Laborlux, Leica Microsystems, Buffalo Grove, IL). We summarized the numbers and identity of zooplankton > 50 µm and the proportion in each taxon. In addition, we measured the first 20 individuals of the most common species for several metrics from photographs taken by a Leica EC3 camera and image analysis software (LAS EZ 1.8.0; Leica Microsystems, Buffalo Grove, IL). Measurements included body length, and body plus spine for zooplankton with spines except for *Bosmina* which had very small spines.

### *Data analysis*

We evaluated the proportion of individuals by major taxon (Cladocera, Copepoda, Rotifera) in preserved samples with generalized linear models to test differences between trials, and replicate tanks within each trial. The mean size and range of selected zooplankton genera in preserved samples was reported. The proportion of live and dead zooplankton in treatment versus control samples was evaluated with chi-square tests of independence. We modeled the drop in pH with carbonation over time in test tanks by fitting a non-linear multi-step regression to explore the model of best fit. Oxygen, conductivity, and turbidity measurements in test and control tanks were analyzed with paired t-tests to determine significant differences before and at the completion of each trial by treatment. All statistical analyses were conducted with SAS version 9.2 (Cary, North Carolina) or TableCurve 2D, Version 5.01(San Jose, California).

## Results

### *Plankton survival and taxa*

The hydroxide stabilization was 100% effective in killing all zooplankton in the test systems (continuity adjusted chi-square = 34,948; $P < 0.001$). Plankton in the test systems at the end of both trials were highly skeletonized by the treatment process (Figure 3 C, D). Fragments of individuals made counting difficult and identification nearly impossible. Mortality occurred during the ballast pumping process during tank filling - the means for Trial 1 and Trial 2 were 65.8% and 54.7%, respectively (Table 2). The mortality in the control tanks at the end of each trial was equivalent or sometimes higher, likely due to the long holding period and absence of light.

There were no significant differences attributed to trial or replicate tank within each trial in the proportion of the three major taxa in samples. Within the major taxa, Rotifera were significantly more abundant than Cladocera, or Copepoda ($F_{2,31}$ = 7,687.4; $P < 0.001$; Table 3).Samples contained representatives of more than 11 genera and 5 families of Rotifera, and one species (*Keratella earlinae),* accounted for 45.1 to 63.7 % of all plankton in samples (Table 4). The body length of rotifers measured in samples ranged from 81 µm to 541 µm, exclusive of spines. Nauplii Copepoda were the smallest arthropods found in the zooplankton (Table 5).

### *Environmental monitoring*

The pH of tank water at filling ranged between 7.8 and 8.4, and was elevated to 11.7 – 11.8 after adding NaOH, and subsequently dropped to 7.2 – 7.6 after carbonation (Figure 4). The neutralization of pH in tanks proceeded rapidly during trials, and the time to neutralization to pH of 7.6 took less than 140 minutes in both trials. The pH depletion from both trials best fit a polonomial non-linear model ($F_{8,31}$ = 1,077.21; adjusted $r^2$ = 0.99; $P< 0.001$). The form of the model prediction equation is provided with Figure 4. The pH in control tanks remained consistent and at the end of trials was 7.7 – 7.8.

The dissolved oxygen ($O_2$) of control tanks before and after tests did not differ significantly ($t_3$= 1.05; $P$ = 0.37); but dissolved oxygen was significantly lower in treated tanks at the end of tests ($t_3$ = 8.40; $P$ = 0.004; Table 6). Conductivity of the ballast water at filling did not vary between trials and tanks over the test periods in the control

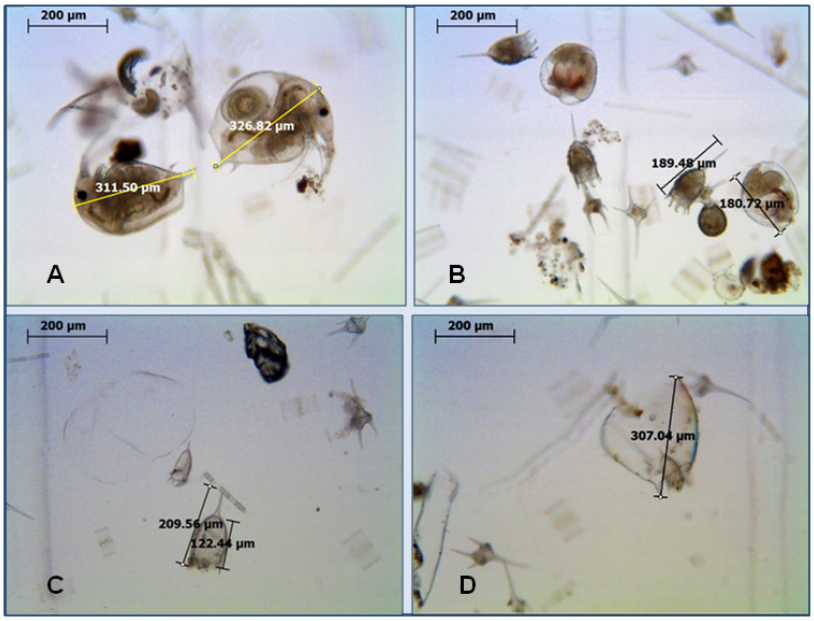


**Figure 3.** Composite of photomicrographs of representative zooplankton species showing method of measurement of size for samples enumerated in control (A, B), and treatment tanks (C, D). Photomicrographs by A. Barenberg.

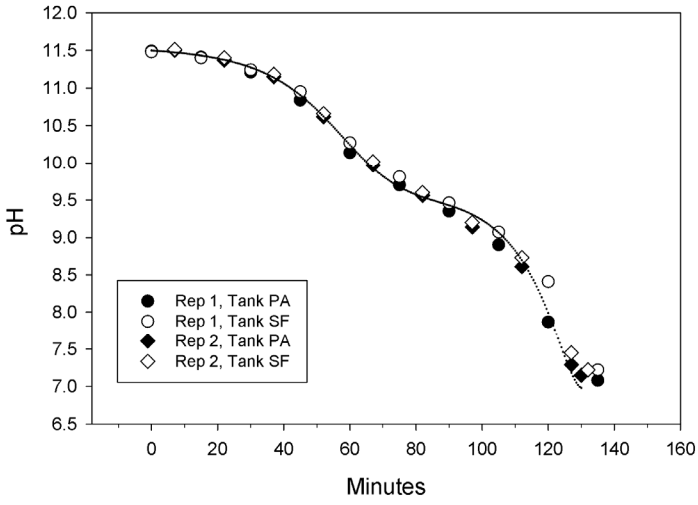


**Figure 4.** Measures of depletion of pH versus time (minutes) during carbonation of replicated treatment tanks, Trial 1 and 2. The depletion best fit a quadratic non-linear regression solution with the following equation: $pH = (a + cx + ex^2 + gx^3 + ix^4)/ (1 + bx + dx^2 + fx^3 + hx^4)$. Predicted line is provided (dotted line) along with the observed data from trials. The pH in control tanks at the end of both trials ranged from 7.65 to 7.76.

**Table 2.** Summary of zooplankton concentration assessed at fill, and in test and treatment tanks at the end of trials on the M/V Ranger III, September 2013. The concentrations (per $m^3$) were extrapolated from sub samples counted with Ward counting wheels to estimate the concentration per cubic meter of total tank volume. The percent mortality is provided. The location of each tank on the deck of the ship is abbreviated: P = port S = starboard, F= forward and A = aft. Locations are also provided in Figure 1.

| Trial | System and location | Total concentration | Live concentration | % Mortality |
|---|---|---|---|---|
| 1 | Fill | 90,277 | 30,902 | 65.8 |
| 1 | Control 1 (PF) | 90,243 | 14,430 | 84.0 |
| 1 | Control 2 (SA) | 67,479 | 16,056 | 76.2 |
| 1 | Test 1 (PA) | 29,471 | 0 | 100.0 |
| 1 | Test 2 (SF) | 31,910 | 0 | 100.0 |
| 2 | Fill | 125,378 | 56,818 | 54.7 |
| 2 | Control 1 (PF) | 92,276 | 23,373 | 74.9 |
| 2 | Control 2 (SA) | 60,162 | 26,626 | 55.7 |
| 2 | Test 1 (PA) | 14,227 | 0 | 100.0 |
| 2 | Test 2 (SF) | 11,788 | 0 | 100.0 |

**Table 3.** Summary of proportionate composition of zooplankton observed in analysis of samples from trials conducted on the M/V Ranger III, September 2013 in samples of live and dead.

| Trial | System | Replicate | Rotifera (%) | Cladocera (%) | Copepoda (%) |
|---|---|---|---|---|---|
| 1 | Pre-fill | 1 | 82.7 | 8.9 | 8.5 |
| 1 | Control 1 | 1 | 88.8 | 5.4 | 5.8 |
| 1 | Control 2 | 2 | 84.5 | 8.2 | 7.4 |
| 1 | Test 1 | 1 | 87.5 | 5.8 | 6.7 |
| 1 | Test 2 | 2 | 87.8 | 6.6 | 5.6 |
| 2 | Pre-fill | 1 | 76.6 | 7.4 | 16.0 |
| 2 | Control 1 | 1 | 83.1 | 8.1 | 8.8 |
| 2 | Control 2 | 2 | 80.6 | 5.1 | 14.3 |
| 2 | Test 1 | 1 | 86.0 | 7.0 | 7.0 |
| 2 | Test 2 | 2 | 92.2 | 1.9 | 5.8 |

**Table 4.** Summary of zooplankton composition by family within phylum Rotifera and subphylum Crustacea recorded in analysis of preserved specimens from fill and control tanks. Counts were made of 100 gridded cells (10% of each sample) using a compound microscope (10X objective). Each sample was analyzed in triplicate with the sum used for proportionate composition.

| Family | Genus and species | Trial 1 | | | Trial 2 | | |
|---|---|---|---|---|---|---|---|
| | | Fill | Control 1 | Control 2 | Fill | Control 1 | Control 2 |
| Asplanchnidae | *Asplanchna priodonta* (Gosse, 1850) | 1.2 | 0 | 0.8 | 3.5 | 0.7 | 0.9 |
| Brachionidae | *Brachionus* sp. | | | | | 0.2 | |
| Brachionidae | *Kellicottia* sp. | | | | | 0.3 | |
| Brachionidae | *Keratella earlinae (*Alhstrom, 1943) | 54.7 | 63.7 | 56.9 | 45.1 | 60.5 | 53.8 |
| Brachionidae | *Notholca* sp. | | | | | 0.3 | |
| Gastropidae | *Ascomorpha ecaudis (*Perty, 1850*)* | | | | 1.1 | 1.7 | |
| Gastropidae | *Gastropus* sp. | 12.3 | 0 | 11.7 | 15.3 | 7.5 | 16.4 |
| Synchaetidae | *Bipalpus hudsoni (*Imhof, 1891*)* | | | | | 0.5 | |
| Synchaetidae | *Ploesoma truncatum* (Levander, 1894) | | | | | 0.3 | |
| Synchaetidae | *Synchaeta* sp. | 4.6 | | 3.5 | 6.4 | 1.4 | 9.5 |
| Trichocercidae | *Trichocerca cylindrical* (Imhof, 1891) | 0.3 | 0.4 | 4.9 | 3.5 | 1.9 | 0.9 |
| | *Undetermined* | 15.6 | 28.5 | 10.9 | 9.2 | 16.1 | 9.7 |
| | **Total Rotifer** | 88.9 | 92.5 | 88.8 | 84.2 | 91.6 | 91.2 |
| Daphniidae | *Daphnia* sp. | 0.9 | 0 | 0.5 | 1.4 | 0 | 0.3 |
| Bosminidae | *Bosmina longirostris* (O.F. Müller, 1776) | 5.7 | 6.4 | 7.1 | 8.5 | 4.1 | 3.2 |
| | **Total Cladocerans** | 6.5 | 6.4 | 7.6 | 9.9 | 4.1 | 3.6 |
| Cyclopidae | Cyclopoid | 2.1 | 0.7 | 2.2 | 1.8 | 0.9 | 1.7 |
| Cyclopidae | Nauplii | 2.6 | 0.4 | 1.4 | 4.1 | 3.4 | 3.5 |
| | **Total Copepods** | 4.6 | 1.1 | 3.5 | 5.8 | 4.3 | 5.2 |

**Table 5.** Summary of mean, and range of body length of selected zooplankton in representative groups in samples filtered from fill and control tanks during both trials. Number of plankton measured is provided (N). Two measures were made for zooplankton with large spines.

| | Body length (µm) | | | Body and spine (µm) | | |
|---|---|---|---|---|---|---|
| Genus and species | Mean | Range | N | Mean | Range | N |
| | | Rotifera | | | | |
| *Asplanchna priodonta* | 415.5 | 173.8 – 541.3 | 20 | | | |
| *Keratella earlinae* | 110.6 | 81.3 – 144.7 | 120 | 166.5 | 99.3 – 211.6 | 120 |
| *Gastropus* sp. | 135.4 | 89.9 – 185.6 | 26 | | | |
| *Trichoccerca cylindrica* | 325.5 | 93.7 – 388.9 | 27 | 568.4 | 113.9 – 674.4 | 23 |
| | | Cladocera | | | | |
| *Bosmina longirostris* | 289.2 | 223.7 – 379.7 | 60 | 296.9 | 227.2 – 426.7 | 45 |
| | | Copepoda | | | | |
| Cyclopoidea | 504.4 | 335.7 – 789.5 | 43 | 657.4 | 428.0 – 1009.8 | 33 |
| Nauplii | 198.4 | 101.5 – 375.8 | 71 | | | |

**Table 6.** Summary of dissolved oxygen, conductivity, and turbidity measured in test and control tanks at the beginning and end of sequential trials of elevated pH on the M/V Ranger III.

| | Trial 1 | | | | Trial 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | Test 1 | Test 2 | Control 1 | Control 1 | Test 1 | Test 2 | Control 1 | Control 1 |
| | | | | Dissolved $O_2$ mg/L, % saturation | | | | |
| Start | 8.68 | 8.64 | 8.64 | 8.69 | 8.63 | 8.58 | 8.64 | 8.71 |
| | 96.6% | 95.9% | 96.0% | 96.4% | 95.3% | 93.5% | 94.4% | 95.0% |
| End | 6.38 | 6.51 | 8.66 | 8.54 | 7.22 | 7.05 | 8.38 | 8.78 |
| | 65.0% | 69.0% | 88.7 | 90.7% | 76.3% | 74.0% | 88.8% | 92.7% |
| | | | | Conductivity µS/cm | | | | |
| Start | 112 | 111.8 | 111.9 | 111.8 | 121.9 | 112.8 | 113.3 | 112.8 |
| End | 1047.0 | 1037.0 | 116.2 | 112.6 | 1041.0 | 1022.0 | 124.3 | 112.8 |
| | | | | Turbidity NTU | | | | |
| Start | 1.93 | 1.42 | 1.32 | 1.43 | 1.65 | 1.43 | 1.49 | 1.36 |
| End | 1.00 | 1.43 | 1.36 | 1.34 | 1.28 | 1.13 | 1.57 | 1.43 |

**Table 7.** Mean and range of measures of engine exhaust monitored before it entered the catalytic converter during ballast neutralization process of both trials, September 2013.

| | Trial 1 | | | Trial 2 | | |
|---|---|---|---|---|---|---|
| Parameter | Mean | Range | N | Mean | Range | N |
| $O_2$ % | 12.3 | 12.1 – 12.6 | 3 | 12.1 | 11.9 – 12.2 | 8 |
| $CO_{ppm}$ | 71.7 | 68 – 75 | 3 | 69.4 | 58 – 85 | 8 |
| $CO_2$ % | 6.5 | 6.2 – 6.6 | 3 | 6.6 | 6.5 – 6.7 | 8 |
| $NO_{ppm}$ | 1,190 | 1,167 – 1,236 | 3 | 1,218 | 1,131 – 1,262 | 8 |
| $SO_{2\ ppm}$ | 0 | 0 | 3 | 0 | 0 | 8 |

**Table 8.** Summary of flow rate through the scrubber system, temperature, and engine operating conditions during neutralization process of trials of ballast treatment on board the M/V Ranger III, September 2013.

| | Trial 1 | | | Trial 2 | | |
|---|---|---|---|---|---|---|
| Measurement | Mean | Range | N | Mean | Range | N |
| Scrubber water flow (L) | 37.0 | 36.71–37.48 | 3 | 36.8 | 36.7–37.1 | 3 |
| Scrubber water temperature (°C) | 23.47 | 21.6–26.6 | 3 | 21.6 | 21.1–22.2 | 3 |
| Gas flow to tank spargers (L/m) | 62.30 | 62.3 | 2 | 61.4 | 59.5–63.7 | 6 |
| Gas bypass flow (L/h) | 11,327 | 11327 | 3 | 11,327 | 11327 | 3 |
| Prop shaft speed (rpm) | 327 | 326–328 | 2 | 328 | 328–331 | 3 |
| Prop pitch (%) | 100 | 100 | 2 | 100 | 100 | 3 |

treatments (mean 116.5 µs/cm, SD 5.47 $t_3 = 1.61$; $P = 0.21$), but increased significantly ($t_3 = 170.40$; $P < .0001$) in the NaOH treatments (mean = 1036.7; SD = 10.65 µs/cm). The turbidity measures in control tanks were not significantly different before and after treatments relative to tank fill conditions ($t_3 = 2.03$; $P = 0.14$; Table 6). The quantities of $O_2$, carbon monoxide (CO), $CO_2$ and nitrogen monoxide (NO) from the engine entering the catalytic converter were similar between trials, and no sulfur dioxide ($SO_2$) was observed (Table 7). The water cooling and flow through the catalytic converter and flow of gas to the tanks for neutralization were consistent between trials (Table 8).

## Discussion

Our replicate trials of hydroxide stabilization as a ballast disinfection tool were 100% effective in killing freshwater ballast organisms > 50 µm, the largest size class of zooplankton. The effectiveness exceeded the standards for ballast discharge in the U.S. The U.S. Coast Guard (USCG) and the U.S. Environmental Protection Agency (EPA) both regulate ballast water discharges and have identical numeric concentration-based standards. These limits appear in the USCG's Standards for Living Organisms in Ships' Ballast Water Discharged in U.S. Waters (US Federal Register 2012) and the EPA's current Vessel General Permit (US Federal

Register 2013) and are separated into three grouping by organism size: 1) < 10 live organisms per $m^3$ that are ≥ 50 μm in minimum dimension; 2) fewer than 10 living organisms / mL ≥ 10 μm and < 50 μm in minimum dimension; and 3) indicator organisms < 10 μm in minimum dimension with *Vibrio cholerae* (Pacini, 1854) at < 1 CFU/100 mL, *Escherichia coli* (Migula, 1895) at < 250 CFU/100 ml, and intestinal *enterococci* at < 100 CFU/100 mL). These regulations are similar, but not identical to those proposed by the International Convention for the Control and Management of Ships' Ballast Water and Sediments (IMO 2004).

Our trials focused on testing the system's efficacy related to the largest size class in the regulations, zooplankton. Note that both USCG and EPA regulations exempt Great Lakes vessels from the requirement to install ballast water treatment systems. However, this assessment uses those regulations as a point of comparison since they are the current currency of ballast treatment efficacy discussion. The standard was clearly met as all zooplankton filtered from treatment tanks at the completion of our trials were dead and highly skeletonized. The measures of density of zooplankton after treatment were reduced because cell fragments and carapace segments of degraded organisms were not counted.

The relative proportion of taxa was similar between fill and the end of trials in control tanks and micro-plankton (Rotifera) were dominant. The distribution of taxa met the requirements of EPA (2010) for land based trials of a minimum of 3 species across 5 phyla. This taxa requirement is an element of challenge conditions that EPA has identified for land-based testing. The goal is to have water quality and organism concentrations during testing that presents a challenge for the system that is reasonably representative of actual waters a system might encounter in shipboard operations. Besides minimum taxa, EPA also requires minimum organism concentrations for zooplankton of $10^5/m^3$ (US EPA 2010). The concentration of zooplankton in Trial 1 was lower than recommended. The density of live zooplankton in control tanks at the end of our trials was lower than that observed at filling, supporting the hypothesis that extending holding time in the dark may induce mortality in the plankton community. However, all concentrations were above the threshold for control samples suggested by EPA of 100 organisms/$m^3$ (EPA 2010). Thus, mortality was due to treatment, not other factors.

We did not evaluate phytoplankton or bacterial survival in elevated pH in this study, but bench studies using NaOH on a suite of 31 bacterial isolates from 15 taxonomic groupings of fish pathogenic and environmental bacteria demonstrated that a pH 12 was 100% effective within 72 h (Starliper and Watten 2013). Effectiveness of elevated pH at pH's targets below 12 was observed in a second similar series of tests conducted using environmental bacteria isolated from ballast samples obtained from the freshwater freighter the M/V Indiana Harbor (Starliper et al. 2015). In addition, previous bench scale studies conducted by the Great Ships Initiative (GSI) at pH 11.5, 12.0, and 12.5, demonstrated the effectiveness of NaOH at killing adult rotifers *Brachionus calyciflorus* (Pallas, 1766), the cladoceran *Daphnia magna* (Straus, 1820), and Eucyclops copepods (TenEyck et al. 2009; 2013). Replicated land-based trials using NaOH conducted by GSI designed to simulate a larger ballast tank system reported high efficacy of NaOH as a biocide. They observed complete mortality in 2 of 4 test tanks, with an overall average of 4.9 live organisms/ $m^3$ for zooplankton (Cangelosi et al. 2011). The GSI group also conducted one field trial aboard the MV Indiana Harbor in Lake Superior that confirmed the effectiveness of the hydroxide stabilization treatment, but live zooplankton at the time of discharge exceeded regulations (Cangelosi et al. 2013). It is not entirely clear how to reconcile these disparate results. In our tests the NaOH treatment process skeletonized all organisms and yet a number of living organisms were found in the shipboard tests conducted by GSI. Differences may be related to site-specific water chemistry and sampling methods as well as the potential, in the GSI tests, for the commingling of treated and untreated water and the presence of settleable solids within the horizontal surfaces of the ballast tanks included in the tests. The latter result from normal ballasting operations and are known to harbor a variety of freshwater invertebrate taxa (Duggan et al. 2005). The potential for commingling of water was related to treatment design in the shipboard test that GSI evaluated, where two of the eight ballast tanks shared a common fill and drain piping system that had not been treated with NaOH before samples were taken.

A ballast treatment protocol must not only be effective but it must meet applicable water quality standards for human and environmental health (IMO 2008a, b). Our study was not designed to test the toxicity of the neutralized ballast water.

However, previous trials conducted by the GSI on the neutralized effluent from a land based and shipboard trial showed promising results with minimum negative effects in whole effluent tests on fathead minnow, *Pimephales promelas* (Rafinesque, 1820), daphnia *Ceriodaphnia dubia* (Richard, 1894) and algae *Selenastrum capricornutum* (Printz, 1914) (Cangelosi et al. 2011, 2013).

The neutralization of the elevated pH with cleaned engine exhaust was effective and safe and implementation of this method could reduce $CO_2$ emissions, thereby providing an additional environmental benefit. Our test scrubbing system provided stable levels of gas flow and temperature with inlet $CO_2$ and $O_2$ concentrations that matched concentrations established for diesel engines used to power typical bulk carriers like the M/V Indiana Harbor operating in the Great Lakes (B. Watten, unpublished data). The exhaust $O_2$ ranged between 12 – 13% by volume or about 60% of inlet air levels as per fuel combustion requirements. As a result, the dissolved $O_2$ in the treated ballast water was lowered with pH during gas sparging due to reduced oxygen partial pressure in the treated exhaust gas (Weiss 1970). The partial pressures measured included the effect of the lowered oxygen mole fraction as well as local hydrostatic pressure. Likewise, $CO_2$ transfer is related to partial pressures along with local dissolved gas deficits (Watten et. al. 2004). In our case of $CO_2$ addition to elevated pH water, gas transfer was accelerated by the reaction of NaOH with $CO_2$ in the liquid film that yields sodium carbonate and then sodium bicarbonate. The net effect on transfer, indicated by the factor $\beta$ (Yoshida and Miura 1963; Watten et al. 2004), was the maintenance of relatively high dissolved gas deficits that provide the driving force for gas transfer. The $CO_2$ transfer rates increase with $\beta$ up to a NaOH normality of about 2.0 N (Onda et al. 1968) which is well above the 0.0108 N dose used in our trials. The $\beta$ effect no doubt contributed to the level of $CO_2$ recovery achieved in the test ballast tanks that provided a working water depth of just 94 cm. Our calculated $CO_2$ application rate equated to 1.56 times the stoichiometric requirement, or in other terms, a $CO_2$ recovery of 64% when we use depletion models (Figure 4) to establish a required sparging time (124.6 min) to achieve pH 8.0.

The depletion model assumes the mean observed $CO_2$ level in the engine exhaust was 6.55% and a mean exhaust sparging rate per tank was 62.9 L/min (given the mean observed $CO_2$ level in the engine exhaust (6.55%), and the mean exhaust sparging rate per tank of 62.9 L/min). Higher recovery rates are expected in deep ballast tank applications known to provide greater sparged gas retention times (Watten and Beck 1985).

Other technologies that meet environmental concerns have been pursued with an interest to find compounds that are effective in seawater and freshwater environments (see reviews by Goncalves and Gagnon 2012; Werschkun et al. 2012). Commonly suggested chemical treatments include chlorine and bromine-based compounds (Raikow et al. 2007). Chlorination systems can generate trihalomethanes, halogenated acetic acids, and bromate (Maranda et al. 2013). Ozonation has also been tested but in seawater this method produces high levels of bromated compounds (Wright et al. 2010). The generated byproducts of many of these methods can be reduced effectively by active carbon filtration, but these steps require additional infrastructure and associated costs. Cangelosi et al. (2007) provided evidence that filtration with 25 or 50 µm filter reduced densities of organisms operating in the Great Lakes, but additional treatment was likely necessary to effectively minimize risk and meet discharge standards associated with organisms of all sizes in the water column.

In summary, our study documents the effectiveness of hydroxide stabilization as a tool for disinfecting ballast systems in freshwater. This method provides the added benefit of potential reduction of $CO_2$ emissions from ships fitted with such a ballast management system. Additional studies need to be conducted by independent parties before this tool could be accepted as an approved ballast management system by regulators.

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