2011

Efficacy of NaCl brine for treatment of ballast water against freshwater invasions

Tony Nan-Chun Wang
University of Windsor

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By

Tony Wang

A Thesis
Submitted to the Faculty of Graduate Studies through the Great Lakes Institute
for Environmental Research in Partial Fulfillment of the Requirements for the
Degree of Master of Science at the University of Windsor

Windsor, Ontario, Canada

2010

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Tony Nan-Chun Wang

APPROVED BY:

_____
Dr. Lynda Corkum
Department of Biological Sciences

_____
Dr. Hugh MacIsaac, Co-supervisor
Great Lakes Institute for Environmental Research

_____
Dr. Sarah Bailey, Co-supervisor
Department of Fisheries and Oceans Canada

_____
Dr. Doug Haffner, Chair of Defense
Great Lakes Institute for Environmental Research

Sept 16, 2010

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Abstract

Currently, all transoceanic vessels entering the Great Lakes must perform ballast water exchange or saltwater flushing. Non-compliant vessels presently have limited and often costly and/or time consuming alternatives available. Treatment with sodium chloride (NaCl) brine as an alternative ballast water management option was examined here.

Six shipboard trials were conducted - three trials each on vessels with residual ballast water and with full ballast tanks - under operational conditions to determine the efficacy of brine ballast water treatment. Results indicate that brine is highly effective at reducing viability of zooplankton.

It took 25 and 5 hours to achieve 100% mortality in ballasted and residual ballast vessels respectively. Brine distributed well in tanks, however, vessel movement was essential to ensure thorough mixing. This method of ballast treatment appears to be cost-effective and safe and it could be implemented to reduce risk of new invasions in the Great Lakes.

Dedicated to my parents Michael and Grace

For all their support and encouragement

Acknowledgements

I would like to thank my advisors, Drs. Sarah Bailey and Hugh MacIsaac, for their support throughout the planning and implementation of my studies. Their assistance and comments during the preparation of this thesis were invaluable. I am also grateful that they supported my travel to many conferences, enabling me to develop my presentation skills and network with international colleagues.

I would like to acknowledge Dr. David Reid, Dr. Tom Johengen, Captain Philip Jenkins, Chris Wiley, Matthew Deneau, Jocelyn Gerlofsma, and Johanna Bradie for their assistance in the field. Without their gracious help, my experiments would not have been possible.

Finally, I would like to acknowledge my parents, Michael and Grace, and my partner, Lauren, for their support and encouragement in my work.

The support and encouragement I received from my friends, my labmates, and my officemates, was very motivational and critical to the timely preparation of this thesis.

Table of Contents

Declaration of Originality

List of Tables

List of Figures

CHAPTER1: GENERAL INTRODUCTION

Biological invasions are a leading cause of species extirpation and extinction, and contribute to global homogenization wherein native species are replaced by non-indigenous species (NIS) (McKinney and Lockwood, 1999; Rahel, 2002; Drake and Lodge, 2004). A NIS is a species that has established outside of its native habitat range. The impacts of biological invasions are evident on almost all landmasses and in aquatic environments (Bright, 1999; Olden et al., 2008; Piola et al., 2009).

Zooplankton are a major concern when it comes to freshwater species invasions, especially since the group is incredibly diverse, capable of rapid reproduction, and have strong ecological effects (Machida et al., 2009). Zooplankton can be transported and released in large numbers through ships' ballast water. Choi et al. (2005) found the abundance of zooplankton on bulk carriers in San Francisco Bay averaged 374 individuals m^{-3} in summer months (June-September). About 1,000 to 800,000 individuals m^{-3} may be transported in unmanaged ballast water and subsequently be released with the discharge of ballast water (Wonham et al., 2001; Verling et al., 2005; McCollin et al., 2008). Not only can NIS greatly affect the environment, they can also negatively impact the economy by affecting equipment maintenance costs and tourism revenue. The cost associated with NIS in Canada alone exceeds \$13 billion $year^{-1}$ (Colautti et al., 2006a). It is therefore necessary to prevent and stop NIS invasions.

Invasive species in the Great Lakes

At least 180 aquatic NIS have established in the Laurentian Great Lakes, and the resulting impacts of some species have been severe (Ricciardi, 2001; Duggan et al., 2003; Ricciardi, 2006). From 1840 to 2006, 13 of the NIS established in the Great Lakes were zooplankton species (NOAA, 2006). This may seem like a small number but NIS can have devastating effects on recipient habitats. An example of a species introduced through ballast water that had negative impact on the environment is *Bythotrephes longimanus.* It is native to Eurasia and was first found in Lake Huron in 1984, but it has since spread to other Great Lakes (Sprules et al., 1990; Hovius et al., 2007). These predatory cladocerans prefer large zooplankton as prey. Defensive mechanisms of *Daphnia*, such as tailspines and helmets, are not effective against these new predators (Schulz and Yurista, 1999) and *Bythotrephes* may prey on native predatory species, such as *Mesocyclops* and *Leptodora*, to near extinction (Hovius et al., 2007). Generalists like *Bythotrephes* have the potential to consume a large portion of zooplankton production (Yurista and Schulz, 1995; Foster and Sprules, 2010).

Invasion through ballast water

Approximately 7,000 species are transported in ballast water around the world each day by commercial vessels (Carlton, 1999) and the movement of ballast water is one of the most important vectors for aquatic NIS transfer today (Ruiz et al., 1997). Some vessels contain much larger volumes of ballast water than previous generations of ships. Consequently, modern vessels can deliver

more propagules at a faster rate than in the past, and thereby increase the propagule pressure associated with ballast water release.

The primary mechanisms of ship-mediated introductions include ballast water, ballast sediments, and biological fouling of ship hulls (Dodgshun et al., 2007). It is important to focus on ballast water rather than other ship-mediated pathways because NIS contained in the water column are more likely to be discharged from vessels than NIS or fouled on ship hulls. Sylvester and MacIsaac (2010) found that hull fouling posed little risk of species invasion to the Great Lakes because few freshwater organisms survive on hull surfaces after transit across the Atlantic Ocean.

Ships with "no-ballast-on-board" are often referred to as NOBOB ships. These ships are loaded with cargo so ballast water is not needed for balance and trim (Transport Canada, 2010). Ballast pumps can remove most of the water out of the tanks but small amounts of water and sediment will typically remain on board as residuals, as in the case of NOBOB tanks (Boylston, 1996). The layer of unpumpable water and sediment may become a more or less permanent layer on the bottom of the tanks supporting many benthic life forms of all life stages.

There are four basic stages to biological invasion: transport, introduction, establishment, and spread (Colautti and MacIsaac, 2004). During the transport stage, NIS must be loaded into the vessel and survive the physical transfer from donor region to recipient region. In the second stage, the NIS that survived the trip must be discharged into the recipient ecosystem. Those discharged must survive the physical and chemical conditions of the new habitat to establish a

reproductive colony. Some individuals will be in such poor health after the release that they cannot establish a reproducing population. The fourth stage begins when the established population grows and individuals begin to disperse and expand the geographical range in the recipient habitat. NIS may cause ecological or economic harm through interactions, such as predation or competition with native species, and may subsequently affect the economy (e.g. fish industries) (Lockwood et al., 2007). Typically, it is only when the NIS is widespread and abundant that it will cause harm to the environment or to the economy (Lockwood et al., 2007).

There are three filters that may affect the transition of NIS between stages: propagule pressure, physico-chemical factors, and community interactions (Colautti and MacIsaac, 2004; Colautti et al., 2006b). Propagule pressure is a measure of introduction effort and includes three main components: propagule size, propagule number, and the condition of propagules (Lockwood et al., 2007; Simberloff, 2009). Propagule size is the number of individuals in a release event and propagule number is the number of release events. As propagule size and number increase, the probability of successful invasion also increases because as more individuals are introduced into one place, it becomes more likely that some individuals will be successful in establishing colonies and thrive in the new environment (Kolar and Lodge, 2001; Lockwood et al., 2007; Sagata and Lester, 2008). The condition of propagules also contributes to the success rate of biological invasions in that the healthier the invaders are, the more likely that they will survive long enough to find food and suitable mates and contribute to the

success rate of establishment (Simberloff, 2009). Propagule pressure acts on all four stages of invasion and it aids the transition of NIS to subsequent stages (Colautti and MacIsaac, 2004).

Physico-chemical factors also impact the ability of NIS to transition through the stages of an invasion (Jones and Ricciardi, 2005; Leung and Mandrak, 2007; Cordell et al., 2010). If factors such as temperature or pH are not tolerable by the NIS, then the NIS may not survive long enough to establish a reproducing population.

Community interactions act on stages two, three, and four (Colautti and MacIsaac, 2004). NIS that survived the transport stage will interact with the native species or other established NIS, after the release. The widely quoted 'Tens rule' states that about 10% of all introduced NIS successfully establish and about 10% of those established species become invasive (Williamson and Brown, 1986). Karatayev et al. (2009) showed that successful NIS are not just random subsamples of species drawn from a native region, but are more robust than the natives and have greater tolerance to pollution. Some examples of harmful invasive species in the Great Lakes are zebra mussels (*Dreissena polymorpha*), round gobies (*Neogobius melanostomus),* and predatory cladocerans such as *Cercopagis pengoi* and *Bythotrephes longimanus.*

Prevention of NIS introductions effectively eliminates the need to develop NIS control and management programs for stages two, three, and four. Therefore, it is important to focus on eliminating NIS in ballast water before they are discharged.

Ballast water regulations

Since 1993, transoceanic vessels with filled ballast tanks have been required to perform ballast water exchange (BWE) with highly saline, open ocean water. Current Canadian BWE regulations state that the exchanged ballast water must have a final salinity of at least 30 parts per thousand (‰) and that the saline water must be taken from mid-ocean at least 200 nautical miles offshore and in depth of at least 2000 meters (Transport Canada, 2010). Despite implementation of these regulations, the number of NIS introductions did not appear to decline (Duggan et al., 2003; Ricciardi, 2006). The International Maritime Organization's (IMO) D-1 regulation for BWE requires vessels to conduct at least 95% volumetric exchange (IMO, 2010). In addition, the IMO's D-2 ballast water performance standard was proposed (but not yet implemented) to reduce the chance of successful invasion by reducing propagule pressure for specific size classes and indicator bacteria in discharged ballast water (Table 1).

BWE can greatly reduce the abundance of freshwater species by purging them from tanks (Gray et al., 2007), and the high salinity level of sea water should induce physiological stress on organisms remaining in the tanks (Wonham et al., 2005; Ellis and MacIsaac, 2009). However, some vessels cannot comply or can only partially comply with the regulations due to various factors such as weather conditions and ship safety.

Even though greater efforts by researchers to identify invasive species may be a factor in an apparent increased rate of discovery of NIS over the past

17 years, continued discoveries of new NIS led some people to question the effectiveness of BWE (Duggan et al., 2003). Ricciardi (2006) reported that recently introduced NIS to the Great Lakes are mostly euryhaline benthic invertebrates, and suggested that the Great Lakes are still at risk of more NIS invasions because these NIS may have the ability to survive BWE.

Treatment of ballast water

Besides BWE, various methods for eliminating NIS in the ballast tanks have been suggested, including biocide, heat, and ultraviolet treatments. There are at least 41 ballast water treatment systems around the world that are at various stages of development and approval (Lloyd's Register, 2010). Most treatment technologies are still in the experimental phase and will not be ready for implementation until around 2016 (California Environmental Protection Agency, 2002).

The filtration technology is perhaps the most environmentally-friendly method at removing organisms from ballast water but it must be coupled with other treatment(s), such as heat or UV radiation, to eliminate NIS quantitatively (California Environmental Protection Agency, 2002; Kazumi, 2007). Preliminary observations showed that biocides are effective at treating ballast water (California Environmental Protection Agency, 2002; Gavand et al., 2007; Kazumi, 2007) but appropriate disposal or neutralization must be considered (California Environmental Protection Agency, 2002; Kazumi, 2007). Research has demonstrated that deoxygenation, UV radiation, and heat treatment are not

effective against zooplankton resting eggs but they are effective against organisms in other life stages (Tamurri et al., 2002; California Environmental Protection Agency, 2002; Kazumi, 2007; Raikow et al., 2007).

Using brine as a treatment method

The addition of 230‰ sodium chloride (NaCl) brine has been proposed as a treatment method for non-compliant vessels by Jenkins (2007). This method of killing ballast water organisms has similar rationale as BWE in that the brine will cause high osmotic stress to the organisms and thus induce mortality. BWE that achieved a final salinity of 30‰ in ballast tanks has an efficiency of >99% mortality for freshwater organisms (Gray et al., 2007), therefore, treatment using brine at 230‰ is expected to be at least as effective as BWE.

Brine is readily available around the Great Lakes basin and is cost-effective (Jenkins, 2007). Jenkins (2007) estimated that the cost of brine treatment per vessel could range between $5,200 and $7,200. The cost includes transportation of the brine, the brine itself, and labour (loading and unloading). Most of the cost of brine treatment is associated with delivery, therefore, to lower the cost, it may be possible to install "brine stations" at various ports for quick delivery of brine to vessels.

Bradie et al. (2010) demonstrated that exposure to NaCl brine at 115‰ concentration was highly toxic for a wide variety of aquatic invertebrates that may be transported in ballast water. Treatment at 77‰ was also highly toxic, however, it took a longer time to fully eliminate invertebrates (Bradie et al., 2010). The

ability to completely mix brine and ballast water in ballast tanks under operational conditions was highlighted as a potential impediment to this management strategy.

This thesis describes the first comprehensive trials to evaluate the efficacy of NaCl brine as a tool to prevent introductions of aquatic invasive species via ballast water. This study evaluates how well NaCl brine is at eliminating organisms in ballast tanks on transoceanic vessels and also evaluates how well brine mixes in tanks under normal operational conditions.

CHAPTER 2: EFFICACY OF NaCl BRINE FOR TREATMENT OF BALLAST WATER AGAINST FRESHWATER INVASIONS

Introduction

Up to five billion m^3 of ballast water, carrying an estimated 7,000 species, are transported daily around the world by commercial vessels (Carlton, 1999; Tsolaki and Diamadopoulos, 2010). Studies indicate that commercial vessels may transport 1,000s to 100,000s of individuals of zooplankton per m^3 in ballast water (Wonham et al., 2001; Verling et al., 2005; McCollin et al., 2008). Propagule pressure theory indicates that the probability of successful establishment of introduced non-indigenous species (NIS) is directly proportional to the density of viable individuals introduced (Lockwood et al., 2005, 2007). Even though attenuation of propagule number is common for most biota during transit in a vessel's ballast tanks, commercial shipping and ballast water release has played a strong role in the introduction of NIS to novel habitats worldwide (Ruiz et al., 1997; Wonham et al., 2001). For example, 34 of 56 (61%) aquatic NIS discovered in the Laurentian Great Lakes since the St. Lawrence Seaway opened in 1959 were introduced by shipping activities, including at least 10 zooplankton species (Kelly et al., 2009; NOAA, 2010).

Ballast water management systems, utilizing filtration, de-oxygenation, biocides, and/or ultraviolet treatment can minimize the risk of ship-mediated aquatic invasions by reducing propagule pressure. However, these systems will not be comprehensively deployed until approximately 2016 according to an

International Maritime Organization (IMO) agreement and timeline (Tsolaki and

Diamadopoulos, 2010; Lloyd's Register, 2010). Until then, ballast water exchange

(BWE) and saltwater flushing are mandatory management practices used to

prevent aquatic NIS introductions in the Great Lakes (Government of Canada,

2006; SLSDC, 2008). BWE involves discharging fully loaded ballast tanks while

the ship is located in mid-ocean, and replacing this water with high salinity water

to achieve ≥ 30 parts per thousand (‰) final salinity. Saltwater flushing, similar to

BWE but with smaller volumes of water, is used for tanks containing only residual

ballast water (i.e., no-ballast-on-board or NOBOB; see Bailey et al., 2010). The

biological efficacy of BWE is variable for coastal marine habitats (Ruiz and Reid,

2007), though it appears to be highly effective (>99%) against freshwater

zooplankton (Gray et al., 2007). BWE and saltwater flushing reduce invasion risk

by decreasing the number of individuals (propagule pressure) and number of

species (colonization pressure) in ballast tanks by physical removal (i.e.,

purging). In addition, exposure to mid-ocean water may provide additional

protection against fresh- and brackish-water taxa sensitive to osmotic stress

(Santagata et al., 2008; Ellis and MacIsaac, 2009).

    Though compliance with regulations is high, approximately 2% of ballast

tanks are non-compliant upon arrival to the Great Lakes, indicating a need for

alternative treatment methods (GLSBWWG, 2009). Currently, vessels can retain

non-compliant ballast water on board throughout their operations on the Great

Lakes or return to an approved offshore location to perform BWE and/or saltwater

flushing. Canadian regulations allow for use of approved treatment technologies,

however, no such technologies have been approved by Canada or the U.S.A. to date, except for in the States of Michigan and Washington (University of California, 2009). I propose here that sodium chloride (NaCl) brine - hereafter called only 'brine' - be used as an alternative treatment method for non-compliant tanks. Brine, at 230‰ salinity, is presently used for de-icing roads during winter around the Great Lakes, and is relatively cheap and readily available (Jenkins, 2007). Laboratory experiments have demonstrated that brine kills both freshwater and oceanic zooplankton over a short time exposure (hours) when applied at a minimum concentration of 77‰ (Santagata et al., 2009; Bradie et al., 2010). Here, I conduct shipboard experiments to determine if ballast water treatment with brine is effective under operational conditions. I test the toxic effect of brine exposure on freshwater invertebrates contained in large volumes and residual volumes of ballast water. Specifically, I test whether zooplankton in experimental tanks experience significantly higher mortality than in control tanks when exposed to brine.

Methods

Study Site Description

   A total of six shipboard trials were conducted on vessels operating on the Laurentian Great Lakes. Three trials were conducted with filled tanks (ballast-on-board vessels) of transoceanic commercial bulk carriers between June and October 2009 during voyages from Toronto, ON to Thunder Bay, ON (Table 2). For each of the trials, paired upper-stool ballast tanks with identical design were

filled with Great Lakes freshwater (0‰) at port in Toronto. One tank served as a control, which had no brine addition, while the other was later treated with brine; the control tank was filled completely while sufficient space was left in the experimental tank to accommodate subsequent addition of brine.

Three additional trials were conducted with residual ballast (no-ballast-on-board vessels) of domestic commercial tankers while moored in Sarnia, ON between November 2008 and December 2009 (Table 2). Paired double bottom ballast tanks of identical design were utilized for each trial, each containing residual Great Lakes freshwater (0‰). One tank served as a control with no brine addition, while the other was later treated with brine. Brine, at a concentration of 230‰, was delivered to vessels by tanker truck (Road Maintenance Equipment & Services Inc., Cobourg, ON). Brine was pumped through a hose from the brine truck directly into the treatment ballast tanks. A flowmeter on the pump indicated the amount of brine pumped into tanks (Table 2).

Assessment of brine distribution

For ballasted tank trials, five self-recording programmable sondes, each with temperature, conductivity, optical dissolved oxygen, and depth sensors, were secured in the treatment tank. Sondes were positioned to cover a range of depths and horizontal coverage to quantify the extent of brine mixing within the tank. One sonde was installed in the control tank to monitor the same conditions. Conductivity sensors were calibrated with NaCl solutions ranging from 0-120‰ prior to each deployment. Specific conductance was used to correct for

temperature effects. A third order polynomial equation was calculated from each set of calibrations, for each sonde, to convert measured specific conductance to equivalent NaCl concentration (‰). These empirical calibration curves were necessary to determine actual concentrations of brine present in tanks during experiments because the sondes are not calibrated specifically to NaCl and reported salinity values from the sondes at conductivities above 60 mS cm^{-1} are not accurate or within manufacturer specifications. In addition, specific conductance output of each sonde was checked post-experiment against a 44±1‰ NaCl solution (as measured with a precision hand-held NaCl refractometer). All measurements were within 1‰ of the expected concentration. Dissolved oxygen sensors were calibrated against air saturation for each sonde prior to each deployment. Vertical profiles of the experimental tank were taken with a hand-held YSI unit at the same time zooplankton samples were collected.

For residual ballast experiments, the multi-parameter sondes could not be used owing to safety issues relating to battery-powered instruments around volatile cargo. Instead, water samples were taken from at least the three tank corner locations farthest from the location of brine addition for subsequent measurement of salinity using a digital salinity refractometer. Samples were collected approximately hourly, from both the top and bottom layer of ballast residuals using a plastic pipette, at the same time as collection of zooplankton samples.

Experimental Design – Ballasted Tanks

To assess biological efficacy of brine treatment of ballasted tanks, zooplankton samples were collected from ballast water in both control and treatment tanks prior to the addition of brine and during the vessel transit following treatment at time points approximately 1h, 6h, 12h, 24h, 48h, and 72h post-treatment. Consecutive, vertical (1.8-m depth) plankton net tows were taken with a 30-cm diameter, 40-μm mesh conical net through an opened deck hatch for the first two vessels; the number of net tows was based on the expected density of zooplankton in the ballast water, thus sample volume increased over time as the density of live animals in treatment tanks decreased (target of ≥ 25 individuals per sample). As the upper stool tanks of the third vessel did not have deck hatches, samples were collected by lowering 1.27-cm inner diameter high density polyethylene tubing, fitted with a stainless steel check valve, into the tank through the sounding tube. Approximately 50-L of water was manually pumped to the deck surface at each sample time point, and filtered through the 40-μm mesh plankton net. After initial samples were collected, brine (230‰) was added to the experimental tanks through opened deck hatches (first two vessels) or sounding tube (third vessel) at Port Weller, ON. Owing to the large volume of water in the tanks, it was not possible to add enough brine to achieve a final concentration of 77‰ for even the short time intervals suggested by previous studies (Jenkins, 2007; Santagata et al., 2009; Bradie et al., 2010). As a result, I decided to examine the effect of 45‰ brine treatment on zooplankton over multiple days. Ellis and MacIsaac (2009) showed that NIS already present in the Great Lakes experience 100% mortality when exposed to 30‰ seawater for 72 hours. A

sufficient volume of brine was added to the tank to achieve the target concentration based on estimates of ballast volume provided by the ships' crew. Using a diesel pump, it took approximately one hour to apply the brine for each experiment and the volume added is shown in Table 2.

Experimental Design – Residual Ballast

The volume of residual ballast to be treated was estimated in consultation with the ships' crew so that brine could be added to the treatment tank in a 1:1 ratio to achieve a final target concentration of 115‰, based upon results of laboratory experiments (see Bradie et al. (2010) and recommendations by Jenkins (2007)). Using an intrinsically safe air-driven pump, which was slower than the diesel pump used for ballasted experiments, it took approximately one hour to apply the brine for each trial and the volume added is shown in Table 2.

To assess biological efficacy of brine treatment of residual ballast water, zooplankton samples were collected from residual ballast water in both control and treatment tanks prior to the addition of brine and following treatment with sampling conducted approximately hourly for up to 5 hours post-treatment. Zooplankton was collected by physically entering ballast tanks; a manual bilge pump was used to collect a measured volume of water in 25-L plastic pails prior to filtration through a 40-μm mesh plankton net. A 1-L sample was initially collected to determine the density of zooplankton in tanks before each trial began. The volume sampled for each trial depended upon the initial density of zooplankton in residual ballast water (target of ≥ 25 individuals per sample).

Samples were collected at the location most distant from brine addition to the tank and a constant volume was sampled for all time points for both control and experimental tanks for each experiment.

Viability Assessment

Samples were filtered through a 40-µm sieve to remove excess water and transferred to a petri dish for observation under a microscope immediately following collection (i.e. on board the vessel). Zooplankton viability was assessed through a combination of physical stimulation with a dissection probe and vital staining with 10-g/L neutral red (Tang et al., 2006). Samples in the petri dish were washed into a 250-ml glass beaker using de-ionized water. One ml of neutral red solution was added to 100-ml of zooplankton sample volume and left for 15 min. Following staining, samples were repeatedly washed with tap water over a 40-µm sieve to remove excess stain and transferred to a small, gridded petri dish for viability assessment. Neutral red stained most live zooplankton, and thus made the organisms much easier to find and check for body movement; however, as the stain was not 100% accurate, care was taken to assess all non-motile organisms that did not stain. Zooplankton which moved or twitched when stimulated by probe were considered live.

Assessment of zooplankton viability was completed within 30 minutes of sample collection. Live and dead zooplankton were divided into separate sample jars and preserved in 95% ethanol for later enumeration in the laboratory; dead individuals were removed from controls by pipette whereas live individuals were

removed from treatment replicates. While the number of individuals transferred by pipette was recorded in the field, the live and dead sample fractions were enumerated more precisely back in the laboratory to determine abundance and proportion of live zooplankton in both control and treatment replicates. Owing to large numbers of zooplankton in some of the control 'live' fractions, three 0.5-ml subsamples were taken from 50-ml total sample volume to estimate abundance. Subsamples were drawn with replacement by Hensen-Stemple pipette, following thorough mixing to ensure uniform distribution of organisms. Samples that had less than 1,000 individuals were counted in entirety.

Statistical Analysis

To assess the efficacy of brine treatment based on changes in live zooplankton abundance, samples collected prior to brine treatment (T_0) were compared to the final samples collected after brine treatment (T_1). Following the methodology of Gray (2007), I calculated the percent change in live zooplankton abundance in each tank as:

$$\%r = (T_1/T_0)*100, \tag{1}$$

where %r represents the percent of zooplankton remaining after brine treatment, T_0 is the initial abundance, and T_1 is the final abundance measured after treatment. Identical calculations were conducted for control tanks ($\%r_C$) at time 0 (C_0) and matching final time point (C_1). Using these values, I then calculated the efficiency of the brine as:

$$Br_{Effic} = [(\%r_C - \%r_T) / (\%r_C)] * 100, \tag{2}$$

where $\%r_T$ is the fraction remaining in the treatment tank and $\%r_C$ is the fraction

remaining in the paired control tank. Paired sample t-tests were conducted to

confirm that zooplankton abundance was similar in the control and treatment

tanks at T_0 for both BOB and NOBOB experiments. The abundance data was

square-root transformed before analysis. One-way analysis of variance with

repeated measures (RM-ANOVA) using SPSS 11.5 was utilized to determine if

there were significant differences in abundance of live zooplankton between

control tanks and treatment tanks following brine treatment.

Results

Brine distribution

Brine distributed well in ballasted tanks, especially after vessels were

underway. The final uniform concentration of brine achieved was within 11-22%

of the target value, reflecting difficulties estimating accurate volumes of ballast

water inside tanks (Table 2). Measurements taken with the hand-held YSI unit

showed that it took approximately 10 hours for the brine to reach uniform

distribution in the tank center at the location of brine addition. However, records

from multi-parameter sondes installed in tanks showed that stratification occurred

during initial brine addition and uniform salinity was achieved 12-37 hours after

brine addition (Figure 1); tanks were presumably stirred by rolling action after the

vessels were underway. Different measurements taken from the instruments

installed in ballast tanks are summarized in Table 3. All of the readings in the

control and treatment tanks remained relatively constant through time, however,

conductivity in treatment tanks increased following the addition of brine.

Brine mixed well with residual ballast water for trials 1 and 3, however, incomplete mixing occurred during trial 2 with visible stratification of brine and freshwater residuals. Salinity in control tanks remained the same for the duration of the trials (Figure 3). The target concentration of 115‰ was reached or exceeded after 5 hours for all three trials (Table 2).

Brine toxicity

The paired sample t-tests showed that there were no significant differences in live zooplankton abundance between control and treatment tanks in either the ballasted tank experiment (t= -0.234, p=0.837) or residual ballast experiment (t=0.638, p=0.589) at the outset of the trials, before brine was applied. In ballasted tank trials, zooplankton consisted mainly of rotifers (Appendix 1). Other organisms such as annelids, molluscs, and insects/arachnids (i.e. spiders) were found in small numbers.

Abundance of live zooplankton in control tanks remained relatively consistent through time, however, abundance dropped significantly in treatment tanks following brine application ($F_{1,2}$ = 335.02, p = 0.003, ANOVA, Table 4). Complete mortality was observed at approximately 25 hours following brine treatment. While the total abundance of live zooplankton remain relatively constant in control tanks through time (Figure 2A), the proportion of viable zooplankton increased through time (Figure 2B).

Rotifers were also the most abundant taxon recorded in residual ballast

trials (Appendix 1). Abundance of live zooplankton remained relatively consistent through time in control tanks, but dropped significantly following brine application ($F_{1,2}$ = 168.05, p = 0.006, ANOVA, Table 4). Both the proportion of total viable and the total abundance of zooplankton in control tanks remained relatively constant through time (Figure 4A-4B), which suggests that there was very little reproduction. Complete mortality of zooplankton in treatment tanks was observed at approximately 5 hours post-brine treatment.

Brine treatment was highly effective at killing freshwater zooplankton, with no live zooplankton recorded from the final samples collected for all six trials.

Discussion

The addition of brine appears to be a highly effective and operationally practical means for treatment of freshwater ballast. It took approximately 25 hours exposure to 45‰ brine (Figure 2), and 5 hours exposure to 115‰ brine, to effectively exterminate freshwater zooplankton (Figure 4) from ballast tanks having full or residual ballast on board, respectively. Brine appears to be an effective interim treatment for non-compliant vessels entering the Great Lakes that could be implemented immediately, although several challenges remain.

The two hour delay in mixing observed for one of the residual ballast trials was likely due to the static trim of the vessel and internal structure of the ballast tank. Internal tank structures, such as longitudinal members and bulkheads, may restrict natural mixing and diffusion for tanks that are completely filled as well as for tanks containing only residual ballast water. I observed that vessel movement,

either as a result of wave action while underway or trim adjustments at berth, greatly facilitated mixing of brine in ballasted and residual ballast tanks, respectively. As a result, care must be taken to ensure complete mixing with ballast water to ensure maximal efficacy. Furthermore, when treating filled ballast tanks, the volume of brine needed to achieve the target concentration, particularly for treatment of freshwater ballast, must be considered since sufficient space must be available in tanks to accommodate the addition of brine. Ballast water may need to be divided among several tanks within a vessel to receive the brine volume without overflow of tanks.

While these tests indicated that brine treatment could completely eliminate freshwater zooplankton transported in ballast water, I acknowledge that individuals surviving brine treatment could have gone undetected due to the small sample sizes utilized in this study. Furthermore, additional tests examining a broader array of taxa are warranted since bacteria, viruses, and phytoplankton were not assessed during this study. I also did not examine efficacy against resistant taxa associated with ballast sediments, such as invertebrate dormant stages (Bailey et al., 2004, 2005; Briski et al., 2010). Previous studies have demonstrated that dormant stages of freshwater zooplankton can withstand exposure to high salinity levels and other chemical treatments (Bailey et al., 2004; Gray et al., 2006; Raikow et al., 2007a, b). Based on these studies, it is likely that brine treatment would be ineffective against dormant stages; however, the risk posed by dormant stages may be offset by high retention rates within tanks (i.e., dormant stages are not easily discharged from tanks)(Bailey et al.,

2006).

Finally, I acknowledge concerns with regards to the environmental impact of brine-treated ballast water being subsequently discharged at Great Lakes' ports. The relatively high concentration of brine used to treat residual ballast water (115‰) would be diluted to approximately 5.5‰ by filling ballast tanks with additional Great Lakes ballast water prior to discharge. In contrast, full tanks treated to 45‰ would have to be discharged directly, since there likely will be no head space available to load additional fresh water into tanks for dilution purposes prior to discharge. In both scenarios, a further immediate dilution of 100x is expected with discharge to a freshwater harbour (see Wells et al., 2010), resulting in brine concentrations of ~55-450-mg L^{-1} (0.10‰-0.81‰). While any addition of brine to freshwater ecosystems is not desirable, the environmental impact of brine treatment would be limited by the fact that the estimated amount of brine needed to treat non-compliant tanks annually (approximately 500 tonnes, or 20 tanker trucks) is far exceeded by the amount of brine already entering the Great Lakes as run-off from winter road treatment. American states bordering the Great Lakes and the province of Ontario use about 5.2 million tonnes of road salt annually (Transportation Research Board, 1991; Environment Canada, 2001). Peak chloride concentrations in small streams draining urban Ontario watersheds can be as high as 5000-mg L^{-1} (9.1‰), while the highest average discharge concentration discharged into Lake Ontario is 332-mg L^{-1} (Kaltenecker and Todd, 2007). Further, British Columbia has suggested that maximum chloride concentrations should not exceed 600-mg L^{-1} to protect sensitive aquatic species

from acute toxicity (Kaltenecker and Todd, 2007). As a result, the environmental impact of brine ballast water treatment would appear minor in comparison to other sources entering the lakes. Furthermore, since it is virtually impossible to eradicate NIS after establishment, small discharges of brine are an obvious choice as the lesser of two evils.

If brine was approved as an alternate method for treatment of non-compliant tanks, brine stations could be set up at strategic points around the Great Lakes, especially in the St. Lawrence River or Seaway, to facilitate treatment and decrease application cost. Instead of having truck delivery of brine, as was the case in this study, vessels could be treated as they entered the Seaway from a common facility. A method to treat non-compliant ballast tanks may be required for the foreseeable future, as ballast water management systems utilizing filtration, de-oxygenation, biocides, and/or ultraviolet treatment are still in the developmental phase and will not be widely employed until approximately 2016 (Tsolaki and Diamadopoulos, 2010; Lloyd's Register, 2010). Brine treatment could also serve as a 'back-up' strategy for cases where ballast water management systems, once approved and implemented, break down during ship operations.

CHAPTER 3: GENERAL DISCUSSION

The addition of brine appears to be a highly effective and operationally practical means for treatment of non-compliant freshwater ballast, which could be implemented immediately to reduce the risk of introduction of pelagic zooplankton. I observed that approximately 25 hours of exposure to 45‰ brine and approximately 5 hours exposure to 115‰ brine effectively exterminated freshwater zooplankton from ballast tanks having full, or residual, ballast on board, respectively. Further, vessel movement appears to be an essential component of successful brine treatment by facilitating mixing in ballast tanks. The movement of vessels in transit swirls ballast water inside ballast tanks and promotes mixing, as documented by videos taken while conducting the experiments. This swirling of water helps distribute brine to the far edges of tanks and helps achieve uniform distribution. Stationary vessels may encounter problems with brine mixing, as occurred during one of the residual ballast trials. Stationary vessels may be able to achieve adequate mixing by adjusting the ships' trim and/or list through shifting of cargo or ballast water.

While brine treatment was very effective against pelagic zooplankton in all of the trials conducted, additional studies with phytoplankton, bacteria, and viruses should be conducted to confirm efficacy for a wider array of ballast-mediated taxa. A microcosm experiment conducted by Greenwald and Hurlbert (1993) found that total zooplankton abundance decreased with salinity. This is consistent with the experiments conducted in Chapter 2. NaCl brine treatment may also be effective on freshwater phytoplankton as a study conducted by Redden and

Rukminasari (2008) found that an increase in salinity reduces phytoplankton abundance. However, high salinity treatment against saltwater phytoplankton may not be effective (Greenwald and Hurlbert, 1993).

In the field experiments conducted here, brine was delivered by tanker truck. This method of delivery may not be effective on a larger scale due to many factors that could delay or prevent brine trucks from reaching their destination. During the field experiments, there were incidents where the brine truck broke down en route and/or became stuck in traffic. To solve this problem, it may be possible to set up a number of brine stations at major Great Lakes ports or at strategic locations, such as the Welland Canal. In doing so, vessels needing brine treatment can receive it immediately, increasing the utility of brine treatment as a back-up method by reducing costs of delivery and time. Jenkins (2007) estimated that cost of treatment, which includes brine, transportation, and labour, could range from $5,200 to $7,200. The amount of brine needed to treat ballast tanks depends on the salinity of the ballast water in those tanks. The higher the salinity in the ballast tanks, the less brine needed to treat the water. In the experiments described here, no more than 20,000L of brine were needed per tank treatment.

One problem that ballast-on-board vessels may encounter is an inability to receive the extra volume required to treat fully loaded tanks with brine. It may be possible to accommodate the addition of brine by dividing fully loaded tanks into multiple empty tanks so that there is room to compensate for the additional volume. no-ballast-on-board (NOBOB) vessels will not have this problem

because treatment would be applied while the tanks are nearly empty of ballast water.

The environmental impacts of brine treatment of non-compliant vessels will be relatively insignificant in comparison to other brine applications around the Great Lakes. The amount of road salt and brine used annually for de-icing roads around the Great Lakes region during winter far exceeds the amount of brine needed to treat non-compliant vessels annually. For NOBOB vessels, the treated residuals will be diluted to approximately 5‰ and thus no acute toxicity effects are expected. In contrast, fully ballasted tanks treated with brine to a salinity of approximately 45‰ will be discharged directly without dilution due to the fact that there is no room in the tanks for the addition of freshwater. While acute toxicity effects are possible, dilution of approximately 100 times is expected when treated ballast water is discharged into the Great Lakes, bringing the brine concentration to 55-450 mg L^{-1} (0.1‰ - 0.81‰). This range is well below the 5,000 mg/L (9.1‰) chloride discharge limit for Ontario (Kaltenecker and Todd, 2007). Each of the eight Great Lakes states have their own maximum permissible chloride discharge limit (Table 5). While the addition of brine into freshwater ecosystems is not ideal, it is less harmful than the possible alternative of new successful invasions.

The International Maritime Organization (IMO) has proposed ballast water discharge standards under the Ballast Water Management Convention that, once ratified, will require all vessels operating in international waters to treat ballast water with approved treatment systems (IMO, 2004). With an expected implementation deadline of 2016, there are at least 41 ballast water treatment

systems that are at various stages of development and approval (Lloyd's Register, 2010), including systems which make use of physical, mechanical and/or chemical treatment processes. All of the treatment methods have their own advantages and disadvantages, each suited to a particular set of operating conditions.

Filtration systems, usually used in combination with other treatment processes, remove organisms and particles above a certain size by forcing water through a filter that will retain target organisms. This method is ideal because the addition of chemicals to ballast water is not required (Kazumi, 2007). The filtered organisms can be stored and disposed of or discharged back into the source environment. A disadvantage to this method is that the flux of water through filters can be immensely reduced as organisms are deposited onto the filter surface (Boylston, 1996), requiring constant cleaning to ensure good water flow through filters.

Treatment using biocides has the advantage of ease of application. Concentrated solid or liquid chemicals such as sodium chloride or hydrogen peroxide may be added directly to ballast tanks or intake lines. Effective biocide concentrations may be maintained by automatic feed systems. One of the disadvantages of this method, however, is the potential for negative environmental impacts due to spills or incomplete neutralization before ballast water is discharged into recipient aquatic ecosystems (Boylston, 1996; Kazumi, 2007). A group of experts on the scientific aspects of marine environmental protection (GESAMP Ballast Water Working Group) was established specifically

to review systems utilizing chemicals or other active substances during the IMO approval process in order to minimize associated environmental impacts.

Heat treatment using waste heat from a ship's propulsion system is ideal for some situations in which sufficient heat can be generated to kill organisms as no chemical products are utilized. For example, temperatures of 38 ºC for over 30 hours was enough to kill all zooplankton and the majority of phytoplankton in shipboard trials conducted by Rigby et al. (1999). Heat treatment, however, may not be practical for short voyages or large volumes of ballast water since there may be insufficient time or energy to heat the ballast water to the desired temperature (Boylston, 1996).

While there are many more treatment methods, there will inevitably be situations where a system has malfunctioned or broken down. While NaCl brine treatment is not practical for use as a primary method of ballast treatment, it will remain a cost-effective and easy-to-apply back-up treatment method as vessels transition to the use of technological treatment systems in the coming years. Ballast water may carry a diverse community of aquatic taxa and is an important vector requiring careful management to reduce future biological invasions. The brine treatment method outlined in this thesis is a promising new tool for ballast water management that can be used to prevent further zooplankton invasions in the Great Lakes.

Table 1. IMO's D-2 standard for maximum density of organisms discharged after ballast water treatment (IMO, 2010). (cfu = colony forming unit).

Organism size or Indicator Microbe	Maximum Permissible Density in Treated Ballast effluent
Organisms ≥ 50µm in minimum dimension	<10 viable organisms m^{-3}
Organisms <50µm ≥ 10µm in minimum dimension	<10 viable organisms mL^{-1}
Toxicogenic *Vibrio cholera* (O1 and O139)	<1 cfu $100mL^{-1}$ or <1 cfu g^{-1} zooplankton samples (wet weight)
Escherichia coli	<250 cfu $100mL^{-1}$
Intestinal enterococci	<100cfu $100mL^{-1}$

Table 2. Experiment locations, date, and amount of brine applied. The initial

concentration of brine was 230‰ and was manufactured by Road

Maintenance Equipment & Services Inc. in Cobourg, ON. Location refers

to initial port where brine was applied, and the destination port,

respectively, unless the ship was stationary in port.

Experiment	Date	Location	Target/Final salinity (‰)	Volume of brine applied
BOB 1	June 16, 2009 to June 20, 2009	Toronto, ON to Thunder Bay, ON	45/50	~20,000L
BOB 2	Sept. 30, 2009 to Oct. 2, 2009	Toronto, ON to Thunder Bay, ON	45/38	24,445L
BOB 3	Oct. 22, 2009 to Oct. 24, 2009	Toronto, ON to Thunder Bay, ON	45/35	20,000L
NOBOB 1	Nov. 22, 2008	Sarnia, ON	115/125	~10,000L
NOBOB 2	May 27, 2009	Sarnia, ON	115/117	~10,000L
NOBOB 3	Dec. 16, 2009	Sarnia, ON	115/158	~10,000L

Table 3. Measurements of temperature, conductivity, dissolved oxygen, and pH in control and treatment tanks for the three ballast-on-board shipboard trials.

1st ballast-on-board vessel					
Parameters	**Control**				
Time (Hours)	**0**	**1**	**10**	**25**	**44**
Temperature (°C)	13.8	13.8	13.8	14.7	15.5
Conductivity (mS cm^{-3})	725	731	733	752	768
Oxygen (mg L^{-1})	10.3	9.8	9.8	9.7	8.8
pH	7.7	7.5	8	7.9	7.9
2nd ballast-on-board vessel					
Parameters	**Control**				
Time (Hours)	**0**	**1**	**10**	**25**	**44**
Temperature (°C)	17.3	17	16.6	16.4	16
Conductivity (mS cm^{-3})	0.4	0.4	0.4	0.4	0.4
Oxygen (mg L^{-1})	6.8	7.3	7.2	6.8	7
pH	7.1	7.4	7.9	7.8	7.8
3rd ballast-on-board vessel					
Parameters	**Control**				
Time (Hours)	**0**	**1**	**10**	**25**	**44**
Temperature (°C)	9	8.7	8.7	9	8.8
Conductivity (mS cm^{-3})	0.5	0.5	0.6	0.5	0.5
Oxygen (mg L^{-1})	11.8	12.2	13	12.9	11.5
pH	7.7	7.6	7.6	7.4	7.5
1st ballast-on-board vessel					
Parameters	**Treatment**				
Time (Hours)	**0**	**1**	**10**	**25**	**44**
Temperature (°C)	14.3	14.4	14.5	15.4	16.5
Conductivity (mS cm^{-3})	914	88565	83815	84230	84196
Oxygen (mg L^{-1})	10.5	7.8	8	7.8	7.6
pH	7.8	7.6	7.6	7.6	7.6
2nd ballast-on-board vessel					
Parameters	**Treatment**				
Time (Hours)	**0**	**1**	**10**	**25**	**44**
Temperature (°C)	17.1	16.4	16.3	16.3	16.3
Conductivity (mS cm^{-3})	0.4	77	68	68	68
Oxygen (mg L^{-1})	7.5	5.2	5.9	5.8	6.1

pH	7.3	6.9	7	7	7
3rd ballast-on-board vessel					
Parameters	**Treatment**				
Time (Hours)	**0**	**1**	**10**	**25**	**44**
Temperature (°C)	9	8	8.2	8.7	8.9
Conductivity (mS cm^{-3})	1.2	76	59	58	56
Oxygen (mg L^{-1})	12.4	10	11.9		
pH	7.8	7.1	7.1		

Table 4. ANOVA with repeated measures demonstrating the effects of brine on

live zooplankton abundance. Significance levels for F-values: * (p<0.05),

**(0.1>p>0.05).

	ANOVA effects		
	F values (df)		
	Treatment	Time	Treatment*Time
Ballast-on-board experiment	335.02* (1,2)	6.69* (4,8)	3.57** (4,8)
No-ballast-on-board experiment	168.05* (1.2)	9.93* (3,6)	20.72* (3,6)

Table 5. Maximum permissible chloride values permitted in ballast water discharge by each of the eight Great Lakes states and Ontario (Gregory and Sindt, 2008; Kaltenecker and Todd, 2007; PWEA 2010; Stollenwerk, 2009).

State and Province	Chloride (mg/L)	Salinity (‰)
Illinois	500	0.91
Indiana	N/A	N/A
Michigan	250	0.45
Minnesota	N/A	N/A
New York	N/A	N/A
Ohio	N/A	N/A
Pennsylvania	250	0.45
Wisconsin	1514	2.70
Ontario	5000	9.10

Figure 1: Changes in salinity (±SE) following addition of brine into treatment tank

for the three ballasted vessels.

Figure 2: Changes in (A) mean (±SE) live abundance of zooplankton and (B) mean (±SE) proportion of total viable zooplankton in control and treatment tanks for ballasted tank experiments following brine treatment.

Figure 3: Changes in mean (±SE) salinity over time in control and treatment tanks in ballast residuals experiments.

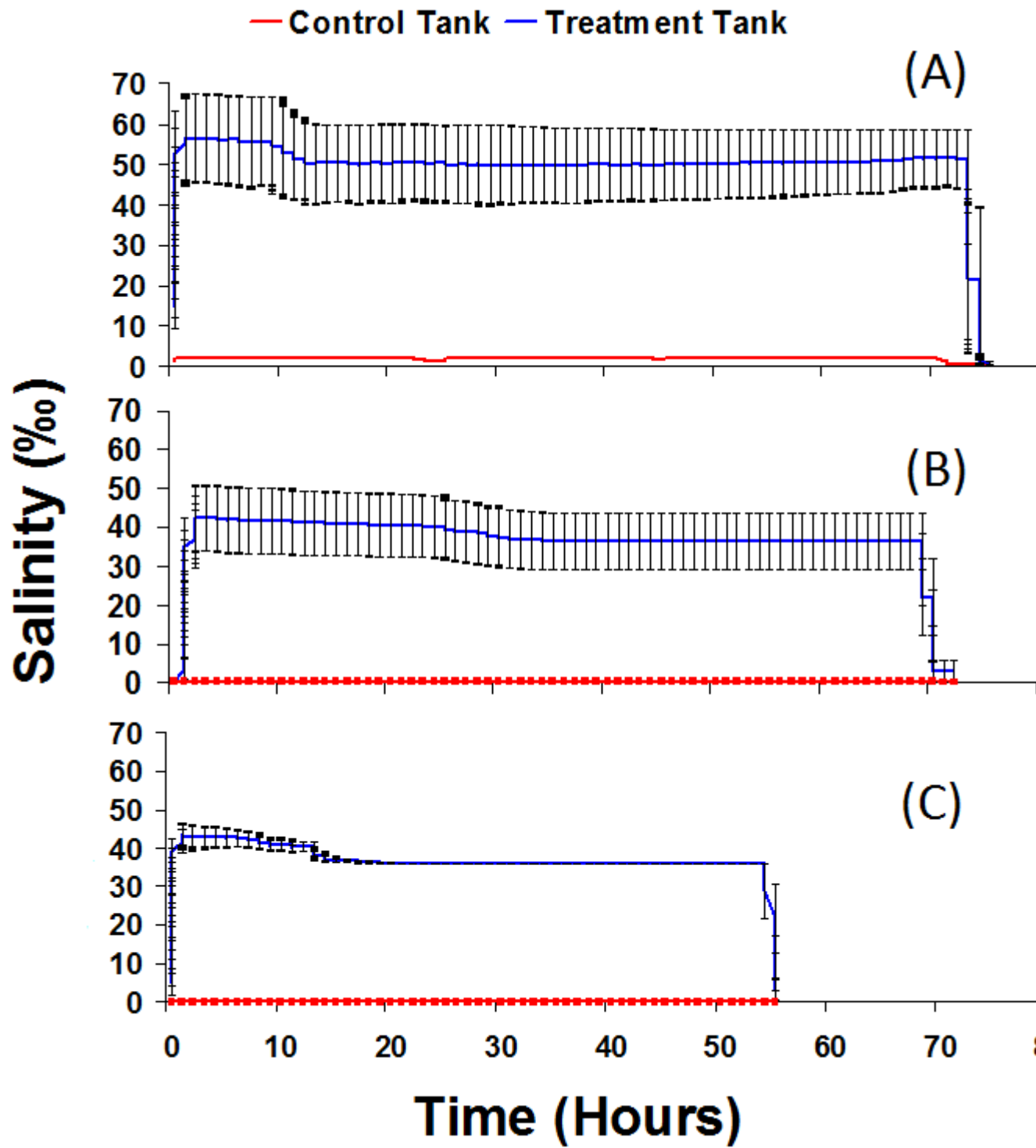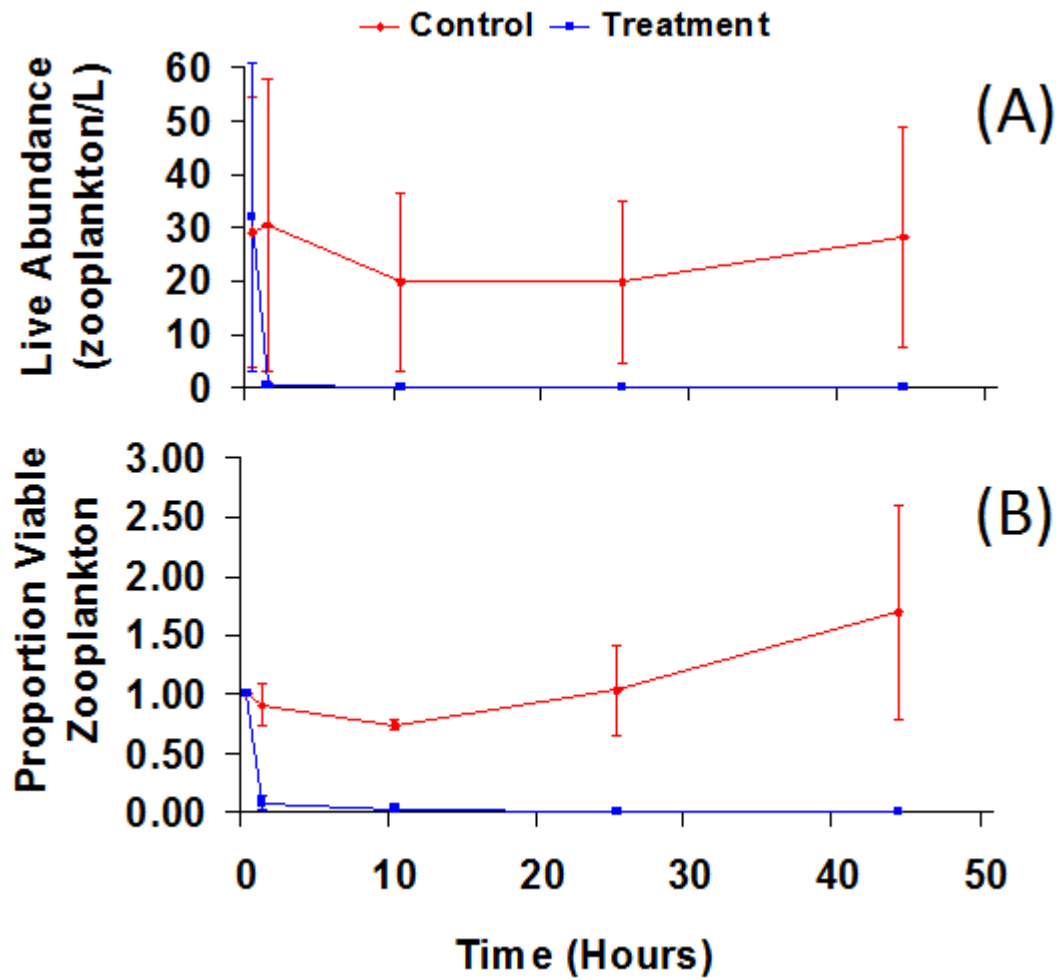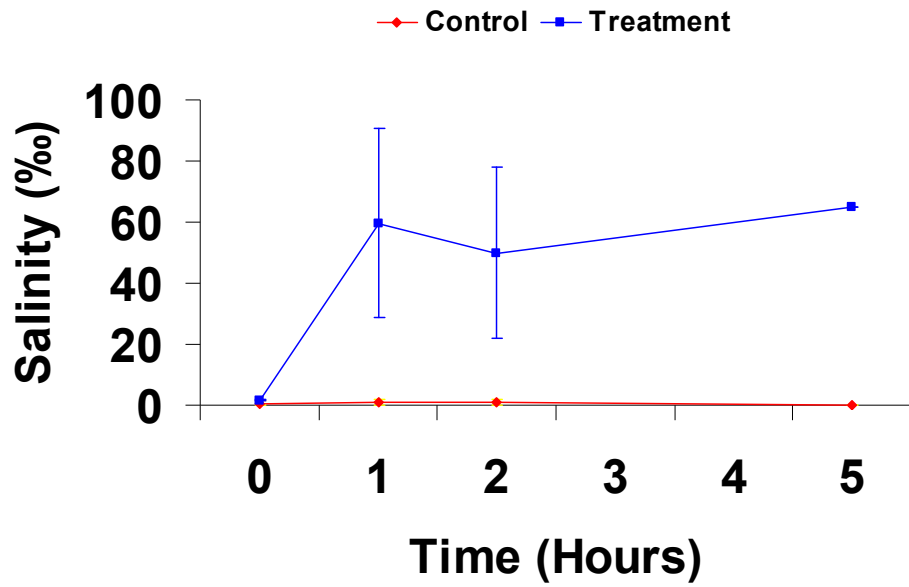**Figure 4**: Changes in (A) mean (±SE) live abundance of zooplankton and (B) mean (±SE) proportion of total viable zooplankton incontrol and treatment tanks for ballast residuals experiments following brine application.
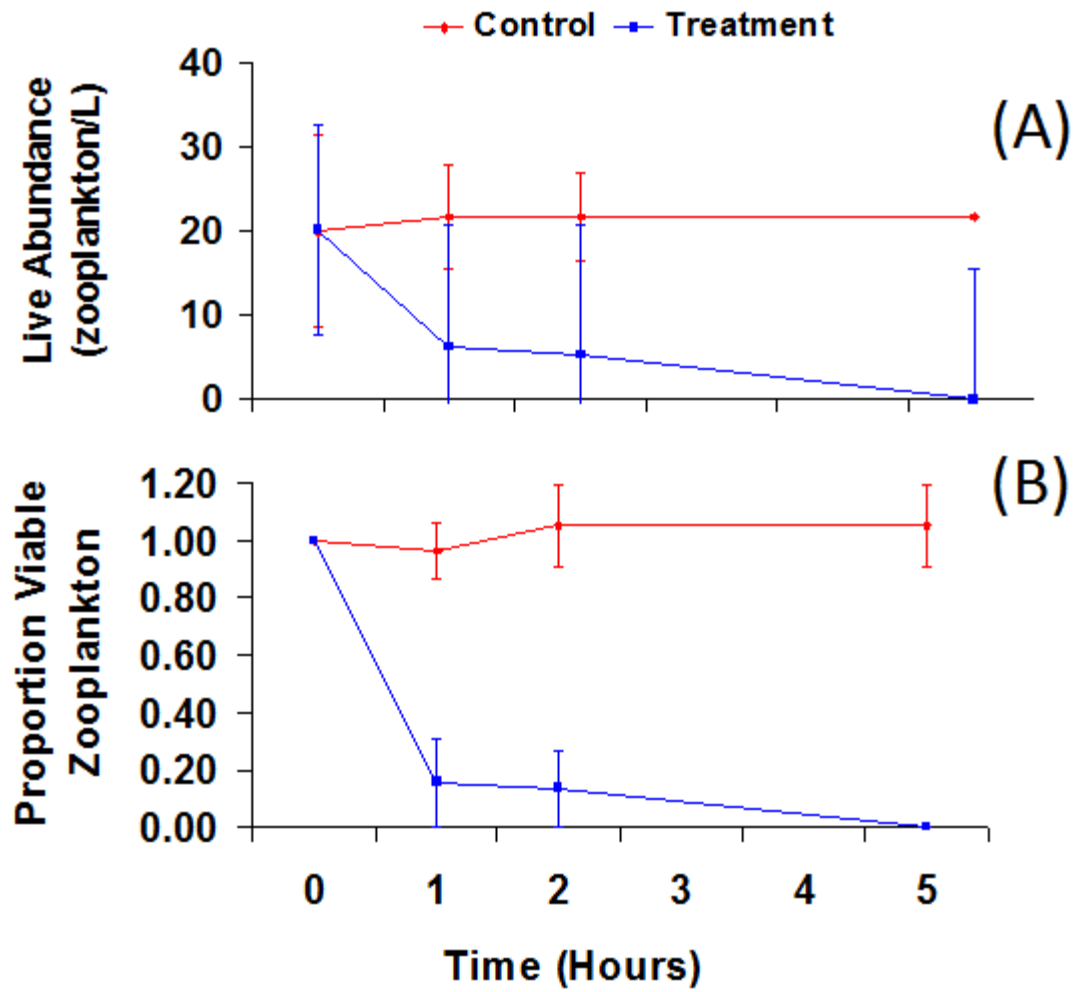
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Appendix 1. List of zooplankton species recorded from ballasted and residual

ballast experiments.

Ballasted vessel 1

Control	0	1	10	25	44
Copepoda					
Calanoid	160	680	770	710	3433
Cyclopoid	110	1040	610	650	4033
Harpacticoid	70	570	310	110	1533
Nauplii	30	230	100	150	167
Cladocera					
Daphnia	10	210	40	190	267
Bosmina	270	1230	520	630	6567
Diaphanosoma	10	40	0	0	0
Bythotrephes	0	0	0	0	0
Rotifera					
All rotifers	290	4530	2130	1970	19800
Other (Annelid, insect, mollusc)	60	70	30	50	33
Treatment					
Copepoda					
Calanoid	115	7	2	0	0
Cyclopoid	132	3	1	0	0
Harpacticoid	41	0	0	0	0
Nauplii	16	0	0	0	0
Cladocera					
Daphnia	12	0	0	0	0
Bosmina	157	5	4	0	0
Diaphanosoma	2	0	0	0	0
Bythotrephes	0	0	0	0	0
Rotifera					
All rotifers	520	16	2	0	0
Other (Annelid, insect, mollusc)	5	0	1	0	0

Ballasted vessel 2

Control	0	1	10	25	44
Copepoda					
Calanoid	20	17	11	12	17
Cyclopoid	37	41	35	26	24
Harpacticoid	19	23	13	13	17

Nauplii	6	10	4	6	5
Cladocera					
Daphnia	6	6	0	0	2
Bosmina	51	62	45	38	48
Diaphanosoma	0	0	0	0	0
Bythotrephes	15	11	12	11	11
Rotifera					
All rotifers	170	142	120	129	134
Other (Annelid, insect, mollusc)	32	27	22	21	24
<u>**Treatment**</u>					
Copepoda					
Calanoid	41	2	0	0	0
Cyclopoid	15	2	2	0	0
Harpacticoid	30	0	0	0	0
Nauplii	8	1	0	0	0
Cladocera					
Daphnia	0	0	0	0	0
Bosmina	11	3	0	0	0
Diaphanosoma	0	0	0	0	0
Bythotrephes	8	0	0	0	0
Rotifera					
All rotifers	78	3	1	0	0
Other (Annelid, insect, mollusc)	5	1	0	0	0

Ballasted vessel 3

<u>**Control**</u>	0	1	10	25	44
Copepoda					
Calanoid	26	3	7	11	22
Cyclopoid	16	4	5	15	27
Harpacticoid	3	1	3	6	13
Nauplii	4	0	1	7	28
Cladocera					
Daphnia	0	1	0	2	2
Bosmina	22	11	12	16	31
Diaphanosoma	0	0	0	0	0
Bythotrephes	4	0	0	3	4
Rotifera					
All rotifers	23	16	16	27	59
Other (Annelid, insect, mollusc)	7	2	7	11	30
<u>**Treatment**</u>					

Copepoda					
Calanoid	7	1	0	0	0
Cyclopoid	10	2	0	0	0
Harpacticoid	2	0	0	0	0
Nauplii	2	0	0	0	0
Cladocera					
Daphnia	1	0	0	0	0
Bosmina	17	2	0	0	0
Diaphanosoma	0	0	0	0	0
Bythotrephes	2	0	0	0	0
Rotifera					
All rotifers	28	6	4	0	0
Other (Annelid, insect, mollusc)	2	1	0	0	0

Residual ballast 1

<u>Control</u>	0	1	2	3
Copepoda				
Calanoid	0	1	0	1
Cyclopoid	2	1	2	2
Harpacticoid	0	0	1	1
Nauplii	2	2	3	2
Cladocera				
Daphnia	0	2	0	0
Bosmina	0	1	2	2
Diaphanosoma	0	0	0	0
Bythotrephes	0	0	0	0
Rotifera				
All rotifers	15	12	14	22
Other (Annelid, insect, mollusc)	3	3	4	4
<u>Treatment</u>				
Copepoda				
Calanoid	0	0	0	0
Cyclopoid	1	0	0	0
Harpacticoid	0	0	0	0
Nauplii	1	0	0	0
Cladocera				
Daphnia	0	0	0	0
Bosmina	0	0	0	0
Diaphanosoma	0	0	0	0
Bythotrephes	0	0	0	0
Rotifera				
All rotifers	6	0	0	0

Other (Annelid, insect, mollusc)	2	0	0	0

Residual ballast 2

Control	0	1	2	3
Copepoda				
Calanoid	9	28	46	
Cyclopoid	14	33	37	
Harpacticoid	7	17	8	
Nauplii	23	21	22	
Cladocera				
Daphnia	2	13	14	
Bosmina	12	117	114	
Diaphanosoma	2	2	1	
Bythotrephes	0	0	0	
Rotifera				
All rotifers	118	190	192	
Other (Annelid, insect, mollusc)	17	31	33	
Treatment				
Copepoda				
Calanoid	0	4	2	0
Cyclopoid	0	4	3	0
Harpacticoid	0	2	0	0
Nauplii	4	11	9	0
Cladocera				
Daphnia	0	0	0	0
Bosmina	5	22	10	0
Diaphanosoma	0	1	0	0
Bythotrephes	0	0	0	0
Rotifera				
All rotifers	31	138	91	0
Other (Annelid, insect, mollusc)	2	26	21	0

Residual ballast 3

Control	0	1	2
Copepoda			
Calanoid	4	6	4
Cyclopoid	3	12	7
Harpacticoid	0	2	2
Nauplii	10	8	9
Cladocera			
Daphnia	2	4	0

Bosmina	52	35	24
Diaphanosoma	0	0	0
Bythotrephes	0	0	0
Rotifera			
All rotifers	146	93	60
Other (Annelid, insect, mollusc)	17	12	13
Treatment			
Copepoda			
Calanoid	6	0	0
Cyclopoid	21	0	0
Harpacticoid	8	0	0
Nauplii	19	0	0
Cladocera			
Daphnia	0	0	0
Bosmina	37	0	0
Diaphanosoma	0	0	0
Bythotrephes	0	0	0
Rotifera			
All rotifers	158	0	0
Other (Annelid, insect, mollusc)	23	0	0

Vita Auctoris

Name:	Tony Nan-Chun Wang
Place of birth:	Tainan, Taiwan
Hometown:	Brampton, Ontario, Canada
Education:	North Park Secondary School, Brampton, Ontario 2000- 2004, Grade 12 Diploma, Ontario Academic Credits
	University of Guelph, Guelph, Ontario 2004-2008, Honours BSc Marine and Freshwater Biology
	Great Lakes Institute for Environmental Research, University of Windsor, Windsor, Ontario, 2008-2010, MSc Environmental Science