The Role of Osmotic Stress (Salinity Shock) in Protecting the Great Lakes from Ballast-Associated Aquatic Invaders

Technical Report

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EXECUTIVE SUMMARY

This Technical Report examines the history and basis for using ballast water exchange as a barrier against aquatic species introductions. It describes the nature of osmotic stress (or salinity shock) and its role in the application of BWE and saltwater flushing to reduce the ballast-related invasion risk to the Great Lakes ecosystem.

In the late 1980s ballast water exchange (BWE) was implemented as an interim policy aimed at protecting the Great Lakes from continued introduction of aquatic nonindigenous species attributed to ballast tank transport. Two benefits were ascribed to ballast water exchange: nearshore water and associated organisms would be flushed out and replaced by open-ocean water and organisms which are less likely to survive in the Great Lakes; freshwater or coastal organisms remaining the ballast tanks would be killed by salinity shock (see below).

In 2004 the International Maritime Organization (IMO) adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments. The Convention will enter into force 12 months after ratification, after which use of BWE will be gradually phased out and on-board treatment systems will be phased in.

In 2010 Canada submitted a proposal to IMO outlining the potential benefits of continuing to use BWE in addition to on-board treatment (BWE+Treatment) of ballast water on ships bound for fresh- and brackish-water ecosystems. The proposal is based on concern that treatment systems alone would allow discharge of a certain number of live organisms (as determined the IMO D-2 performance standard) during each ballast discharge event. Live fresh- or brackish-water organisms in the discharge, even within the IMO limits, would pose a risk to any fresh- and/or brackish-water ecosystem receiving the discharge. Conceptually, if a ship is required to conduct BWE and tank flushing, as well as treatment, the resulting treated discharge could contain the same number of live organisms, but they likely would not be freshwater species. Salinity exposure would have killed most, if not all larval and adult freshwater species.

In 2011 USEPA issued a draft 2013 Vessel General Permit that includes a requirement for ships bound for the Great Lakes to conduct BWE+Treatment if they have ballasted with water <18 ppt within a month prior to entry. In 2012 Canada confirmed its plan to require BWE+Treatment for vessels using an on-board treatment system and entering Canadian waters with ballast having a salinity of 2 psu (~2 ppt) or lower. The plan to require BWE+Treatment has been criticized because there has been no conclusive scientific verification of the enhanced protection conceptualized in the Canadian IMO proposal, and there hasn't been an assessment of practicability of this procedure.

Osmoregulation is a mechanism by which organisms maintain internal cellular stability by regulating or adapting the salt content of their body fluids to changes in the salt content of their external environment. Osmoregulation is well studied for many classes of organisms, and there is a large body of literature and a wealth of information available. Most organisms have a limited range of environmental salinity they can manage. The range of salinity over which a particular species is able to survive is its *salinity tolerance*, which is closely related to its osmoregulatory capability. The basis for using BWE as an invasive species management tool for the Great Lakes is that freshwater organisms subjected to sudden salinity increase, such as produced by mid-ocean ballast water exchange or tank flushing, experience significant life-

threatening osmotic stress resulting in high mortality. Unfortunately, some organisms are capable of surviving exposure to the full range of salinity between freshwater and ocean water, so BWE will not necessarily be 100% effective against all freshwater-tolerant organisms. The response of aquatic organisms to a sudden change in salinity depends on many factors: the salinity range they generally experience in their present habitat, the rate of salinity increase or decrease during their exposure to change, the end point (how high or low the endpoint is, compared to their normal habitat range), the amount of time they're exposed, temperature, life stage, and their osmoregulatory capability. The effectiveness of BWE and salinity exposure will depend on the nature of the organisms inhabiting the ballast tank.

Studies performed with appropriate controls to correct for non-exchange effects in ballast tanks suggest that in general, BWE can achieve 80 to >95% removal of coastal planktonic marine organisms, mostly through flushing unless the original ballast water is fresh- or low-salinity water. In the latter case, salinity shock can cause high mortality (>99%) of freshwater and low-brackish water organisms not flushed from the tank. BWE appears to be more effective at reducing or eliminating coastal planktonic invertebrates in ballast tanks than for reducing or eliminating microbial organisms. Experimental evidence suggests that when carried out according to regulations and established procedures, BWE can be highly effective at reducing invasion risk, especially for freshwater systems. However, the continued discovery of new invaders in the Great Lakes after mandatory BWE was established was interpreted by many regulators, environmental groups and policy-makers as evidence that BWE doesn't work.

After a number of years of applying BWE and still finding new invaders in the Great Lakes, it was realized that over 80% of foreign vessels trading in the Lakes were not being required to conduct BWE because their tanks were empty, that is, they contained no pumpable ballast water (no-ballast-on-board, or "NOBOB"). As documented by research during the early 2000s, such tanks contain small amounts of residual water and sediment that house live aquatic organisms and dormant eggs, so entry of NOBOB vessels into the Great Lakes was a significant gap in the protection framework intended when BWE was implemented. This was resolved by regulations in 2006 and 2008 that implemented mandatory flushing of empty tanks and strict enforcement of a salinity requirement (≥30 ppt) for water carried in ballast tanks, even residual unpumpable water. Since then several scientific studies have experimentally documented the biocidal effects of salinity, which causes osmotic shock, against a wide range of freshwater and estuarine invertebrate taxa, both in laboratory experiments and via shipboard studies.

The primary mechanism by which BWE reduces invasion risk is to flush coastal water and organisms out of ballast tanks, with varying degrees of completeness. When the original ballast is also low-salinity or fresh water, salinity shock-induced mortality of low-salinity species is an additional mechanism that can enhance BWE efficacy. Regardless of the original ballast water, the BWE process brings replacement ballast water with live saltwater organisms, which when discharged pose less risk to receiving coastal marine ecosystems, and should pose much less risk to freshwater ecosystems.

Since 2006 (through October 2012) no new aquatic invaders have been discovered in the Great Lakes. While this is good news, it must be interpreted with caution. It is clear that tank flushing closed a major loophole in the protection framework and, in combination with BWE, has contributed to a significant reduction in ballast-associated risk. Although the added mortality attributed to salinity (osmotic) shock can effectively eliminate many, if not most live freshwater taxa, it does not kill viable dormant eggs and cysts. Salinity exposure does prevent them from hatching in ballast tanks and tank flushing reduces the amount of residual sediment, where eggs and cysts accumulate. Dormant eggs and cysts can be discharged during deballasting, and with appropriate environmental cues, they can hatch into live organisms in recipient ecosystems. Their presence in ballast tank sediment limits the protection BWE and tank flushing can provide for freshwater systems like the Great Lakes. It has been observed that organisms with broad salinity tolerance and the ability to produce resting eggs dominated the composition of new invaders discovered in the Great Lakes since mandatory BWE was implemented in 1993. These are also the organisms most likely to be carried in the residual ballast transported by NOBOB vessels. Most scientists will not be surprised if new ballast-implicated invaders are discovered in the future.

A deficiency in our understanding of the efficacy of BWE and tank flushing is lack of sufficient data related to organisms other than large (>50µ) invertebrates, especially small invertebrates, phytoplankton, and microorganisms. So while BWE and tank flushing appear to be highly beneficial and protective of the Great Lakes ecosystem, vital information about the introduction rates and effects of salinity on other freshwater-tolerant organisms is lacking.

In principle, the use of BWE in combination with on-board treatment (BWE+Treatment) could enhance reduction of the risk associated with ballast water discharges to fresh- and brackish-water ecosystems. BWE would potentially eliminate freshwater-tolerant (i.e., high-risk) species from the ballast water prior to treatment, leaving less risky marine and high-brackish species in the discharge. The efficacy against high-risk species will be dependent on the salinity tolerance of the organisms. There are some, mainly estuarine, organisms that have tolerance over the full range of salinity, although their ability to survive a rapid change from low salinity to almost full-strength ocean water is another factor. Using BWE as a precursor to on-board treatment would also provide a form of insurance in case of undetected or unexpected treatment system failure, especially since these systems are very new and at present we don't have much actual operational experience with them to accurately gauge their reliability and performance.

There is ample scientific evidence to support <u>the conceptual principles</u> presented in the Canadian BWE+Treatment proposal. However, the practicability of the proposed procedure under shipboard operating conditions has not yet been evaluated. Also, the effectiveness of BWE+Treatment compared to treatment alone needs to be verified by direct experiments. At least one series of experiments using simulated exchange+treatment vs. treatment alone has been completed at a land-based test facility, but the results have not yet been published, although they are expected soon. In addition, a shipboard test program is also underway by Canadian scientists.

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1. INTRODUCTION

In 2004 the International Maritime Organization (IMO) adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments (IMO 2004). Once the Convention enters into force (12 months after ratification criteria are met), it allows continued use of ballast water exchange (BWE) only until discharge standards established by Regulation D-2 (Ballast Water Performance Standard) become effective. Regulation D-2 establishes size-based numeric discharge limits for viable organisms that BWE alone will not meet, and is scheduled to be phased in based on vessel ballast capacity and build date. In 2010 Canada submitted a proposal to IMO (IMO 2010) for ships bound for fresh- or brackish-water ports to use BWE in combination with on-board IMO-approved treatment systems designed to produce ballast water discharges meeting the IMO D-2 standard. This concept (BWE+Treatment) is based on research suggesting that osmotic stress induced mortality as a result of BWE can frequently reduce invasion risk from ballast discharge to fresh- and brackish-water ports to a level at least as protective as the D-2 standard. For freshwater recipient ecosystems, it may be possible to achieve a discharge of relevant organisms[1] even less than the D-2 standard. Use of a treatment system alone (and meeting the IMO D-2 standard) would still allow discharge of up to 9 viable high-risk freshwater organisms per m^3 (≥50μ size range) into freshwater ecosystems like the Great Lakes. Under the BWE+Treatment scenario, it is possible that most, if not all freshwater taxa would be killed prior to treatment, leaving mainly, if not exclusively low-risk marine taxa to comprise the (up to) 9 viable organisms allowed in the discharge.

The Canadian proposal is mirrored in the draft 2013 Vessel General Permit (USEPA 2011a, Section 2.2.3.7) specifically for ships entering the Great Lakes. Additionally, several Great Lakes States, including New York, Michigan, Wisconsin, and Minnesota have included BWE+Treatment requirements in their draft and/or final Clean Water Act Section 401(a) Certifications of that permit. Croot (2012) argues that there have not been scientific studies that conclusively demonstrate the benefits of BWE+Treatment. There is also criticism that the practicability of the proposed BWE+Treatment combination has not been demonstrated and the added cost to ship operations has not been evaluated[2]. A recent paper discussing Canadian plans for implementing the IMO Convention notes that in addition to minimizing risk to freshwater ecosystems, an additional benefit expected under a BWE+Treatment strategy is that BWE would provide backup in case of undetected or unexpected treatment system failure (Transport Canada 2012). Scientific studies to better quantify the effectiveness of BWE+Treatment are planned by Transport Canada (C. Wiley, Transport Canada, personal communication), and at least one independent field study was started during 2012 led by University of Windsor (H. MacIsaac, personal communication).

This Technical Report examines the history and basis for using ballast water exchange as a barrier against aquatic species introductions to coastal ecosystems. It describes the nature of osmotic stress (or salinity shock) and its role in the application of BWE and saltwater flushing to

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[1] Organisms that pose high invasion risk to freshwater ecosystems because they have a high probability of survival in freshwater habitats.

[2] As part of its 2011 economic analysis for the VGP, EPA evaluated the additional operating costs of the ballast water exchange requirement for vessels entering the Great Lakes (USEPA 2011b, Table 4-12).

reduction of ballast-related invasion risk to the Great Lakes ecosystem. It shows that there is ample scientific evidence to support the conceptual principles presented in the Canadian BWE+Treatment proposal (IMO 2010). This report does not address practicability or cost concerns, or reliability of ballast water treatment systems.

2. BACKGROUND: BALLAST WATER EXCHANGE AND SALTWATER FLUSHING

2.1 BALLAST WATER EXCHANGE

BWE to reduce the risk of aquatic species invasions to North American waters, especially the Great Lakes, is a well-known ballast management practice aimed at reducing the presence of aquatic nonindigenous species (ANS) in ballast water (MEPC 1991, USCG 1993). It was initially developed in Canada in the early-to-mid 1980s, not for management of ANS, but to protect the aquaculture industry of the Magdalen Islands from sewage-contaminated ballast water (Wiley, personal communication; SLSDC 2012). By 1988 the International Joint Commission and Great Lakes Fishery Commission had become increasingly alarmed by continuing discoveries of ANS in the Great Lakes, as illustrated by three new (at that time) aquatic invaders (ruffe, *Gymnocephalus cernua*; spiny water flea, *Bythotrephes longimanus*; and zebra mussel, *Dreissena polymorpha*). They determined the most likely source for many Great Lakes aquatic invaders was ballast water discharged from foreign vessels trading within the Lakes. In consultation with scientists, shipping experts, and U.S. and Canadian federal scientific and regulatory agencies, they proposed implementing ballast water exchange (IJC & GLFC 1990) as a means of reducing risk of continued ballast-related species introductions. In 1989 Canada published voluntary ballast water management guidelines for vessels entering the St. Lawrence River and the Great Lakes, and in 1993 the U.S. Coast Guard established mandatory ballast management requirements, including mandatory use of BWE on ships originating from beyond the EEZ and entering the Great Lakes or Hudson River (USCG 1993). Also in 1993, the first IMO guidelines concerning ships' ballast water and harmful aquatic organisms were adopted (IMO 1993), and included ballast water exchange and sediment removal at sea as one of several possible approaches.

Two benefits were ascribed to ballast water exchange (Carlton 1990, IJC & GLFC 1990, Locke *et al*. 1991, USCG 1993): nearshore water and associated organisms would be flushed out and replaced by open-ocean water and organisms which are less likely to survive in the Great Lakes; freshwater or coastal organisms remaining the ballast tanks would be killed by the saltwater (i.e., the result of salinity shock). Two main BWE processes have been used: 1) *empty-refill exchange*, in which ballast water is pumped out until the ballast tank is empty (contains only unpumpable ballast water), and then refilled with new water (mid-ocean or from an approved coastal exchange zone site), and 2) *flow-through exchange*, in which new water (mid-ocean or from an approved coastal exchange zone site) is pumped into a ballasted tank, generally at the bottom, and the tank is allowed to overflow (usually at deck level, see *Figure 1*) until at least three times the total tank volume has overflowed from the tank.

One of the earliest biological studies of ballast tanks in ships trading in the Great Lakes was conducted in 1980 by Bio-Environmental Services (1981), but BWE was not a practice at the time and therefore none of the ships had conducted BWE. Salinity of the sampled ballast water ranged from 0-36 ppt, with 65% of samples classified as marine, 31% estuarine and 4% freshwater. Over 150 distinct genera and species of phytoplankton and 56 distinct aquatic invertebrate fauna were identified in the samples, including freshwater forms.

Figure 1: Water overflowing through a deck hatch on a commercial cargo vessel during flow-through exchange. (Photo courtesy of Smithsonian Environmental Research Center and the Great Lakes NOBOB Project).

Locke *et al*. (1991, 1993) were the first to evaluate the effectiveness of BWE in eliminating freshwater-tolerant organisms on ships specifically entering the Great Lakes. Their results were based on the presence/absence of live freshwater-tolerant taxa in ballast water of ships sampled while up-bound to the Great Lakes in the St. Lawrence River or at dock in Montreal, Quebec, and they did not actually conduct exchange experiments. They calculated BWE effectiveness of 67-86% based on live freshwater taxa found in 24 vessels originally ballasted in fresh or brackish-water ports prior to mid-ocean BWE; only 14 of the 24 had achieved the BWE-required ≥30 ppt final salinity. These results were instructive, but not definitive, given the lack of controls and test protocols. However, their work was an important early attempt to evaluate the effects of exchange on ballast water coming into the Great Lakes, and in particular, they exhibited considerable foresight when they noted the frequent occurrence of ships entering the Great Lakes with unpumpable ballast water. Such ships were not subject to BWE requirements, and Locket *et al*. expressed concern that organisms carried in unpumpable residual water could be released during subsequent (ballasting and then) deballasting operations as these ships visited multiple ports within the Lakes. They recommended strategies be developed to treat ‘unpumpable’ residual water, such as flushing of such ballast tanks with saltwater (Locke *et al*. 1991, Recommendation (4)).

Numerous studies since the early 1990s attempted to measure the efficacy of ballast water exchange for flushing out coastal water and organisms. Ruiz *et al*. (2007) discussed the methodological difficulties of evaluating the effects of BWE and the limitations associated with many of the studies published through 2004. Such studies used different experimental methodologies and were conducted on ships with different ballast tank configurations; most did not address conditions specific to Great Lakes concerns, i.e., freshwater taxa survival. In addition, many studies did not control for effects unrelated to exchange, such as natural mortality in the tanks (Ruiz and Reid 2007).

Wonham *et al*. (2001) conducted empty-refill exchange experiments on saltwater ballast, with control tanks to correct for natural mortality. Natural mortality in the absence of exchange reduced live plankton densities by >98%, and diversity[3] (number of taxa) was reduced by >50% in the unexchanged control tanks over the course of the 16 day voyage. Open ocean exchange replaced an estimated 96-100% of coastal water and 80-100% of live coastal organisms, but the total population of plankton in exchanged tanks increased significantly, presumably reflecting the influx of mid-ocean organisms. Due to the relatively small change in salinity, osmotic stress/salinity shock was not a factor, and the effectiveness of exchange against encysted phytoplankton, bacteria, and very small plankton was not evaluated. They concluded "*Open-ocean exchange represents an additional selective filter in the ballast invasion pathway that reduces but does not eliminate coastal taxa.*" (Wonham *et al*. 2001, p. 10).

Ruiz *et al*. (2007) reported results from over two dozen BWE experiments with controls for non-exchange effects. Calculated exchange efficacies, based on dye measurements, ranged from 88-99% for replacement of coastal water and 80-95% for removal of coastal planktonic organisms, depending on the type of ship (*Figure 2*) and parameters measured. They found no significant differences between flow-through and empty-refill or high vs. low starting salinities.

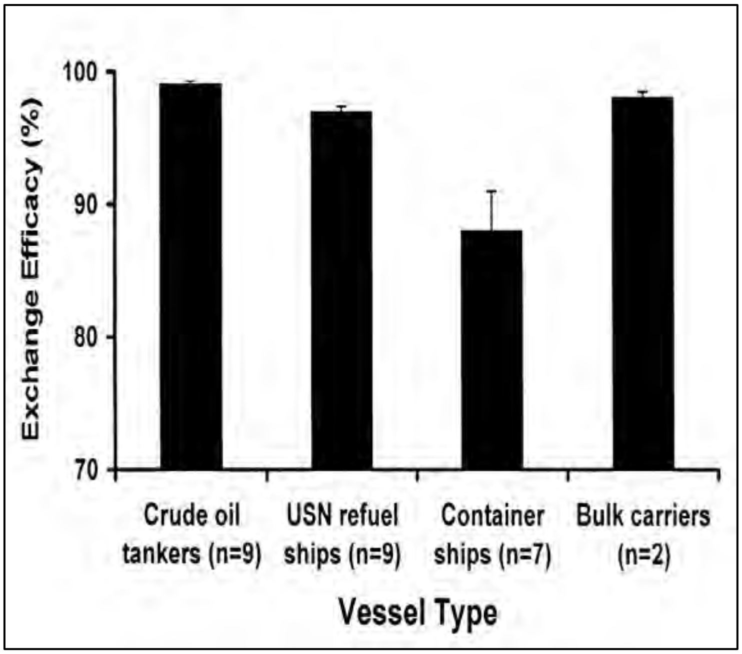

Figure 2: Efficacy of Empty-Refill BWE for removing original ballast water on different ship types determined by Ruiz et al. (2007) using Rhodamine Dye as a tracer. (Figure reproduced from Ruiz et al. 2007)

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[3] Number of taxa

Although they concluded that BWE significantly reduces total propagule supply of coastal organisms, especially for waterborne life stages, they cautioned that residual ballast and microorganisms and cysts posed an unknown and understudied risk.

Gray *et al.* (2007) conducted controlled BWE exchange experiments in ballast tanks filled with Great Lakes water. They found BWE to be >99% effective at eliminating the freshwater zooplankton in the ballast tanks. They concluded that BWE is particularly effective against freshwater organisms and can provide strong protection to freshwater ecosystems against invasions by freshwater taxa.

Klein *et al.* (2010) focused on BWE effects on diatoms, a highly abundant form of phytoplankton in ballast water. Their experiments were conducted using saltwater ballast and included unexchanged tanks as controls. They found BWE introduced new diatom species to exchanged ballast tanks and several freshwater diatom species survived BWE and were still viable at the conclusion of the voyages. While BWE effectiveness was ~87% for reducing total abundance of diatoms in the ballast tanks, they concluded that the density of viable diatoms present at discharge represented a high risk for establishment at receiving ports.

Simard *et al.* (2011) conducted exchange experiments on two cruises between Europe and North America, both of which controlled for natural mortality. Their results, which measured the flushing efficacy of the exchange process, ranged from 23-54% for zooplankton and 29-40% for microplankton when adjusted for natural mortality. Both experiments were conducted on saltwater ballast and did not involve effects of salinity shock, as the organisms contained in the water were not exposed to a significant change in salinity.

Briski *et al.* (2012) found live macroinvertebrates[4] in ~10% of ballast tanks sampled on ships arriving to the Atlantic coast of Canada, including tanks that had undergone BWE. Most were marine species and some are known to be highly invasive. One live crab specimen (*Rhithropanopeus harrisii*) was a gravid[5] female. This species has been reported in freshwater reservoirs in Texas, suggesting it could pose an invasion risk to the Great Lakes if introduced. BWE did not affect macroinvertebrate occurrence, although more than half of those found were in ballast tanks of coastal voyages NOT required to use BWE, and the majority of discoveries were found in ships after short voyages (<7 days).

Many studies of BWE efficacy have targeted invertebrates, which are common invaders in many aquatic ecosystems. Ruiz *et al.* (2000) pointed out that the biology of many microorganisms may facilitate their invasion potential: high capacity for asexual reproduction, ability to form dormant resting stages, and broad environmental tolerances, such as salinity and temperature. Since 2000 several studies have examined microorganisms (bacterial and/or virus-like-particles) in ballast water and/or attempted to evaluate the effects of BWE on microbial communities.

Ruiz *et al.* (2000), Drake *et al.* (2001, 2007) and Sun *et al.* (2010) measured microorganism concentrations in ballast water but did not conduct BWE efficacy tests. Ruiz *et al.* (2000) was one of the first studies of microorganisms (bacteria, virus-like-particles and *Vibrio cholerae*

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[4] Macroinvertebrates are invertebrates that can be seen with the naked eye.

[5] Carrying eggs

bacteria O1 and O139) in ballast water and found concentrations were 6-8 orders of magnitude greater than population densities of other taxonomic groups reported in ballast water. Drake *et al*. (2001) broadly characterized microorganism communities (bacteria and virus-like-particles) in ballast water of ships arriving to Chesapeake Bay. They found considerable variation among vessels, but mean abundances of bacteria and viruses were less than typically found in Chesapeake Bay water and similar to densities reported for open-ocean surface waters. There were some data in common to both Ruiz *et al*. (2000) and Drake (2001) (same ship and tank samples) and neither study differentiated results from exchanged vs. unexchanged tanks.

Drake *et al*. (2007) sampled both exchanged and unexchanged ballast tanks in ships arriving to Chesapeake Bay over a 7 y period and found no significant differences in mean microorganism (bacteria or virus-like-particle) concentrations between exchanged and unexchanged tanks. They did, however, subsample different in-tank habitats - water, water and sediment residuals, and biofilm - and found concentrations of microorganisms varied over 1000-fold among these habitats, with ballast water >> sediment and water residuals >> biofilms[6].

Similarly, Sun *et al*. (2010) did not conduct actual BWE experiments, but reported bacterial abundances from ballast water of ships arriving to Vancouver, British Columbia. Sampled water included a mix of mid-ocean exchanged, intracoastal exchanged and intracoastal unexchanged ballast. Bacterial abundances were significantly higher in Vancouver port water than in any of the ballast water samples, but this should not be a surprise. It is not unusual for water in coastal ports, where ballast water is most often loaded, to contain bacterial pollutants from local activities, such as sewage and agricultural run-off. Also not surprising, unexchanged coastal ballast water had significantly higher bacterial abundance compared to mid-ocean exchanged ballast water. No microbial community composition data were obtained.

Drake *et al*. (2002) conducted microbial experiments using two exchange and two control (no exchange) ballast water holds. They observed microbial constituents (bacteria and virus-like-particles) decreased throughout the voyage, but found no significant differences in microbial concentrations between exchanged and unexchanged holds in their final samples, taken 5 days after exchange was completed. They measured microorganism cell abundance and biomass, but not community composition. Since naturally occurring bacterial communities have distinct estuarine, coastal, and oceanic populations (Seiden *et al*. 2011), the microorganism community composition in a ballast tank could change during BWE as coastal water is replaced by mid-ocean water, but this wouldn't necessarily be reflected in abundance.

Quilez-Badia *et al*. (2007) also conducted mid-ocean exchange experiments using paired exchanged tanks and unexchanged (control) tanks. Total bacterial concentrations generally decreased over time in both control tanks and exchanged tanks, but total bacterial abundances in the exchanged tanks decreased significantly right after exchange and then became constant thereafter. An incubation effect was observed in a few tanks (control and exchange), as revealed by bacterial abundances increasing over time.

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[6] A biofilm is a complex microbial community adhering to surfaces that are regularly in contact with water, consisting of colonies of bacteria and usually other microorganisms such as yeasts, fungi, and protozoa that secrete a mucilaginous protective coating in which they are encased (www.dictionary.com).

Seiden *et al*. (2011) conducted similar experiments, using two ballast tanks for mid-ocean exchange experiments paired with two unexchanged ballast tanks as controls. They found mid-ocean exchange did not significantly reduce bacterial abundances and final abundances in the exchanged and unexchanged tanks were not significantly different at the end of voyages. Further, ballast water had significantly higher abundances than receiving port waters.

BWE appears to be more effective at reducing or eliminating coastal planktonic invertebrates in ballast tanks than for reducing or eliminating microbial organisms, although the effects of BWE on the latter have only been characterized by changes in abundance. Changes in microbial community composition may occur during BWE due to differences between microbial communities typically found in open-ocean surface water vs. coastal and port water.

While BWE can be effective at removing coastal water and organisms, it is not a perfect filter. Results of BWE efficacy studies have been quite varied, and although some of the variance can be attributed to differences in methodology and/or differences in tank architecture, efficacy is also affected by various other factors, such as ship type, adherence to procedural guidelines, and the environmental conditions ballast organisms encounter during the voyage. Significant natural mortality of ballast tank organisms prior to or without having conducted BWE has been observed.

The primary mechanism by which BWE reduces invasion risk is to flush coastal water and organisms out of ballast tanks, with varying degrees of completeness. When the original ballast is also low-salinity or fresh water, salinity shock-induced mortality of low-salinity species is an additional mechanism that can enhance BWE efficacy. Regardless of the original ballast water, the BWE process brings replacement ballast water with live saltwater organisms, which when discharged pose less risk to receiving coastal marine ecosystems, and should pose much less risk to freshwater ecosystems.

2.2 SALTWATER FLUSHING – THE NOBOB PROBLEM

Continued discoveries of new ANS in the Great Lakes after mandatory BWE regulations were implemented in 1993 (see GLANSIS 2012) raised doubts that BWE is effective in protecting the Lakes from aquatic invaders, although the effect of salinity exposure on organisms most likely to survive and establish in the Great Lakes was expected to be helpful, if not significant (Carlton 1990; Locke *et al*. 1991, 1993). The scientific and regulatory communities soon realized that BWE requirements did not apply to vessels entering the Great Lakes with empty (no pumpable ballast water) ballast tanks, even though such tanks were known to contain small amounts of residual ballast water and sediment. Reeves (1997) revisited the problem of unpumpable ballast water raised by Locke *et al*. (1991, 1993), referring to vessels that reported "no ballast on board" as "NOBOBs". He stated "*The problem with the NOBOBs, representing between 75 and 95 percent of the vessels entering the system, is that some 40 percent of those vessels engage in a cross-transfer of ballast inside the Great Lakes…..This is probably the most serious limitation of our current regulatory regime.*" (Reeves 1997, p. 291-292)

Ricciardi (2006) calculated that from 1960 (the first year the Saint Lawrence Seaway operated over a full shipping season) through 2003 the rate of new ANS reported in the Great Lakes was 1.8 per year, an average of one new species reported every 28 weeks (~7 months), whereas the rate from 1840 through 2003 was only 1.1 new species per year (an average of one reported every 11 months). This was similar to findings by Holeck *et al*. (2004) that the rate of new ANS reported in the Great Lakes and attributed to ballast water more than doubled after the implementation of ballast water controls (comparing 1959 to 1988 vs. 1989 to 2000), although they acknowledged a number of potential drivers not related to ballast water that might explain the apparent increase. Ricciardi (2006) also noted that euryhaline benthic[7] organisms that can produce a resting stage[8] dominated the composition of new invaders reported since mandatory BWE was implemented in 1993. These species generally have broad salinity tolerance and thus may survive exposure to salinity over 30 ppt resulting from BWE. The presence of such species limits the protection BWE can provide for freshwater systems like the Great Lakes. These are also the organisms most likely to be carried in the residual ballast in NOBOB vessels. Since BWE requirements still did not apply to NOBOBs during the period covered by his study, Ricciardi (2006) mirrored the concerns expressed by Locke *et al*. (1991, 1993): "*NOBOB ships may represent an active vector that plays a role in introducing benthic organisms, especially those with resting stages*" and "*the effectiveness of BWE (note added by author: referring to BWE regulations) has been undermined by the increasing proportion of inbound foreign vessels that are not subject to regulation*" (Ricciardi 2006, p. 426).

Johengen *et al*. (2005) investigated the characteristics and biology of residual water and sediments in NOBOB ballast tanks. They documented significant numbers of live taxa, as well as viable resting stages, including marine and some freshwater species not native to the Great Lakes. Numerous NOBOBs trading into the Lakes were found to repeatedly ballast in low-salinity or fresh water ports in Northern Europe (as well as within the Great Lakes Basin), but were not subject to BWE regulations. As a result of this study, and early results from a follow-on study (Reid *et al*. 2007), in 2005 the U.S. Coast Guard established a best management practice policy stating that vessels operating outside the Great Lakes should conduct saltwater flushing of their empty tanks, as well as mandatory BWE on their ballasted tanks, before entering the Saint Lawrence Seaway (USCG 2005). This was not proposed as a regulation because in the U.S. federal policy can be implemented relatively quickly while regulations can take years for approval. Canada quickly followed with regulations making ballast water management mandatory for all ships entering Canadian waters (Transport Canada 2006). Management options provided for in the regulations included mid-ocean BWE, retention of ballast water onboard, transfer of unexchanged ballast water to a reception facility, or use of an approved ballast water treatment technology. Ships bound for the Great Lakes and choosing to use BWE for ballast water management were also required to flush their empty (no pumpable water) tanks. In 2008 the Saint Lawrence Seaway Development Corporation harmonized its regulations with the new Canadian regulations, requiring all ocean-going ships originating from beyond the EEZ to undertake saltwater flushing (SLSDC 2008). The U.S. EPA established a national regulation for saltwater flushing in their 2008 Vessel General Permit (USEPA 2008).

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[7] These are animals and plants that live on or in the bottom of an ocean or lake (benthic) and are tolerant of a wide range of salinity (euryhaline).

[8] "Resting stage" or "diapausing stage" refers to a reproductive body (embryo, egg, spore, cyst) in a period of dormancy – a strategy to help the organism survive unfavorable or extreme environmental conditions (e.g., temperature extreme, desiccation, even digestion); resting stages can become active and produce juveniles of a species when conditions become more favorable.

A joint *Ballast Water Working Group* (BWWG) was created in 2006, consisting of representatives of the U.S. Coast Guard, Transport Canada-Marine Safety, Saint Lawrence Seaway Development Corporation (United States) and the St. Lawrence Seaway Management Corporation (Canada). The BWWG coordinates enforcement of ballast water management rules for the Great Lakes. It established a Joint Ballast Water Management Exam Program to conduct detailed inspections of foreign vessels entering the Great Lakes from outside the EEZ. Inspections included detailed review of ballast water reports, logs, records, and ballast water management plans. Inspectors interview crew to assess their understanding of the vessel's Ballast Water Management Plan and operational procedures. Ballast tanks are sampled for salinity or the presence of mud that would suggest a satisfactory management practice was not employed. Initially, detailed inspections were focused on a vessel's first voyage into the Lakes, but in 2008 the policy expanded to examine 100% of ballast tanks on all vessels on all transits regardless of prior entries. Since 2007, 94-99% (*Table 1*; BWWG 2008, 2009, 2010, 2011, 2012) of ballast tanks were found in compliance with salinity requirements. Ships with ballast water not in salinity compliance are issued a Letter of Retention which states that the water may not be discharged in the Great Lakes Seaway system. When the vessel departs the system, the Letter is rescinded after retention of the water is confirmed. Thus, starting in late 2006, the discharge of unmanaged low-salinity ballast water was essentially eliminated by strong regulations and strict enforcement.

Table 1: Results of Ballast Tank Enforcement Inspections by Agencies in the Great Lakes Seaway Ballast Water Working Group (BWWG 2008-2012).

Tanks in Compliance with Salinity Requirement	
2007	96%
2008	99%
2009	98%
2010	94%
2011	97%

2.3 THE DISCOVERY RATE RECORD

Bailey *et al*. (2011) evaluated the annualized discovery rate of new ANS in the Great Lakes attributed to shipping (*Figure 3*, after Bailey *et al*. 2011 Fig. 4(b).) and found a general increase in the mid-1980s, but then a rapid decrease starting in the mid-to-late 1990s, more or less coincident with U.S. implementation of mandatory BWE. In addition, since 2006 there have been no new ballast-associated ANS reported. The last time a gap of more than five years between new species discoveries occurred in the Great Lakes was from 1952-1958, just before the Seaway opened (GLANSIS 2012).

The observation that no new species have been reported since 2006 is coincident with two significant policy changes: 1) inclusion of NOBOBs and most coastal traders in mandatory ballast water exchange/flushing and 2) strict enforcement of the salinity standard, with monitoring of all tanks for evidence of sediment build-up and to exclude discharge of low-salinity ballast water. The latter ensured that organisms in ships bound for the Great Lakes will be exposed to sudden high physiologic stress, whether they're in pumpable or residual ballast water, or accumulated sediments. Exposure of freshwater ballast organisms to high salinity via BWE, and exposure of mid-ocean saltwater organisms to sudden low salinity when discharged into the freshwater of the Great Lakes induces "salinity" or "osmotic" shock, a potentially deadly form of physiologic stress.

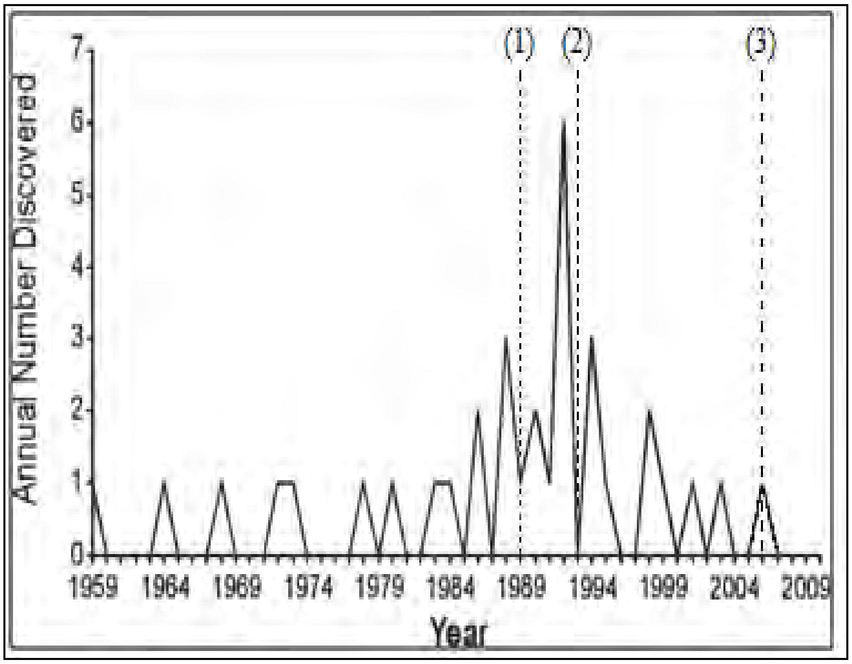

Figure 3: Annualized number of ship-related ANS discovered in the Great Lakes from 1959 through 2010, inclusive. Dashed lines mark (1) voluntary BWE (1989, Canada), (2) mandatory BWE (1993, U.S.) and (3) mandatory BWE and flushing of all tanks (2006, Canada). (After Bailey et al. 2011, reprinted with permission. Copyright 2011, American Chemical Society).

The observed discovery rate record <u>may</u> reflect the protective effects of both the original implementation of BWE in 1993 and the NOBOB policy implementations of 2006. Interpretation of *Figure 3* must take into account that scientific interest in invasion ecology grew dramatically starting in the late 1980s (Ricciardi and MacIsaac 2008). Increased scientific interest led to increased awareness and more activities to look for aquatic invaders in the Great Lakes, and those efforts likely contributed to some of the increase in discovery rate seen between 1984 and 1992. Also, Sturtevant *et al*. (2007) calculated that economic changes during the 1980s led to a decline in both the number of vessels carrying pumpable ballast water (ships could no longer afford to carry partial or full loads of ballast water and little or no cargo) and in the annual number of foreign ships trading in the Great Lakes during the period 1994 to 2004 (compared to 1978-1988). These changes translated into a potential reduction of up to 76% in average annual ballast water volume carried into the Great Lakes when comparing these same periods. A reduction in ballast water volume would produce a lower risk of new species introductions and thus could have been a contributing factor in the decline of ship-related ANS discoveries after 1994 (*Figure 3*).

Another complication with interpreting the discovery record of ANS is that once a species successfully invades an ecosystem, it may not immediately be discovered, and the length of time it takes to be discovered (lag time) can depend on how big it is, how rapidly its population increases, how it reproduces, what habitat it occupies, and how much effort is made to look for that type of organism. The length of time a newly discovered invader has actually been in an ecosystem is often not known with certainty. For example, three nonindigenous testate amoebae were found in Great Lakes beach sands in 2002 (Nicholls and MacIsaac 2004). Even though two of these species were found at multiple locations in several of the Great Lakes, they had not been previously reported anywhere in the Great Lakes basin. While it is highly probable that these species were introduced via ballast water, there is no way to determine when they were first established. *Echinogammarus ischnus* was first reported in the Laurentian Great Lakes from samples collected in 1995 (Witt *et al.* 1997). Archived samples later revealed *E. Ischnus* was present in western Lake Erie in 1994, and based on size distribution of the 1994 population, it was likely present at least as early as 1993 (van Overdijk *et al.* 2003). Similarly, viral hemorrhagic septicemia (VHS) was first identified in Great Lakes fish collected from the Bay of Quinte, Lake Ontario, Canada in 2005 (USDA 2006), but subsequent examination of archived samples suggest it has been present since at least 1999 (GLANSIS 2012). Some benthic invertebrates, such as those noted by Ricciardi (2006) as dominating the composition of new Great Lakes invaders discovered since 1993, may be quite small, making immediate discovery difficult and resulting in potentially large lag times before they are discovered; some reproduce asexually, producing resting eggs that can sit dormant in sediment for decades (Hairston *et al.* 1995) until appropriate environmental cues stimulate them to hatch.

According to the discovery record, new shipping-related invasions dropped to zero immediately after implementation of the Canadian tank flushing regulations (Transport Canada 2006), yet it took several years to achieve full enforcement of these regulations (*Table 1*). It is not likely that the 2006 regulations alone produced such an immediate result; it is more likely that the discovery rate reflects cumulative effects over time of the entire ballast water management framework and the increasingly strict enforcement of regulations. The discovery rate record must be interpreted with caution. It may be entirely coincidental that in the Great Lakes, the discovery rate of new invaders dropped dramatically around the same time mandatory BWE was originally implemented and has been zero since tank flushing was also made mandatory.

Bailey *et al.* (2011) assessed the efficacy of ballast water policies enacted for the Great Lakes and based on four criteria concluded that "*risk of ship-mediated aquatic NIS introductions has been markedly reduced*" (Bailey *et al.* 2011, p. 2559) since ballast management regulations and strict enforcement were implemented. It is clear that tank flushing closed a major loophole in the protection framework and has contributed to a reduction in ballast-associated risk. Even though the recent discovery rate has been zero, and all ballast water being discharged from foreign sources is saline, the future invasion risk is not zero. It will not be too surprising if new ballast-implicated invaders are discovered in the future.

3. THE BENEFITS AND LIMITATIONS OF OSMOTIC (SALINITY) SHOCK

In developing and evaluating the effectiveness of BWE for protecting the Great Lakes from species invasions, osmotic or physiologic stress (salinity shock) has been acknowledged as an important factor. For example, Carlton (1990) summarized fifteen alternative ballast water preventive options. In the case of freshwater ballast the option he outlined was to pump-in seawater, for which he stated "*This treatment presumes that sufficient addition of saltwater to the freshwater would lead to the mortality of the freshwater organisms (via disruption of physiological, osmoregulatory processes).*" (Carlton 1990, p. 7). Canada's proposal (IMO 2010) stated that ballast water exchange is particularly effective in protecting brackish and freshwater habitats because it imposes an environmental salinity barrier: "*Any fresh water or brackish coastal taxa entrained in ballast tanks that are not purged from tanks by ballast water exchange will be subjected to osmotic stress when euhaline ocean water is used to replace coastal ballast water.*" (IMO 2010, p. 2). In this section concepts of osmoregulation and osmotic stress are outlined.

(Note: The following sections (3.1 and part of 3.2) are based on general information and discussions about physiology widely available in many references. For this document, four main references were used: Wilson 1979, Randall *et al*. 2001, Marshall and Grosell 2006**,** and Hickman *et al*. 2007, unless otherwise noted).

3.1 OSMOREGULATION

French physiologist Clause Bernard (1813-1878) proposed that all living organisms exist in a "*milieu intérieur*" that ensures a stable internal environment for their metabolic functions and cellular infrastructure. In the early 20th century Harvard physiologist W.B. Cannon gave a name to the tendency of organisms to regulate their internal fluid composition and maintain this stability: *homeostasis*.

Cells are the fundamental structural units of living organisms. All cells have a *cell membrane* (aka plasma membrane), which defines the cell. Some cells also have a *cell wall* that surrounds the cell membrane. Cell walls are stronger than cell membranes and provide structural support to protect the cell against excessive swelling when water diffuses into the cell. Cell walls are found in plants, bacteria, fungi, algae, and some single cell microorganisms. Both the cell membrane and cell wall help maintain homeostasis. Two types of processes challenge homeostasis: 1) metabolic processes within cells that involve materials essential for survival (e.g., oxygen, salts, water, nutrients) and which generate waste products that must be removed; and 2) external changes in the environment surrounding the organism. For aquatic organisms, both temperature and salinity of the surrounding environment are of critical importance and can pose great challenges to organism survival, as well as place limits to their geographic distribution. However, the following discussion is limited to the effects of salinity.

3.2 OSMOSIS, OSMOCONFORMERS AND OSMOREGULATORS

Water diffuses across a semipermeable membrane in the direction of lower water concentration by a process called *osmosis*. In aqueous salt solutions, the concentration of water decreases as the concentration of dissolved salt increases. Two solutions with different salinity will have different *osmolality*[9], and if separated by a semipermeable membrane, osmosis causes water to move from the lower salinity (greater concentration of water) to the higher salinity (lower concentration of water) solution. Cells are selectively permeable (semipermeable) to water, and are thus subject to osmosis, but limit or exclude passage of other molecules. Osmosis across a cell membrane and/or cell wall in a living organism is accompanied by a net gain or loss of water molecules inside the cell until flow of water molecules in both directions is in equilibrium. At *osmotic equilibrium* no further net gain or loss or water occurs. Essential ions, other compounds, and waste products can be transported across cell surfaces by various biochemical/physiological transport mechanisms that vary by species.

The force that moves water in response to different solute concentrations across a semipermeable membrane is called *osmotic pressure*. It is approximately equivalent to the hydrostatic pressure needed to achieve osmotic equilibrium between two solutions of different osmolality. J.H. van't Hoff, first Nobel laureate in Chemistry, described osmotic pressure in his 1901 Nobel lecture: "*What is osmotic pressure? When a solution, e.g. of sugar in water, is separated from the pure solvent - in this case water - by a membrane which allows water but not sugar to pass through it, then water forces its way through the membrane into the solution. This process naturally results in greater pressure on that side of the membrane to which the water is penetrating, i.e. to the solution side. This pressure is osmotic pressure.*" (van't Hoff 1901, p. 5). Osmotic pressure is hydrostatic pressure linked to difference in the concentration of solutes between solutions separated by a semipermeable membrane, such as the inside and outside of a cell. It is solute driven. This can be contrasted with *turgor pressure*, which is pressure exerted on a cell wall from inside by an excess of water within the cell. Turgor pressure is directly linked to water accumulation caused by osmosis. The term "turgor pressure" applies to plants, bacteria, and fungi cells and some protists because they have cell walls. Extreme internal osmotic pressure and uncontrolled accumulation of water in a cell can result in swelling (excessive turgor) of the cell and rupture the cell wall and membrane, causing death. Alternately, uncontrolled diffusion of water out of a cell by osmosis can lead to desiccation of the cell fluids, loss of turgor pressure, and collapse of the cell membrane, also causing death.

In aquatic ecosystems, when an organism is faced with changes in the salinity of its environment, it either avoids the change, it adapts physiologically, or it dies. Some species have little or no physiological ability to regulate their internal osmotic variables (water and solute concentration), so osmotic concentration in their body fluids surrounding and/or within their cells changes to conform to external salinity. These are referred to as "*osmoconformers*" and the internal osmotic concentration in their body fluids will be approximately equal to (isotonic with) that of the surrounding water. Marine animals may be osmoconformers, but not freshwater animals, because the molality of freshwater is much too low to sustain cellular processes. Osmoconformers have no defense against externally-driven significant osmotic changes. Some species are able to employ various mechanisms to regulate their body fluid at constant or nearly constant osmotic values different from the surrounding water. These species are called "*osmoregulators*" and the processes and mechanisms by which they do so are collectively called *osmoregulation*. Osmoregulation is a means for these organisms to maintain internal homeo-

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[9] The terms "osmolarity" and "osmolality" are used to describe the relative osmotic strength of a solution, which depends on the amount solute (e./g., salt) per liter or kg of solution.

stasis, which includes managing intake and output of water as well as maintaining an acceptable solute concentration. Some osmoregulators maintain their internal osmotic concentration above that of the surrounding water (*hyperosmotic regulator*) and some maintain it below the surrounding water (*hypoosmotic regulator*). For example, all freshwater animals are hyperosmotic regulators. Teleosts (bony, ray-finned fishes, which are the predominate fish in ocean and freshwater ecosystems) maintain the salt concentration of their body fluids at about 1/3 that of seawater (Karnaky 1999), yet are found widely distributed from freshwater to saltwater. Freshwater teleosts are hyperosmotic regulators - they maintain the osmolality of their body fluids well above the osmotic concentration of freshwater. In these fish, osmosis produces a constant influx of water and diffusive loss of important ions, for which their osmoregulatory system compensates by eliminating excess water through generation of large volumes of very dilute urine and reabsorbing electrolytes from the surrounding water through the gills. In contrast, seawater teleosts are hypoosmotic regulators, since the osmotic concentration of their body fluid is lower than that in seawater. These fish suffer constant osmotic loss of water across their gills, for which they compensate by drinking large volumes of seawater and producing only small amounts of urine. Drinking seawater brings an excess of salt ions, which are eliminated through the gills and skin.

The detailed cellular-level physiological and biochemical mechanisms by which various types of aquatic organisms respond to and control osmotic challenges are quite varied and complex, and are the subject of a large body of research and literature well beyond the scope of this discussion.

3.3 SALINITY TOLERANCE (PHYSIOLOGICAL STRESS, OSMOTIC SHOCK, SALINITY SHOCK)

All aquatic organisms have limitations in their ability to survive changes in salinity. The range of salinity over which a particular species is able to survive is its *salinity tolerance*, which is closely related to its osmoregulatory capability. Organisms in water outside their salinity tolerance range can lose their ability to maintain homeostasis, resulting in physiological stress ("osmotic shock" or "salinity shock"), diminished or disrupted metabolic functions (for example, ionic regulation, respiration rate, nutrient intake, and oxygen requirements), and potential death. The outcome of osmotic stress depends on the suddenness and severity of the change, the age or life stage, temperature, and possibly other abiotic factors. Aquatic organisms with narrow salinity tolerance are called *stenohaline* and have little ability to maintain homeostasis outside a narrow salinity range. Alternately, *euryhaline organisms* have developed osmoregulatory mechanisms that allow them to survive in areas with broad and sometimes rapidly changing salinity ranges, such as estuaries. Stenohaline and euryhaline organisms can be osmoconformers or osmoregulators and some appear to transition from one to the other depending on salinity. The European shore crab (*Carcinus maenas*) is an osmoregulator in the lower end of its salinity tolerance range, but as salinity increases above ~22 ppt the osmotic concentration of its body fluid increases and it appears to become an osmoconformer.

A large body of research exists about salinity tolerance, with a wealth of detailed field and laboratory results on salinity tolerances for many aquatic species. A survey of relevant literature reveals that salinity tolerance within different groups of aquatic organisms (e.g., marine, estuarine, and freshwater fishes, invertebrates, phytoplankton, and microorganisms) can be highly variable.

Brand (1984) tested the salinity tolerance (based on reproductive/growth rate) of 46 species of coastal, estuarine, and oceanic phytoplankton. He found wide salinity tolerance ranges for many of the species, although the salinity tolerance of the estuarine and oceanic species was generally reflective of the salinity in their source habitats. Most oceanic species were unable to reproduce below 25 ppt, although a few could still reproduce at 15 ppt. All were killed by 5 ppt and 0 ppt treatments. Estuarine species were all able to reproduce in salinity as low as 5 ppt and as high as 45ppt, and four were also able to reproduce in 0 ppt water. One of these species, *Thalassiosira pseudonana*, was described as "*extremely euryhaline*" and is often found in lakes and rivers. Coastal species exhibited reduced growth at 45 ppt and were capable of growth in salinity as low as 15 ppt; most, but not all, died in 5 ppt water and all were killed by 0 ppt exposure.

Most stenohaline marine teleosts can live in full-strength seawater and also survive in salinities as low as ~12 ppt, which approximates the osmotic concentration of their body fluids. Stenohaline freshwater teleosts can survive in fresh- and brackish-water up to salinity ~12 ppt. Euryhaline marine and euryhaline freshwater teleosts have broad salinity tolerances and can survive the full range of aquatic environments from freshwater to seawater (and also hypersaline conditions), although the rate at which salinity changes can be important. Marshall *et al*. (1999) reported freshwater teleosts with euryhaline capability (e.g., tilapia) readily adapt to increasing salinity if the change is gradual and occurs over days. They also state "*Unlike anadromous fishes that change salinities a few times during their life cycle (and generally do not survive direct transfers from fresh water to full-strength sea water), estuarine-resident teleosts such as killifish must readily adapt to high and low salinity extremes and are capable of surviving direct transfer from fresh water to full-strength sea water.*" (Marshall *et al*. 1999, p. 1536). Some euryhaline organisms are adapted to the low end of the salinity range (low-brackish to freshwater), while some are adapted to the upper end (high brackish to full-strength seawater), and some are widely euryhaline and can adapt across the full salinity spectrum typical of most

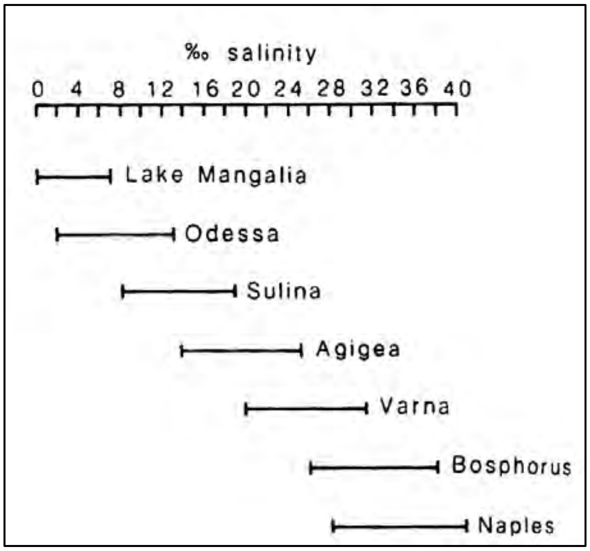

Figure 4: Salinity tolerance of different populations of the European rock shrimp (Palaemon elegans) from various locations in the Mediterranean and Black Seas (Barnes 1989; reproduced by permission of The Royal Society of Edinburgh and R.S.K. Barnes).

aquatic ecosystems. BWE, whether empty-refill or flow-through exposes fresh or low-salinity species in ballast tanks to a rapid increase in salinity to almost full-strength seawater, which should produce the most stress and result in greatest mortality to stenohaline and low-euryhaline species.

Populations of the same species in geographically separated aquatic environments and different species of the same genus can exhibit widely different salinity tolerances (*Figure 4*, *Table 2*). However, there is general confirmation in the literature that salinity tolerance of many aquatic organisms reflects to a great degree the salinity range of their existing habitat.

Table 2: Salinity tolerance of five Gammerid species. Only G. duebeni is capable of living across the full range of salinity from freshwater to seawater (data from Morrissey and Sumich 2012, Figure 8.8).

Gammarid Species	Approximate Salinity Range
G. pulex	<1 - 6
G. zaddachi	4 - 17
G. salinas	8 - 24
G. locusta	22 - 35
G. duebeni	<1- ~35

It would be useful if freshwater, brackish-water and marine ecosystems had clearly defined and non-overlapping salinity boundaries to which aquatic organisms are restricted, but salinity in natural waters is a continuum from ~0 (e.g., Great Lakes, Lake Baikal) to >100 ppt (e.g., Great Salt Lake, Dead Sea). Williams (1980) defined freshwater as <3 ppt dissolved solids, but noted this was an arbitrary selection. More germane to consideration of salinity tolerance is the classification (i.e., fresh, brackish, marine) of ecosystems based on biology.

Remane (1934, as discussed in Barnes 1989, see also Locke *et al.* 1991) compiled data from the North Sea and Baltic Sea on relative species diversity of freshwater, brackish water, and marine species vs. salinity over the range 0-35 ppt (*Figure 5*). His analysis showed freshwater species not present above 15 ppt, relatively few marine and brackish water species at salinities less than 5 ppt, and a minimum in species abundances occurs between ~5 and ~8 ppt. Khlebovich (1968) examined chemical data from estuaries and suggested that sharp changes occur in the ionic composition associated with seawater in the 5-8 ppt range as seawater is diluted with river water. He concluded that this zone marks a physico-chemical boundary between freshwater and marine fauna. Deaton and Greenberg (1986) reexamined Khlebovich's data. They found changes in ionic ratios are much larger below 2 ppt and disputed the concept that 5-8 ppt is a defining boundary between marine and freshwater fauna. They suggested that the species minimum is due to few animals having the physiological mechanisms to survive in the highly variable estuarine habitat, and those that can have low rates of speciation. Bulger *et al.* (1993) also disputed the validity of Remane's analysis that salinity is the defining variable based on information that Remane's analysis did not actually include any freshwater forms, and the salinity gradient across the Baltic Sea parallels a significant latitudinal temperature gradient which was not accounted for. They used multivariate analysis of biological data to identify key estuarine salinity zones (*Table 3*) and identified the lowest significant salinity zone as 0-4 ppt based primarily on stenohaline freshwater fish. This zone also overlaps with the lower end of an "Inner Estuarine Zone" ranging from 2-14 ppt.

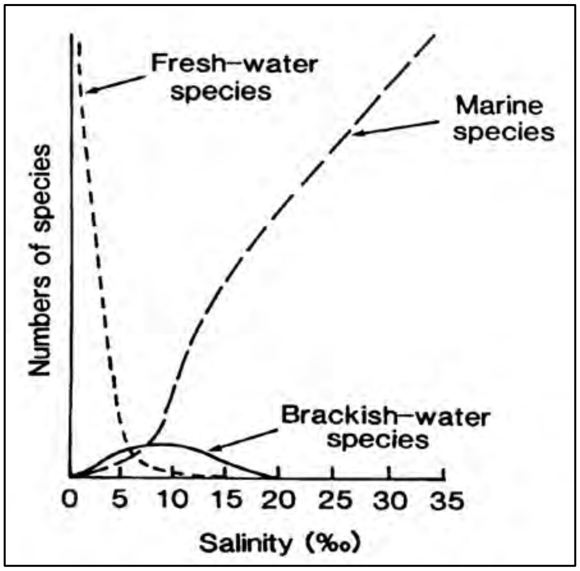

Figure 5: Relative species diversity of freshwater, brackish and marine species found at various salinities from freshwater to saltwater according to Remane (1934) as cited by Barnes (1989; reproduced by permission of The Royal Society of Edinburgh and R.S.K. Barnes).

Table 3: Salinity zones identified through Principal Component Analysis of field data primarily from Chesapeake and Delaware Bays (data from Bulger et al. 1993).

Salinity Zone Name	Salinity Range
1: Freshwater to 4 ppt	0-4
2: Inner Estuarine	2-14
3: Mid-Estuarine	11-18
4: Outer Estuarine	16-27
5: 24 ppt to Marine	≥24

Based on *Table 3* one might conclude that freshwater could be defined as water with salinity <2 ppt, but it would be an arbitrary endpoint and ignores that the 0-4 ppt zone was based on biological data, not just an arbitrarily selection (as per Williams 1980). Further, the significance of this zone would be overestimated, since Bulger *et al*. (1993) noted in their discussion that these zones are not exclusive, that is, organisms typically associated with, for example, Zone 2, are not necessarily excluded from Zone 1.

Ultimately, the response of aquatic organisms to a sudden change in salinity depends on many factors: the salinity range they generally experience in their present habitat, the rate of salinity increase or decrease during their exposure to change, the end point (how high or low the endpoint is, compared to their normal habitat range), the amount of time they're exposed, temperature, life stage, and their osmoregulatory capability. For examples, bivalves exposed to salinity outside their normal range can close their shells and lower their metabolic rate for short periods of time, effectively isolating themselves from a source of osmotic stress. Freshwater stenohaline organisms subjected to sudden salinity increase, such as produced by mid-ocean ballast water exchange or tank flushing, experience significant osmotic stress and likely high mortality. Estuarine organisms may be better equipped to survive BWE because of osmoregulatory abilities, but most estuarine organisms are stenohaline (Morrissey and Sumich 2012) and not likely to survive across the full range of salinity. Stenohaline estuarine organisms adapted for the lower salinity range would suffer osmotic shock after BWE, whereas estuarine organisms adapted for the upper salinity range would suffer osmotic shock after discharge into the Great Lakes. Estuarine organisms that are strongly euryhaline, such as *Thalassiosira pseudonana* (Brand 1984) and *Eurytemora affinis* (USGS 2012) will continue to pose a risk to freshwater systems like the Great Lakes even with BWE.

Salinity shock will not be a 100% reliable barrier to all freshwater-tolerant invaders that could threaten the Great Lakes. Locke *et al*. (1991) reported live freshwater-tolerant species in three ships' ballast tanks, even though salinity was >30 ppt due to BWE. Previous ports for these vessels were Ghent, Rotterdam, and Philadelphia. All three ports are located on estuaries with tidal salinity zones, although Philadelphia is far enough into the Delaware River estuary that salinity is usually less than 4 ppt (Smullen *et al*. 1984). The live species found in these tanks is illustrative of the limitations of salinity shock as a ballast-mediated barrier to ANS, especially for euryhaline estuarine species.

4. RECENT FIELD AND LABORATORY STUDIES OF SALINITY SHOCK ASSOCIATED WITH BALLAST WATER EXCHANGE FOR THE GREAT LAKES

There have been many studies of osmotic regulation and salinity shock for various aquatic organisms, but most have been general physiological studies not linked to BWE. Over the past decade some studies have directly examined salinity tolerance relevant to BWE and the Great Lakes. These studies fall into two categories: effect of BWE on diapausing invertebrate eggs, and salinity tolerance experiments on aquatic invertebrates that mimicked either flow-through BWE or empty-refill BWE. In addition, several studies have examined the effect of NaCl (brine) on aquatic organisms in ballast tanks, but these are only indirectly relevant to BWE as brine is not the same as seawater and it is not utilized as part of BWE.

4.1 EFFECT OF BWE ON DIAPAUSING EGGS (GREAT LAKES)

Bailey *et al*. (2004) extracted diapausing eggs of three invertebrate species native to the Great Lakes from ballast tank sediments in transoceanic ships entering the Great Lakes between 2000 and 2002 and tested them for hatching under ballast exchange conditions. The eggs were placed in freshwater and then exposed to stepwise salinity increments simulating partial-to-complete BWE. The proportion of eggs successfully hatched declined for all three species as salinity increased, and none hatched at full salinity (32 ppt). Some eggs from two of the three species hatched when reintroduced to 0 ppt water after 10 days of exposure to 32 ppt salinity, mimicking a sequence of BWE followed by discharge into the Great Lakes. They also observed partial, but incomplete embryo development in eggs exposed to 8 ppt, suggesting that low-brackish salinity may allow hatching cues to initiate embryo development in some species, but is too high for the embryos to survive. While BWE significantly reduced successful hatching of test animals, it did not provide complete protection against hatching after removal of eggs from the saline conditions, as would happen with discharge into the Great Lakes after BWE.

Gray *et al*. (2005) conducted similar experiments on diapausing eggs collected from both natural freshwater habitats and ballast tank sediments. They compared only two treatments: 0 and 32 ppt, followed by reintroduction to 0 ppt after 10 days to determine if exposure to salinity as experienced during BWE affected the viability (ability to hatch after exposure) and/or taxonomic richness after hatching. They did not find a significant reduction in viability (total hatched) or species richness after exposure to salt water.

Bailey *et al*. (2006) revisited the issue of diapausing eggs, varying the temperature (10°, 20°, 30°C) during salinity treatments (0, 8, 35 ppt). They observed some inhibition of hatching during salinity exposure at 8 and 32 ppt, but also significant initiation of hatching once the eggs were reintroduced to freshwater. In general efficacy of salinity in reducing viability was related to temperature, but not in a consistent manner.

Gray *et al*. (2007) conducted controlled BWE exchange experiments in ballast tanks filled with Great Lakes water and then subjected to BWE in the North Atlantic; they also placed *in situ* incubation chambers to evaluate 1) BWE effects on two sentinel freshwater benthic invertebrates isolated in the chambers, which also contained a deep layer of sediment and 2) BWE effects on animals hatched from diapausing eggs during transit. They found BWE to be >99% effective at eliminating freshwater pelagic organisms in the ballast tanks. Sentinel organism mortality was almost 100%, with indications that saltwater does penetrate into the pore spaces of sediment, thus exposing buried organisms. They also found BWE significantly reduced survival of organisms that hatched during transit. They concluded that BWE is effective at reducing discharge of live freshwater species, and is particularly effective against freshwater organisms (invertebrates).

Gray and MacIsaac (2010) conducted *in situ* experiments in operational ballast tanks to determine effects of BWE on diapausing egg viability using incubation chambers mounted on the floor of the ballast tanks. They ran parallel laboratory tests for comparison. Their conclusions supported previous lab-based findings that saltwater exposure does not significantly reduce viability of diapausing eggs in ballast tanks.

Briski *et al*. (2010) evaluated ballast tank sediment accumulation and conducted hatching experiments on ballast sediments from both transoceanic and coastal-trade ships entering the Great Lakes between 2007 and 2008, the first two years after implementation of strict BWE and flushing regulations by Canada (Transport Canada 2006). Comparison of these post-regulation results with similar pre-regulation data (Bailey *et al*. 2005) revealed post-regulation total sediment accumulation was ~2/3 less and mean density and abundance of diapausing eggs per ship was 80-90% less. They conducted hatching experiments on diapausing eggs separated from sediments and also on whole sediment samples. Total abundance of eggs of high risk species (defined as nonindigenous species that hatched in freshwater treatments) per ship was significantly lower in post-regulation samples. The potential contribution of viable individuals to exchanged ballast water by in situ hatching was estimated to be insignificant compared to the typical concentration of live organisms already in exchanged ballast water. Ballast management regulations enacted in 2006, appear to have markedly reduced the probability of introduction of NIS via dormant eggs carried in ballast sediments by both reducing sediment accumulation and preventing hatching of diapausing eggs of freshwater invertebrates in ballast tanks.

Briski *et al*. (2011) evaluated the effect of mid-ocean exchange on invertebrate dormant egg density and viability in ballast tanks. They compared data from ships that performed BWE with those exempt from BWE and found no significant difference in taxonomic composition or abundance of invertebrates or their dormant eggs and also, BWE had no discernible effect on dormant egg viability in ballast sediments. Reducing the amount of sediment in ballast tanks was determined to be a critical factor for reducing abundance and species richness of both invertebrates and their dormant eggs in ballast sediment.

In summary, all recent experiments to assess the effectiveness of BWE (and saltwater flushing of NOBOB tanks) on diapausing eggs suggest that exposure to seawater does not kill diapausing eggs, even those of freshwater organisms, but it does reduce the likelihood of in-tank hatching and/or causes mortality of freshwater species hatched in ballast tanks during transit. Further, saltwater flushing reduces invasion risk by reducing accumulated sediment and thus potential propagule pressure from diapausing eggs. While exposure to saltwater severely reduces or eliminates the *in situ* hatching success of diapausing eggs, it is not clear what the level of invasion risk is to an ecosystem receiving repeated small inoculations of a few viable eggs of asexually-reproducing invertebrates. This is likely to occur, at least intermittently, because small amounts of ballast sediment remain in tanks even after mid-ocean flushing. Such residual sediments and any eggs they contain can be resuspended and discharged during deballasting, resulting in a potentially small but repetitive inoculation of the recipient ecosystem with viable eggs of nonindigenous species.

4.2 SALINITY TOLERANCE EXPERIMENTS – TESTING EFFECTS OF BWE ON FRESHWATER AND ESTUARINE ORGANISMS.

Santagata *et al*. (2008) simulated both empty-refill (E-R) and flow-through (F-T) BWE to test the salinity tolerance/response of 54 freshwater and estuarine larval and adult crustaceans. Test organisms were collected from freshwater and mesohaline habitats adjacent to ports of the Baltic Sea, North Sea, Great Lakes, Chesapeake Bay, and San Francisco Bay. In the E-R experiments animals experienced instantaneous salinity increase from their ambient salinity to 34 psu (~34 ppt), while in F-T tests the organisms were subjected to stepwise increases of salinity (0, 14, 24, 34 psu) every hour until 34 psu was reached. The effectiveness of both treatment types decreased as the ambient salinity of the collection sites increased (*Figure 6*). Organisms collected from freshwater habitats (0-2 psu) experienced the most mortality: all individuals in 82% of the F-T treatments and 88% of the E-R treatments were dead after 48 hours. They found both types of BWE were about equally effective at the end of 48 hours, but E-R treatments, in which test animals experienced sudden and extreme salinity shock, required less exposure time in 43% of all cases. Santagata *et al*. (2008) concluded that salinity shock does not completely prevent the transfer of all low-salinity biota, but BWE is still a useful management tool to reduce species transfers, especially considering the combined effects of removal and mortality. Current management practices of BWE and saltwater flushing serve to reduce ship-mediated transfer and subsequent risk of introduction of non-indigenous species to the Great Lakes and other low-salinity recipient systems.

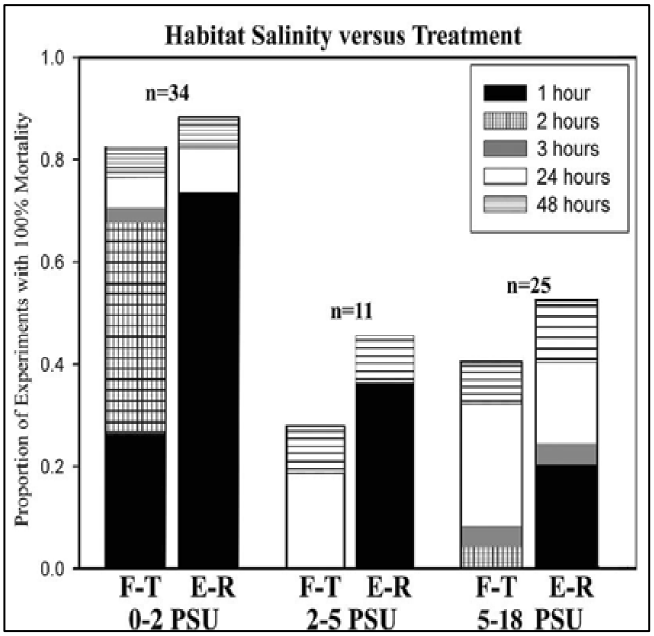

Figure 6: Effectiveness of flow-through (F-T) and empty-refill (E-R) salinity treatments grouped by the habitat salinity observed at the time of species collection. The number of experiments (n) is listed above each salinity range. Bars represent the proportion of experiments within a given salinity category that yielded 100% mortality by treatment. (Figure reproduced from Santagata et al. 2008).

Ellis and MacIsaac (2009) conducted short-term exposures of nine organisms (8 adults, 1 larva) that had already invaded the Great Lakes; five discovered prior to mandatory BWE regulation and three after. As with Santagata *et al*. (2008), experimental protocols mimicked both F-T (stepwise increases: 0, 4, 8, 14, 24, 30 ppt) and E-R (instantaneous increase: 0 to 30 ppt) BWE. Salinity exposure by either BWE method reduced survival of all species, and, comparable to Santagata *et al*. (2008), E-R produced mortality more rapidly. Five test organisms exhibited 100% mortality within 5 hours of initial exposure, regardless of BWE treatment, while adult dreissenid mussels (quagga: *Dreissena rostriformis bugensis*; zebra: *Dreissena polymorpha*), the round goby (*Neogobius melanostomus*), and the amphipod *Echinogammarus ischnus* all took much longer, although 80-90% mortality was observed after 48 hours. Ellis and MacIsaac (2009) concluded that BWE is an effective management tool for preventing the introduction of freshwater species into the Great Lakes.

Karsiotis *et al*. (2012) conducted short- and long-term salinity tolerance experiments on round gobies (*Neogobius melanostomus*) from Lake Erie. Their short-term experiments immersed gobies directly from freshwater into water with salinities ranging in 5 ppt steps from 0 to 40 ppt.

This simulated E-R ballast water exchange (immediate immersion in water ≥30 ppt salinity) and also release into ports having a range of salinities (immediate immersion into water with 5, 10, 15, 20, or 25 ppt salinity). Test animals exhibited significant mortality at salinities ≥25 ppt and none survived in salinities ≥30 ppt. They concluded that this species cannot withstand fully completed BWE (i.e., final salinity >30 ppt).

Matheson *et al*. (2007) examined the efficacy of NaCl brines of various concentrations up to ~70 ppt to kill a range of freshwater test fish, snails, and macrophytes. Santagata *et al*. (2009), Bradie *et al*. (2010), and Wang *et al*. (2012) all tested NaCl brines up to 115 ppt as a potential ballast water/tank treatment. NaCl in solution is known to be more toxic to freshwater aquatic organisms than seawater at equivalent concentrations (Santagata *et al*. 2009). Since the present paper is concerned with the ability of ocean-water to induce osmotic shock and death in ballast tank organisms, the use of NaCl brine is noted but will not be discussed.

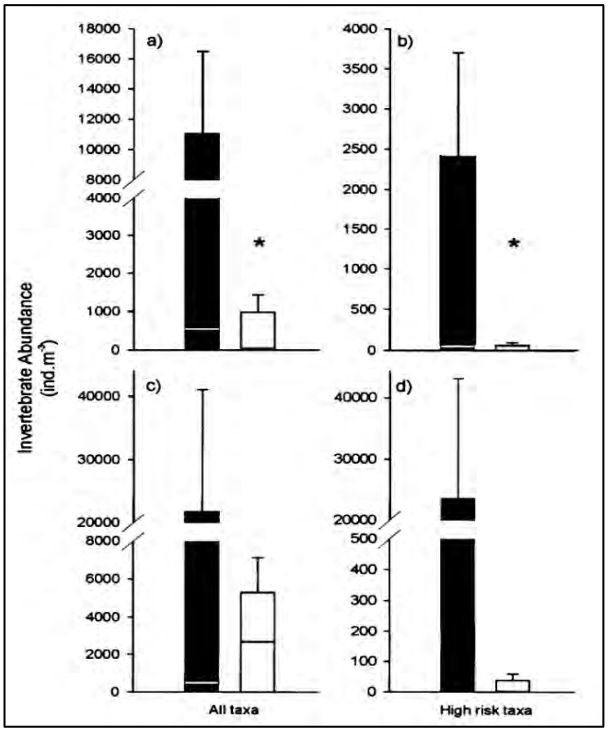

Figure 7: Mean (+/-S.E.) abundance of invertebrates in NOBOB residual ballast (upper panels) and water from ballasted (lower panels) ships, before (black bars) and after (white bars) the introduction of saltwater flushing and ballast water exchange, respectively. Thin horizontal lines on bars are median values. (Bailey et al. 2011, reprinted with permission. Copyright 2011, American Chemical Society).

As part of a program to evaluate the effectiveness of the 2006 expanded ballast management regulations for the Great Lakes, Bailey *et al*. (2011) sampled NOBOB residuals and water from ballasted tanks coming into the Great Lakes between 2005 and 2008. They compared these results ("post-regulation") with data obtained "pre-regulation" and found abundances of all invertebrates in NOBOB residuals was significantly lower, and mean and maximum density of invertebrates in ballasted tanks were also lower (*Figure 7*). When only high-risk taxa (tolerant of salinity up to 18 ppt) were considered, NOBOB residual ballast water had a median density of 0 individuals/m^3 (note: this is not the same as 0 individuals in every sample) and the median density in ballast water was 1 individual/m^3. They concluded that strict enforcement of BWE and flushing has significantly reduced the probability for rare, high propagule ballast water discharges and nearly eliminated high-risk taxa from incoming ballast. Although BWE and tank flushing will not provide complete protection against all aquatic invasions, they suggest that the Great Lakes ballast management regime may frequently reduce the effective invasion risk for freshwater ecosystems receiving ballast tank discharges to approximately the same level expected under the IMO D-2 standard.

Strictly speaking, data to support Bailey *et al.*'s supposition are only available for zooplankton in the >50µ size category. Exchanged ballast water typically carries thousands to millions of other viable organisms (microbes, viruses, protists, small zooplankton, phytoplankton, perhaps the occasional fish, etc) per m^3. Even so, the general concept they highlighted is that freshwater tolerant species, regardless of size range, pose the greatest risk to the Great Lakes and salinity shock brought on by BWE could reduce the abundance of such organisms to at or below the IMO D-2 target level, depending on the salinity tolerance of the particular organisms in a ballast tank.

5. CONCLUSIONS

Salinity shock is caused by osmotic stress, a well documented physiologic response in organisms unable to adequately control their internal osmotic condition in the presence of external changes in salinity. Osmotic stress affects the ability of organisms to carry out required metabolic functions, often leading to death, although some organisms can adapt if the change in salinity is gradual and exposure is short-lived. Few larval and adult freshwater (and low-salinity brackish water) organisms can survive a sudden large change in salinity, such as happens during BWE in ballast tanks containing low-salinity water, or when saltwater organisms are discharged into freshwater.

Flushing out coastal water and organisms is the primary mechanism by which BWE reduces invasion risk when the original ballast is saltwater or brackish water in the upper-salinity range. However, it is not a perfect filter for removing potentially invasive species from ballast water discharges because there is little if any change in salinity.

For protection of fresh- and lower-salinity brackish-water ecosystems, BWE includes the added advantage of salinity shock for enhanced risk reduction. The combination of physical flushing of ballast tanks (water and sediment) and salinity shock can be very effective for reducing risk from organisms adapted to fresh- and lower-salinity brackish habitats.

A deficiency in our understanding of the efficacy of BWE and tank flushing is lack of sufficient data related to organisms other than large (>50µ) invertebrates, especially small invertebrates, phytoplankton, and microorganisms. So while BWE and tank flushing are highly protective of the Great Lakes ecosystem, vital information about the introduction rates and effects of salinity on other freshwater-tolerant organisms is lacking.

Some organisms can reproduce asexually and produce resting eggs (e.g., benthic invertebrates) or cysts (e.g., dinoflagellates, bacteria, protists) which are able to survive harsh conditions until appropriate environmental cues initiate continuation of development. Studies have shown that BWE and tank flushing can reduce the invasion risk associated with resting eggs and cysts by resuspending and flushing out ballast tank sediment where they accumulate, but salinity has little or no effect on long-term survival of these dormant stages.

In principle, the use of BWE in combination with on-board treatment (BWE+Treatment) could enhance reduction of the risk associated with ballast water discharges to fresh- and brackish-water ecosystems. BWE would potentially eliminate freshwater-tolerant (i.e., high-risk) species from the ballast water prior to treatment, leaving less risky marine and high-brackish species in the discharge. The efficacy against high-risk species will be dependent on the salinity tolerance of the organisms. There are some, mainly estuarine, organisms that have tolerance over the full range of salinity, although their ability to survive a rapid change from low salinity to almost full-strength ocean water is another factor. Using BWE as a precursor to on-board treatment would also provide a form of insurance in case of undetected or unexpected treatment system failure, especially since these systems are very new and at present we don't have much actual operational experience with them to accurately gauge their reliability and performance.

There is ample scientific evidence to support the conceptual principles presented in the Canadian BWE+Treatment proposal. However, the practicability of the proposed procedure under shipboard operating conditions has not yet been evaluated. Also, the effectiveness of BWE+Treatment compared to treatment alone needs to be verified by direct experiments. At least one series of experiments using simulated exchange+treatment vs. treatment alone has been completed at a land-based test facility, but the results have not yet been published, although they are expected soon. In addition, a shipboard test program is also underway by Canadian scientists.

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