Final Report

Identifying, Verifying, and Establishing Options for Best Management Practices for NOBOB Vessels

Principal Investigators
David F. Reid, NOAA Great Lakes Environmental Research Laboratory
Thomas H. Johengen, University of Michigan
Hugh MacIsaac, University of Windsor
Fred Dobbs, Old Dominion University
Martina Doblin, Old Dominion University
Lisa Drake. Old Dominion University
Greg Ruiz, Smithsonian Environmental Research Lab
Phil Jenkins, Philip T. Jenkins & Associates Ltd.

June 2007 (Revised)

Acknowledgements

Financial support for this collaborative research program was provided by the Great Lakes Protection Fund (Chicago, IL) and enhanced by funding support from the U.S. Coast Guard and the National Oceanic and Atmospheric Administration (NOAA). Furthermore, without the support and cooperation of numerous ship owners, operators, agents, agent organizations, vessel officers and crew, this research could not have been successfully completed. In particular, Fednav International Ltd, Polish Steamship Company, Jo Tankers AS of Kokstad, Norway, and Operators of Marinus Green generously consented to having their ships to participate in the study. To that end we thank the Captain and crew of the following participating ships for their excellent cooperation and assistance: MV/ Lady Hamilton; MV/ Irma; MV/ Federal Ems; and MV/ Marinus Green. GLERL Contribution #1436.

Contributing Authors by Section

Task 1

Objective 1.1: T. Johengen[1], D. Reid[2], and P. Jenkins[3]
Objective 1.2: D. Reid[2], T. Johengen[1], and P. Jenkins[3]
Objective 1.3: P. Jenkins[3], T. Johengen[1,] and D. Reid[2]

Task 2

Objective 2.1: F. Dobbs[4], Y.Tang[4], F. Thomson[4], S. Heinemann[4,] and S. Rondon[4]
Objective 2.2: Y. Hong[1]
Objective 2.3: H. MacIsaac[5], C. van Overdijk[5], and D. Gray[5]
Objective 2.4: H. MacIsaac[5], C. van Overdijk[5], and D. Gray[5]

Task 3

Objective 3.1: G. Ruiz[6] and S. Santagata[6]
Objective 3.2: G. Ruiz[6] and S. Santagata[6]
Objective 3.3: G. Ruiz[6] and S. Santagata[6]
Objective 3.4: S. Santagata[6], D. Reid[2], T. Johengen[1], and S. Ellis[5]

Author Affiliations:
1 – Cooperative Institute for Limnology & Ecosystems Research, University of Michigan
2 – NOAA Great Lakes Environmental Research Laboratory
3 – Jenkins and Associates, Ltd.
4 – Old Dominion University
5 – University of Windsor
6 – Smithsonian Environmental Research Laboratory

Revisions

October 2007: Page 31, text corrected to remove statement indicating that Enterococci form cysts, which they do not.

Table of Contents

Executive Summary

A “Code of Best Practices for Ballast Water Management” was adopted by the Shipping Federation of Canada and the (U.S.) Lake Carrier's Association in 2000. In 2002 the St. Lawrence Seaway corporations adopted rules making compliance with the Code mandatory for entry into the Seaway. The Code promotes the maintenance of relatively clean ballast tanks through a program of regular inspection and cleaning, combined with a precautionary approach to ballasting with the objective of limiting or avoiding the uptake of ballast under specified conditions. To our knowledge, however, there has never been an assessment of the extent to which commercially operating ships can realistically apply these practices or the effectiveness of the stated practices for reducing the risk of new species introductions. We therefore conducted this scientific study to 1) test and evaluate the effectiveness of the current Best Management Practices (BMPs), focusing on a subset that are specifically applicable to ballast management for reduction of invasion risk associated with empty ballast tanks on ships entering the Great Lakes with no pumpable ballast-on-board (NOBOB), and 2) test a set of enhancements to the existing BMPs, focusing on flushing of tanks with deep ocean water, either when the ship is in NOBOB condition or as an intermediate step in deep ocean exchange. In particular, we wanted to examine whether BMPs are effective at reducing the abundance and viability of live organisms and resting stages.

To complete this objective we conducted detailed biological assessments of microbial, phytoplankton, and invertebrate communities present within both sediment and water ballast residuals for two participating ships during each entry into the lakes and compared results against ballasting history and any BMPs applied. Lastly, we attempted to more thoroughly assess the extent to which salinity toxicity, whether through open-ocean ballast water exchange (BWE) or saltwater flushing, can prevent the transfer of low-salinity species to the Great Lakes.

Constraints on our Experimental Design

Despite substantial effort by the project team, the vagaries of the shipping trade were such that the ships we engaged were unable to assist us as originally proposed. Efforts to overcome these limitations included working with multiple ships instead of the proposed single ship, and conducting two years of field studies instead on the proposed single year. Still, as a consequence of the ships’ unexpected altered trading patterns and voyages and their limited ability to consistently apply BMPs, the character of our experimental design (one predicated on sampling “paired-tanks”) was altered completely. Instead of sampling “control” and “treatment” tanks, we collected a chronological series of samples as best we could. In response to these limitations, we also modified our sampling approach to incorporate the use of our emergence traps to provide for a direct test of our enhanced saltwater flushing BMP. This modification also provided the opportunity to conduct experiments on the effectiveness of BWE when the ballast originated from a freshwater port.

Task 1: Assess the effectiveness of specific ballast management practices on sediment accumulation and characteristics within ballast tanks.

We used in situ water quality instruments to help define the timing and quantity of ballasting as well as the overall quality of source water. In particular, instrument data provided direct confirmation of when BMPs were applied to the treatment tanks. In addition, adding sensors at various locations and heights throughout the tank provided insight to patterns of sediment accumulation observed during direct tank sampling. The structural complexity within the tank and the nature of the ballast intake and stripping system create a general pattern of thicker sediment accumulation in the forward and outer bilge areas of the tanks. The size of the area of significant resuspension and discharge will be affected by the tank design, deballasting flow rate, and nature of the sediment, all of which can vary widely among ships. Qualitative estimates based on visual observations in a limited number of NOBOB tanks suggest that significant resuspension and removal of sediment occurs during discharge, affecting between 30% and 80% of the bottom area, depending on the previous ballasting and sediment management history of the tank. Again, due to the experimental design limitations and a relatively infrequent application of specific BMPs, we could not quantitatively assess the potential effectiveness of BMPs to reduce sediment accumulation. Instrument data did confirm that saltwater flushing, or ‘swish and spit’, can resuspend a portion of the resident sediment and increase the likelihood of eliminating this sediment on subsequent discharge. A single ballasting event in highly turbid ports can result in significant addition of sediment that, if not flushed out almost immediately, can quickly settle, coagulate and become difficult to eliminate.

This study also revealed that the consistent implementation of the Code can be problematic, especially for the environmental precautionary actions (Item 6), because application is very much dependent on local conditions – working rules of the dock (24/7 vs. daylight), rainy season vs. dry season, river berth vs. sheltered harbor or deep water harbor. Acceptance and implementation of the Code by the shipping industry must be understood as a commitment to make a “good faith” effort which if regularly and consistently conducted may somewhat lower overall risk of introductions, but will not completely eliminate it. The practical realities and limitations associated with vessel operations makes the existing BMPs inadequate as the lone strategy for reducing the risk of nonindigenous species introductions from NOBOB vessels. The designation and routine use of saltwater flushing as an official BMP would greatly improve the protection framework for the Great Lakes, if aggressively implemented by the shipping industry.

Task 2: Assess the effectiveness of specific ballast management practices to reduce the density and viability of organisms and resting stages.

Microbiology

Given the deviations from our intended experimental design, we cannot state much about the efficacy of BMPs to reduce the quantities and diversity of pathogenic microorganisms. However, summary data for the microbiological analyses certainly would argue for the consistent application of best management practices. In every tank sampled over the course of this study (total of 20 tanks), at least one of the potential pathogens or indicator species for which we assayed was present. In one case there were 8 such taxa present, with most samples containing between 2 and 6. These may be regarded as “model” organisms; had we assayed for more such species, we believe we would have found them in some samples. We reiterate, therefore, a point expressed in our previous NOBOB study that "it seems prudent to regard all NOBOB ships entering the Great Lakes as potential carriers of pathogens".

Phytoplankton

As a part of BMPs for application to the management of NOBOB vessels, the regular use of saltwater flushing can minimize delivery of viable freshwater phytoplankton to the Great Lake. Short-term changes in salinity can cause problems in osmotic regulation for freshwater phytoplankton. Our results showed that switching from freshwater to saltwater conditions reduced phytoplankton community diversity and restricted phytoplankton growth in lakewater media. The effects of the saltwater exposure/exchange were greater on the water samples than sediment samples; flushing muddy sediment out of tanks may be important steps to minimize the risk of phytoplankton via NOBOB operation. Variations in phytoplankton composition and growth occurred in response to BWE and saltwater flushing, however it was not possible to directly relate differences in populations or viability to any given management activity given the alteration of our experimental design.

Invertebrates

Ballasting events changed (increased or decreased) the number of organisms found in association with both water and sediment residuals, whether ballast was with fresh, brackish, or saltwater. However, there was no consistency to the changes, either in the types of organisms present, or the densities at which they were present. Due to the multiple ballast events between each sampling opportunity, we are unable to associate particular changes with specific ballast events or practices.

It is difficult to directly assess the effects of BMP's on fauna living in residual water from this study as water could not be collected on each sampling date and BMPs were not applied in a consistent or controlled manner. Furthermore, high densities of animals were detected in residual water from both fresh- and salt-water sources. However, our salinity toxicity studies and ballast water exchange experiments show that many taxa from low-salinity ports are eradicated from ballast tanks relatively quickly through exposure to full-strength seawater.

Due to the confirmed presence of viable organisms within water and sediment residuals following ballasting events in overseas freshwater ports, it should be recommended that all ships complete a flushing/exchange in the mid-ocean during voyages to the Great Lakes as the potential risk for introducing saltwater animals to the Great Lakes is much lower than those from potential freshwater sources.

Ballast Water Exchange Experiments

Open ocean ballast exchange (BWE) proved to be a highly effective method to reduce the concentration of zooplankton in the ballast tanks studied. Freshwater animals were completely absent from the exchanged ballast tanks of vessels 1 and 4, while low concentrations remained in the exchanged tanks of vessels 2 and 5. Overall, sequential (empty-refill) exchange resulted in a decrease in total zooplankton abundance by >99% for the four ships for which we were able to assess exchange efficiency. The results from our study suggest that the effectiveness of BWE for freshwater organisms is less variable than that for marine organisms. The reduced variability of

BWE effectiveness in our study may result from pronounced osmotic shock experienced by freshwater animals remaining in ballast tanks after BWE. Vessels transiting between marine ports must rely on purging and dilution of ballast water to eliminate coastal organisms. Vessels transiting between freshwater ports can expect decreases in zooplankton density due both to purging of organisms and to salinity effects. However, it should be noted that this subset of experiments was performed in upper wing tanks and the efficiency of water exchange in these tanks may be greater owing to their structural design and location.

Benthic Invertebrates

To evaluate the effect of BWE on benthic invertebrates, 30 *Echinogammarus ischnus* amphipods and 30 *Brachiura sowerbyi* oligochaetes collected from the Great Lakes were placed with sediments inside an incubation chamber placed within the control and experimental tanks of vessels 4, 5, and 6. Most oligochaetes in the control tanks survived their intercontinental voyages, with mortalities of 16.6%, 0%, and 20%, for vessels 4, 5, and 6, respectively. However, nearly all individuals perished in the exchanged ballast tanks, with mortalities of 100%, 100%, and 96.6% (one live individual out of 30). The survival of one of the oligochaetes in the hatch-out chambers in the exchanged tank highlights one of the potential problems with BWE. The lone live individual was found at the very bottom of the sediment layer, suggesting that saline water may not have been able to penetrate through the sediment. If individuals can survive below the sediment:water interface then they could represent an invasion risk if sediments are disturbed during subsequent ballasting activities. *Echinogammarus ischnus* mortality in the control tanks was higher than that for the oligochaetes at 40%, 60%, and 53.3% for vessels 4, 5, and 6, respectively. In the treatment tanks that had undergone exchange, 100% of *E. ischnus* individuals were deceased at the end of each experiment. These results suggest that saltwater exposure during BWE is likely to be lethal for many species found above the sediment:water interface.

Invertebrate Resting Eggs

The effect of saltwater exposure on diapausing invertebrate eggs was evaluated both directly in the tank and in follow-up laboratory-based hatching experiments. Ballast sediment (300g) previously collected from vessels operating on the Great Lakes was placed inside each of the chambers. Following incubation within the tank and exposure to BWE, sediments were retrieved from the traps and returned to the laboratory to conduct a follow-up hatching viability experiment. The number of animals recovered from hatch-out chambers in control tanks (mean = 0.5 – 3.25 ind/trap) was significantly higher than that from chambers in the exchanged tanks (mean = 0 - 0.25 ind/trap). Three possible explanations for the lower abundance in exchanged tanks are: 1) saltwater exposure may have killed animals that hatched during the pre-exchange period; 2) the presence of saltwater in the chambers could have prevented further recruitment from diapausing eggs in the sediment since environmental conditions would not cue hatching; or 3) environmental conditions inside the incubation chambers deteriorated to conditions unsuitable for hatching. We conducted experiments in which instrument sondes were embedded inside separate incubation chambers of the same design used here. Results showed that exchange between ambient water and water trapped in the chamber can be limited, depending on ship motion, and hence biochemical oxygen demand from sediment can lead to hypoxic or anoxic conditions inside the chambers. Such conditions would prevent most diapausing eggs from hatching.

In follow-up post-BWE laboratory viability experiments, neither the total abundance of hatched individuals nor the species richness of hatched individuals differed significantly between sediments collected from hatch-out chambers in the exchanged versus control ballast tanks. These results suggest that diapausing invertebrate eggs may be largely resistant to saltwater exposure, and that BWE may not mitigate the threat of species introductions posed by this life stage. Previous experiments by our team on diapausing eggs that were isolated from sediment did report significant differences in viability after exposure to saline water. This difference may suggest that eggs embedded within sediment are less vulnerable to saltwater exposure.

Task 3: Characterize source invertebrate populations and assess salinity toxicity as a barrier to prevent transfers of "high risk" species to the Great Lakes in ballast tanks.

Characterizing Source Populations

The Great Lakes and low salinity ports of the east coast of the U.S. and Canada share an invasion threat from the North Sea and Baltic Sea. Of the 269 species reviewed, the Great Lakes and port systems of the North Sea and Baltic Sea have at least 100 species (37%) in common, with 18 of these considered exotic to the Great Lakes region. At least 5 of these species are considered to have negative impacts on the indigenous fauna (invasive). In particular, commercial ships from ports of the Netherlands, Belgium, Germany, Finland, and Russia may represent the greatest threat of invasive species to the Great Lakes and estuarine ecosystems of the eastern United States. Based on trends of temperature, salinity, and ship traffic, the ports of Rotterdam, Antwerp, Ghent, Brake, Bremen, Klaipeda, Kotka, and St. Petersburg have been classified as high invasion risk donor ports. Based on species diversity and environmental tolerances, the most likely taxonomic groups to invade the Great Lakes are the amphipods, isopods, harpacticoid copepods, cladocerans, mysids, and mollusks. During the last 50 years, several long-term shifts in zooplankton composition and abundance have occurred within the North Sea and may potentially increase the invasion rate of Ponto-Caspian species into adjacent freshwater port systems and hence possibly to the Great Lakes.

Salinity tolerance experiments

Salinity tolerance experiments, designed to mimic both flow-through and empty-refill methods were carried out in several different regions known for high invasion rates and commercial ship traffic. Experiments were conducted in the Chesapeake Bay (Maryland), San Francisco Bay (California), and in the European ports of Curonian Lagoon, Klaipeda, Lithuania, Vistula River (Poland), and Rotterdam (The Netherlands) located within the Baltic Sea and North Sea. Over 70 experiments were conducted using 43 invertebrates identified to the species level, four invertebrates identified to the genus level, and ten experiments that included unidentified species of bivalve veligers, barnacle nauplii, cladocerans, polychaetes, flatworms, and copepods. All of the cladocerans in our experiments were eliminated by either 14 or 24 ppt seawater. There are marine cladocerans that can survive in salinities greater than 24 ppt such as species of *Podon*, *Pseudoevadne*, *Evadne*, *Penilia*, and *Pleopsis*. However, these species are rarely found within freshwater habitats or cannot survive in constant freshwater systems. The majority of copepods in our experiments were not tolerant of full-strength seawater, but considering the ability of some species to recover from short-term exposures to dramatic salinity shifts, exposure duration should be at least a day to assure mortality of all copepod species. The larvae of crabs, shrimps, barnacles, and bivalves as well as adult amphipods, isopods, cumaceans, and mysids were generally tolerant of full-strength seawater (or higher salinities). For these taxa, it is a better discriminator of 'invasion risk' for the Great Lakes region to determine species that are capable of establishing populations within a constant freshwater habitat. However, all of these euryhaline species do pose a significant invasion threat to estuarine systems.

In addition, salinity tolerance experiments were conducted in the Great Lakes by both SERC (western Lake Erie; Grand Traverse Bay, MI) and the University of Windsor. In the SERC experiments the common native cladoceran species; *Bosmina longirostris*, *Leptodora kindtii*, and *Daphnia retrocurva* were all eliminated in the initial exposure to 14 ppt seawater. This was also true for the highly abundant rotifer, *Asplachna priodonta*. Two of the most problematic invasive species in the Great Lakes, the predatory cladocerans *Cercopagis* and *Bythotrephes*, were slightly more tolerant of higher salinities and survived until the 24 ppt treatment. This was also true for the widely distributed cladoceran species of *Polphemus*, *Alona*, and *Eurycercus*. However, late stage juveniles brooded within adults of *Bosmina longirostris* and *Eurycercus lamellatus* survived in some of these short-term salinity treatments when returned to ambient water. The only full-strength salinity tolerant species encountered were the abundant quagga and zebra mussel veligers. However, as a final check of viability we transferred these animals to freshwater at the end of the experiment and left them overnight, and no individuals survived the full-seawater treatment and return to freshwater.

With regard for ballast water exchange methods, the greater risk for the Great Lakes lies with species or particular life stages that can tolerate full-strength seawater for at least two days and also establish viable populations within a constant freshwater system. Clearly, this is not the case for the adult forms of the invasive cladocerans *Cercopagis pengoi* and *Bythotrephes longimanus*. Their may be several reasons for their establishment in the Great Lakes, including that these species were introduced prior to ballast water exchange practices, that ballast water exchange practices had not been followed rigorously, or that their resting stages have more physiological resistance than the adults. Previous experiments designed to test the efficacy of ballast water exchange on the hatching success of resting stages of other species of cladocerans from the Great Lakes yielded mixed results, but suggest that saltwater exposure is unlikely to significantly reduce the risk from this potential source of propagules.

Though not a complete barrier against all exotic species, these experiments clearly show that many taxa that originate from low-salinity ports can be eradicated from ballast tanks relatively quickly through exposure to full-strength seawater (34 ppt). This is especially true for several species of rotifers, cladocerans, and copepods that are more likely to occur in freshwater or oligohaline habitats (0-2 ppt). It is not surprising that our experiments with animals from habitats with higher average salinities (2-5 and 5-10 ppt) exhibit greater resistance to treatments of full-strength seawater. These findings support similar conclusions drawn from previous ballast water exchange experiments conducted in the Chesapeake Bay and San Francisco Bay. Invertebrates from our experiments identified as salinity-tolerant species (34 ppt) include mysid shrimps, amphipods, isopods, harpacticoid copepods, bivalve veligers, and decapod zoea. Members of these taxonomic groups often experience dramatic fluctuations in salinity and temperature as part of their normal life histories and these factors have contributed to their ability to invade estuarine habitats. Of these estuarine animals, only a subset of salinity-tolerant species are capable of surviving and reproducing in a constant freshwater habitat such as the Great Lakes. Identifying species and populations with these characteristics from the port systems of the east coast of the U.S. and Canada, North Sea, and Baltic Sea is paramount for preventing problematic species from invading the Great Lakes region via the operations of commercial ships.

Conclusions

In summary, based on our previous work with NOBOBs and the results of direct salinity experiments conducted both on board ships and in the lab, we strongly support the new Canadian ballast management regulations adopted in 2006 that require, and the policy statement issued by the United States Coast Guard in 2005 that encourages mid-ocean tank flushing. Specifically, we recommend that vessels operating outside of the Great Lakes conduct saltwater flushing of their empty (NOBOB) tanks prior to each entry. Flushing is accomplished by allowing a limited amount of saltwater water to slosh around in an individual ballast tank as a result of the ship's rolling and pitching motion during passage, to agitate and resuspend trapped sediments and provide a salinity shock to biota, which is then discharged in the open ocean.

We further emphasize that many of the recommendations put forth in Item 6 of the Code of Best Management Practices require information on local water quality conditions that is not generally available to the shipping industry, or are often not practical to conduct due to cargo loading and unloading requirements. Therefore, while BMPs, if consistently and repeatedly applied, can reduce the risk of introductions from NOBOB vessels by minimizing the amount of sediment and associated organisms that are transported within ballast tanks, the practical realities and limitations associated with vessel operations makes the existing BMPs inadequate as the lone strategy for reducing the risk of nonindigenous species introductions from NOBOB vessels. The designation and routine use of saltwater flushing as an official BMP would greatly improve the protection framework for the Great Lakes, if aggressively implemented by the shipping industry.

A complete copy of the report and associated appendices can be downloaded from the project web site at: http://www.glerl.noaa.gov/res/Task_rpts/2004/aisreid04-1.html

Introduction

The discovery of the zebra mussel, the river ruffe and two gobies in the Great Lakes in circa 1990 sounded the alarm about the danger to the ecosystem of nonindigenous species being introduced through ballast water carried by ocean-going ships trading in the Great Lakes. After consultation with both industry stakeholders and the marine scientific community, in 1989 Canada introduced guidelines for the voluntary exchange of ballast water during transoceanic passages as an immediate ballast management measure to reduce the risk of invasion by fresh and coastal water species that might establish populations in the Great Lakes ecosystem. In 1993 U.S. regulations made the use of ballast water exchange (BWE) and the reporting of the on-board ballast condition mandatory for all ships planning to enter the Great Lakes after operating outside the U.S. EEZ (U.S. Coast Guard 1993).

However, while the initial rules to manage ballast water were being implemented in 1989, the nature of the ocean trade into the Lakes was changing. Economic factors dictated that for the trade to be viable under most circumstances, it was necessary for the ships to have both an inbound and outbound cargo, thus the number of ships entering with pumpable ballast on board (BOB) slowly diminished. By the mid 1990's more than ninety percent of the ships entering the Seaway are estimated to have been carrying no pumpable ballast on board (NOBOB; Colautti et al., 2003). This trend has continued to the present day, and there is no indication that it will change in the foreseeable future.

The reality is that very few ships are capable of completely evacuating all of their ballast for a variety of technical and operational reasons. While the latest bulk carriers are more efficient in stripping their tanks of ballast water, even small volumes of residual water in individual tanks can amount to tonnes of unpumpable ballast on each ship (Johengen et al., 2005). In addition, unless the ship is very efficiently managed, there will be varied accumulations of sediment, some of which can become resuspended during any ballasting operation. With the majority of trade moving between Western European ports and the Great Lakes in NOBOB condition, many ships can go through an entire season without experiencing ballast water exchange, and, until 2006, could have been carrying brackish or fresh water residuals in both directions (Johengen et al., 2005). In 2006 Canada passed new regulations and now requires that all water in ballast tanks of ships arriving from overseas (including the residual water in NOBOBs), as well as non-Canadian coastwise vessels, must be at salinity >30 ppt in order for those ships to discharge their ballast water in the Great Lakes.

Bio-Environmental Services (1981) recommended that sediments in ballast tanks be evaluated, noting that they "may contain the majority of aquatic organisms in a ballast tank. Eggs, cysts, benthic organisms, and settled plankton, because of lack of light in the ballast tank, may reside in the bottom of the tank." Locke et al. (1993) warned of "the possible resuspension in ballast water of organisms carried in 'residual' water or tank bottom sediments." A two-year study commissioned by Transport Canada (Aquatic Sciences Inc. et al., 1996) also identified the probability that NOBOB ships represent a significant threat to the Great Lakes ecosystem through the commingling of their unpumpable residual ballast water and sediment with fresh water from the Lakes. NOBOBs take Great Lakes water as ballast when they offload their inbound cargo and subsequently discharge their new ballast water back into the Lakes at ports at which they load their outbound cargo. Several recent studies confirmed that residual ballast water and sediments in NOBOB ballast tanks often contained adults, larvae and resting stages of many taxa (Niimi and Reid 2003, Johengen et al., 2005), some of which originate from foreign ports having similar environmental conditions to the Great Lakes.

The NOBOB phenomenon is largely unique to the Great Lakes trade, and while this threat will be essentially eliminated when ballast water discharge standards are enforced for all ships, that time is still years away. In the meantime, NOBOBs were not covered by the 1993 ballast water exchange requirements. In the late 1990s the Michigan legislature, unhappy with the lack of progress towards stricter and more comprehensive, ecosystem-protective ballast water management legislation at the federal level, proposed strict and controversial ballast-related State legislation. The threat of legislative action by Michigan elevated the issue and led to a negotiated agreement with the shipping industry to implement a “Code of Best Practices for Ballast Water Management” (‘the Code,’ Shipping Federation of Canada 2000; Appendix 1) in return for removing the more onerous sections of the proposed legislation. The Code was developed by the Shipping Federation of Canada and the (U.S.) Lake Carrier's Association in 2000 and implemented in Michigan legislation in 2001.

In 2002 the St. Lawrence Seaway corporations adopted rules making compliance with the Code mandatory for entry into the Seaway. As implemented the Code did not specifically address NOBOBs, but did generalize the application of management practices to ballast tanks instead of targeting just ballast water. The Code promotes the maintenance of relatively clean ballast tanks through a program of regular inspection and cleaning, with commensurate records, combined with a precautionary approach to ballasting with the objective of limiting or avoiding the uptake of ballast under specified conditions. With the establishment of the Code and the acceptance by both the State of Michigan and the St. Lawrence Seaway authorities, *best management practices* (BMPs) have come into the forefront of AIS risk reduction methods for ballast tanks on ocean-going ships operating in the Great Lakes. Unfortunately the Code does not include the flushing of tanks with deep ocean water, either when the ship is in NOBOB condition or as an intermediate step in deep ocean exchange.

To our knowledge, there has never been an assessment of: 1) the extent to which commercially operating ships can realistically apply the Code practices, or 2) the effectiveness of the stated practices for reducing the risk of new species introductions. The comprehensive ballast management survey that was conducted for the Great Lakes NOBOB Assessment (NOBOB-A; Johengen et al., 2005) involving more than one hundred NOBOB ships made it clear that many ships experience situations that require them to load significant amounts of poor quality water ballast despite the Code goal to avoid ballasting in such situations. This often results in sediment intake that if not dealt with expeditiously results in accumulations that can be difficult to remove.

The goals established for this project (NOBOB-Best Management Practices) were to 1) test and evaluate the effectiveness of the current BMPs, focusing on a subset that are specifically applicable to ballast management for invasion risk reduction of empty (NOBOB) ballast tanks on ships entering the Great Lakes, and 2) test a set of enhancements to the existing BMPs based on results from our NOBOB-A study. The enhancements added the following new or modified practices: 1) when in locations that appear undesirable for ballasting (BMP #6a-f), use the minimum possible ballast in the fewest tanks possible and complete ballasting after transit out of the undesirable conditions; 2) when carrying ballast, discharge and replace poor quality ballast water with cleaner water as soon as possible to minimize the amount of sediment accumulation; and 3) regularly perform a saltwater flush of all empty (NOBOB) ballast tanks when transiting the ocean.

We developed the following three interrelated Tasks to accomplish our objectives:

- **Task 1: Assess the effectiveness of specific ballast management practices on sediment accumulation and characteristics within ballast tanks.**
- **Task 2. Assess the effectiveness of specific ballast management practices to reduce the density and viability of organisms and resting stages.**
- **Task 3: Characterize source invertebrate populations and assess salinity toxicity as a barrier to prevent transfers of "high risk" species to the Great Lakes in ballast tanks.**

The expected outcome was to establish a verified and enhanced subset of the existing "BMPs" that have been scientifically tested for effectiveness at controlling sediment accumulation and minimizing delivery of viable freshwater biological propagules to the Great Lakes.

Study Design: Proposed versus Realized

Our original approach was to work with a single vessel that traded regularly (every 5-6 weeks) into the Great Lakes from overseas, preferably Western Europe. The key criteria for this arrangement were: 1) agreement by vessel owner-operator and Master to work with us over the course of the entire 2004 Great Lakes shipping season; 2) designation of four (paired) ballast tanks (two control, two test tanks) with access unrestricted by cargo storage; 3) agreement by Master and owner to maintain detailed and accurate ballasting records for the tanks involved in the project and make those records available on each new entry trip to the Great Lakes; 4) agreement to allow us to place one or more instrument systems in each of the tanks; 5) agreement to allow a team of scientists to access each of the tanks every time the vessel returns to the Great Lakes; and 6) agreement to follow specific ballast management practice procedures, and contingency alternate procedures, to be mutually developed by a planning team consisting of NOBOB Project Team representatives, the ship's owner/operator, and ship's officers. Prior to the onset of this study we had obtained confirmation from two vessel owner/operators of the availability of vessels that would meet these criteria. It was expected that the participating ship would apply the specified management practices whenever practical and at every opportunity, even if the vessel was not bound for a port where such a procedure was required (BMP #1). We recognized, however, the need to maintain a level of flexibility in our sampling and experimental procedures to accommodate safety and practical limitations imposed by the uncertainty of the ship's trade.

The vagaries of the shipping trade were such that the ship we originally chose to work with was unable to follow the procedures as originally proposed, and was pulled from its Great Lakes rotation on its first voyages after we initiated the on-board experiments. As a result we added a second ship from a different owner/operator and extended the field study an entire extra year when it became apparent that the return rate of both vessels into the Great Lakes was substantially lower than planned. We also modified our sampling approach and added the use of emergence traps to provide a direct test of the effectiveness against freshwater organisms of the enhanced saltwater flushing BMP. This last modification also provided additional scientific verification of the effectiveness of BWE when ballast originates from a freshwater port.

Due to the ships' altered trading patterns and long voyages away from the lakes, the character of our experimental design, which had been predicated on sampling "paired-tanks", was altered completely. Instead of sampling “control” and “treatment” tanks, we collected a chronological series of samples from each tank as best we could. See Table 1.

Table 1. A summary of residual sampling for both participating ships for the study period of July 2004 – December 2005. ND = not determined.

Date	Port	Ship	Tank	Sample ID	Temp	Salinity
29-Jul-04	Cleveland	Irma	5s	B-04211-01wa	ND	2.5
29-Jul-04	Cleveland	Irma	5s	B-04211-01ws	ND	ND
29-Jul-04	Cleveland	Irma	4s	B-04211-02wa	ND	28
29-Jul-04	Cleveland	Irma	4s	B-04211-02ws	ND	ND
29-Nov-04	Cleveland	Irma	4s	B-04334-01wa	ND	14
29-Nov-04	Cleveland	Irma	4s	B-04334-01ws	ND	31
29-Nov-04	Cleveland	Irma	5s	B-04334-02wa	ND	27
29-Nov-04	Cleveland	Irma	5s	B-04334-02ws	ND	42
26-Apr-05	Toledo	Irma	4s	B-05116-01wa	8.2	1
26-Apr-05	Toledo	Irma	4s	B-05116-01ws		1
26-Apr-05	Toledo	Irma	5s	B-05116-02wa	8.1	7
26-Apr-05	Toledo	Irma	5s	B-05116-02ws		5
26-Apr-05	Toledo	Irma	4s	B-05116-01net	8.2	0.2
26-Apr-05	Toledo	Irma	5s	B-05116-02net	8.1	0.2
17-Aug-05	Cleveland	Lady Hamilton	5s	B-05229-01wa	ND	15
17-Aug-05	Cleveland	Lady Hamilton	5s	B-05229-01ws	ND	
17-Aug-05	Cleveland	Lady Hamilton	3s	B-05229-02wa	ND	24
17-Aug-05	Cleveland	Lady Hamilton	3s	B-05229-02ws	ND	
3-Sep-05	Sorel	Lady Hamilton	5s	B-05246-01wa	ND	3
3-Sep-05	Sorel	Lady Hamilton	5s	B-05246-01ws	ND	ND
3-Sep-05	Sorel	Lady Hamilton	3s	B-05246-02wa	ND	2
3-Sep-05	Sorel	Lady Hamilton	3s	B-05246-02ws	ND	ND
6-Sep-05	Cleveland	Irma	5s	B-05249-01wa	22.7	30
6-Sep-05	Cleveland	Irma	5s	B-05249-01ws		ND
6-Sep-05	Cleveland	Irma	4s	B-05249-02wa	23.1	10
6-Sep-05	Cleveland	Irma	4s	B-05249-02ws		ND
6-Sep-05	Cleveland	Irma	5s	B-05249-01net	22.7	28
6-Sep-05	Cleveland	Irma	4s	B-05249-02net	23.1	10
10-Nov-05	Hamilton	Lady Hamilton	3s	B-05314-01wa	11.8	38
10-Nov-05	Hamilton	Lady Hamilton	3s	B-05314-01ws		
10-Nov-05	Hamilton	Lady Hamilton	5s	B-05314-02wa	12.1	38
10-Nov-05	Hamilton	Lady Hamilton	5s	B-05314-02ws		
10-Nov-05	Hamilton	Lady Hamilton	3s	B-05314-01net	11.8	38
2-Dec-05	Cleveland	Irma	5s	B-05336-01wa	7.2	16
2-Dec-05	Cleveland	Irma	5s	B-05336-01ws		
2-Dec-05	Cleveland	Irma	4s	B-05336-02wa	7.3	16
2-Dec-05	Cleveland	Irma	4s	B-05336-02ws		
2-Dec-05	Cleveland	Irma	5s	B-05336-01net	7.2	15
2-Dec-05	Cleveland	Irma	4s	B-05336-02net	7.3	16

Note: Two additional sets of samples were obtained from the MV Irma by the University of Windsor in April and July 2006. These are discussed in Task 2, Objective 2.3.

Our strategy for data analyses reverted to investigation of potential correlations and trends between the biological measures and the ships' ballasting history. Similarly for the objectives related to measuring the effects of BMPs on sediment accumulation we were limited to a more qualitative general description of our observations over time.

In spite of the difficulties a great deal of information was obtained that is scientifically useful, and may also be useful from management perspective as well. Task 3 objectives were not impacted by these issues and studies were completed as proposed. The failures related to our original experimental plans simply highlight the difficulties of conducting planned ballast research on commercial ships whose primary mission is to conduct trade, and for which supporting science can only be accomplished on a noninterference, no-guarantee basis. However, we appreciated the cooperation and assistance of the ships' owners, and Masters and crew in helping us attempt this research.

References

Aquatic Sciences Inc., Philip T Jenkins & Associates Ltd., and RNT Consulting 1996. Examination of Aquatic Nuisance Species Introductions to the Great Lakes through Commercial Shipping Ballast Water and Assessment of Control Options: Phase 1 & Phase II. Final Report. Aquatic Sciences Inc., St. Catherines, Ont., CA.

Colautti R., A. Niimi, C.D.A. van Overdijk, E.L.Mills, K. Holeck, and H.J. MacIsaac. 2003. Spatial and temporal analysis of shipping vectors to the Great Lakes. In: Invasive Species. Pathways, vectors and risk assessment. In, Global Invasive Species Program, edited by G. Ruiz and J.T. Carlton. pp. 227-246.

Johengen, T., D.F. Reid, G.L. Fahnenstiel, H.J. MacIsaac, F.C. Dobbs, M. Doblin, G. Ruiz, and P. T Jenkins. 2005. A Final Report for the Project "Assessment of Transoceanic NOBOB Vessels and Low-Salinity Ballast Water as Vectors for Non-indigenous Species Introductions to the Great Lakes." National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, and University of Michigan, Cooperative Institute for Limnology and Ecosystems Research, Ann Arbor, 287 pp.

Locke A, D.M. Reid, H.C. van Leeuwen, W.G. Sprules, and J.T. Carlton. 1993. Ballast water exchange as a means of controlling dispersal of freshwater organisms by ships. Canadian Journal of Fisheries and Aquatic Sciences, 50: 2086–2093.

Niimi, A. J., and D. M. Reid. 2003. Low salinity residual ballast discharge and exotic species introductions to the North American Great Lakes. Mar. Pollut. Bull. 46: 1334-1340.

Shipping Federation of Canada. 2000. Code of Best Practices for Ballast Water Management. September 28, 2000 URL:

(http://www.shipfed.ca/eng/library/other_subjects/ballats_water/BallastWaterBestPractices.html)

United States Coast Guard. 1993. Ballast water management for vessels entering the Great Lakes. Code of Federal Regulations 33-CFR Part 151.1510.

Task 1. Assess the effectiveness of specific ballast management practices on sediment accumulation and characteristics within ballast tanks.

Objective 1.1. Verify the times and dates of ballast operations and characterize the quality of ballast water during intake and discharge.

Overview

Two bulk carriers, the MV Lady Hamilton, operated by Fednav International Ltd of Montreal, and the MV Irma, operated by Polish Steamship Company of Szczecin, each committed a pair of tanks (combined double bottom hopper side tanks) to be used in the study. One tank was to act as a control tank while the second tank was to serve as the test tank to which various ballast management practices would be applied. In situ water quality monitoring instruments were deployed within designated ballast tanks of these vessels to verify and quantify the timing and quality of ballast water intake and discharge. Two types of commercially available multi-parameter sondes were used during the study; the YSI 6600EDS and the In Situ Troll 9000E (Fig 1.1). Both types of instruments were equipped to measure temperature, conductivity (salinity), dissolved-oxygen, and turbidity. YSI sondes were also equipped with a pressure sensor to measure depth of water in the ballast tank, and a chlorophyll-a sensor.

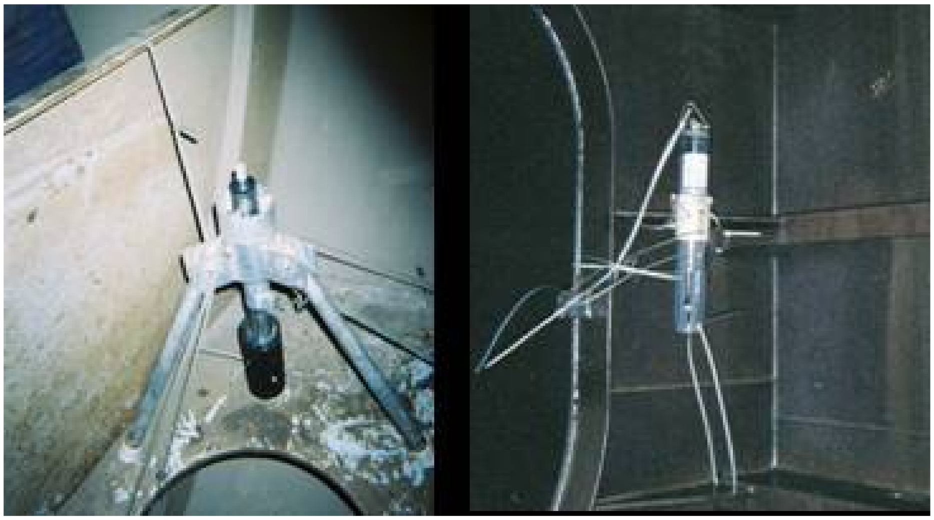


Fig 1.1 Photograph of multi-parameter sondes deployed within ballast tanks (left, In Situ Troll 9000E; right, YSI 6600EDS).

Instruments were deployed within the designated tanks during each entry into the Great Lakes and programmed to record data every 15 minutes for the duration of the voyage. Instruments were retrieved at the next entry into the Great Lakes and data downloaded for processing. Whenever possible multiple instruments were placed throughout the tank to capture spatial dynamics in water quality and particle movement. Locations were selected to compare the following areas within the tank: near and distant to the bellmouth, inner and outer bilge, and at and 2m above the bottom flooring (see Table 1.1). A GPS logging unit was also placed onboard and synchronized with the instrument clocks to record ship position.

Table 1.1. A summary of the deployment record for all of the water quality sondes used in the NOBOB BMP study.

Ship	Start Date	End Date	Tank	Location
Irma	7/28/04	11/30/04	4S	Bellmouth
			5S	Bellmouth
			hand-held	Over-the-side
Irma	4/26/05	9/6/05	4S	Bellmouth
			5S	Bellmouth
Irma	9/6/05	12/2/05	4S	Bellmouth
			5S	Bellmouth
Irma	12/2/05	4/28/06	4S	Bellmouth
			5S	Bellmouth
			5S	Mid-tank, inner, floor
			5S	Aft, outer bilge, floor
			5S	Aft, outer bilge, high
			5S	Forward, outer bilge, floor
			5S	Forward, outer bilge, high
Irma	4/29/06	9/01/06	5S	Bellmouth
			5S	Mid-tank, inner, floor
			5S	Aft, outer bilge, floor
Lady Hamilton	6/3/05	8/17/05	3S	Bellmouth
			5S	Bellmouth
			5S	Mid-tank, inner, floor
			5S	Mid-tank, inner, high
			5S	Mid-tank, outer bilge, floor
			5S	Mid-tank, outer bilge, high
Lady Hamilton	8/17/05	11/10/05	3S	Bellmouth
			3S	Mid-tank, outer bilge, floor
			3S	Mid-tank, outer bilge, high
			5S	Bellmouth
			hand-held	Over-the-side
(added to existing)	9/2/05	11/10/05	5S	Mid-tank, outer bilge, floor
			5S	Mid-tank, outer bilge, high
Federal Ems	9/29/2005	12/28/2005	5S	Aft ladder, by traps
(IETrap Experiment)			5P	Aft ladder, by traps
Marinus Green	9/1/06	9/14/06	1S	Aft ladder, by traps
(IETrap Experiment)			1P	Aft ladder, by traps

Results and Discussion

Instrument Deployments

Participating vessels provided excellent cooperation with regards to allowing access to the designated ballast tanks to deploy and retrieve instruments, and collect residual samples. Consequently, over the course of the study we conducted 8 instrument deployments on the two participating vessels (Table 1.1). In addition, on two occasions instruments were deployed in support of experiments under Objective 2.3 and 2.4 (see Task 2 section). The deployment duration for the instruments was typically on the order of 3 months, with 1-5 sondes deployed in each test tank. While the instruments generally functioned well, the degree of mud present, and the continual cycle of submergence and air exposure proved challenging to the performance of the optical sensors. Erratic data, along with the extreme amounts of data collected, make it difficult to present more than a summary of instrument results. We therefore simply highlight the types of information that was gathered by the sondes to illustrate how they can be used to verify ballasting activities and to capture water quality dynamics of the incoming source water and the ballast contained within the tank. Lastly, the data records obtained by the GPS units were of poor quality and not usable in conjunction with the instrument records. We used ballasting history logs and direct interviews with the ship's officers to compile a record of ballasting operations to compare with the instrument records from each deployment.

Our attempts to have the ship's crew use an independent hand-held version of the water quality sensors to directly characterize water quality conditions adjacent to the ship at the time of ballast intakes were also unsuccessful. The crew aboard the Irma used the instrument on one occasion, but then during a second attempt the instrument was damaged. Even though we quickly supplied a replacement, no further attempts were made to use the water quality sonde. We then supplied the crew of Lady Hamilton with a sensor, but again the crew failed to use the instrument. The combination of the ship activity required during port operations and unfamiliarity with the scientific equipment prevented them from using the equipment as intended.

Verifying Ballasting Events

Time series graphs of both salinity and depth recorded by the instruments (Fig. 1.2) were compared to the information recorded on log sheets that we provided to the ship to record all ballasting activities for the designated tanks. Several interesting features in the time series data are worth noting. First, it requires an examination of both depth and salinity to identify and fully understand all of the ballasting activities that were conducted. For example, in Figure 1.2, five different ballasting activities occurred during this deployment, and inspection of both time series is needed to differentiate between general port-side ballast events, mid-ocean ballast water exchange, and saltwater flushing. On September 10 there is a rapid decrease and then increase in depth but no change in salinity. These data indicate a mid-ocean ballast exchange event where the original ballast was also at seawater salinity. In contrast, the event on October 6 showed very little change in depth but a sharp change in salinity. These data indicate a saltwater flush was performed on the tank while it was essentially empty (i.e., in NOBOB condition).

Table 1.2 is a comparison of instrument data events and the ballast operation log for the MV Irma for the period of July – November 2004. All records for both vessels are provided in Appendix 2 at the end of this report. The ships logs were generally quite accurate and could be easily matched to the instrument records. However, specific times of ballasting events do not match because ship logs record local time and the instruments maintain a running clock based on Eastern Standard Time and sometimes ship logs will select a single time when ballasting occurs in stages. On a few occasions we did note discrepancies that could be easily explained by minor activities, such as adjustments of trim and heel that may have been omitted from the manual records as it not customary to log such events. In addition, some instruments were placed in locations close to the bellmouth where residual water will pool when the ship is trimmed by the stern, resulting in a record that is unrelated to additional ballasting activities, instead caused by cargo operations in port or weather conditions at sea. It should also be noted that the depth sensor on instruments is not zeroed to sea level, so in the example provided in Table 1.2, a depth reading on 08/02/04 of 33.4 represents an empty tank, while the depth reading of 51.0 represents the addition of ~17.6 feet of water. In Table 1.2 different ballasting events are highlighted in colors that denote the salinity of the source water. The red indicates ballasting with freshwater (conductivity <1000) and the green ballasting with seawater (conductivity > 50,000). The blue color designates ballast exchange or flushing that was either required by regulation or was part of our recommended BMPs. For the voyage shown here, the only time the vessel ballasted with freshwater was during her off-loading in the Great Lakes. All other ballasting events prior to her return were with saltwater, and twice she conducted a mid-ocean exchange or flush. Consequently the residual water in her tanks should have posed a minimum risk for potential transfer of freshwater organisms.

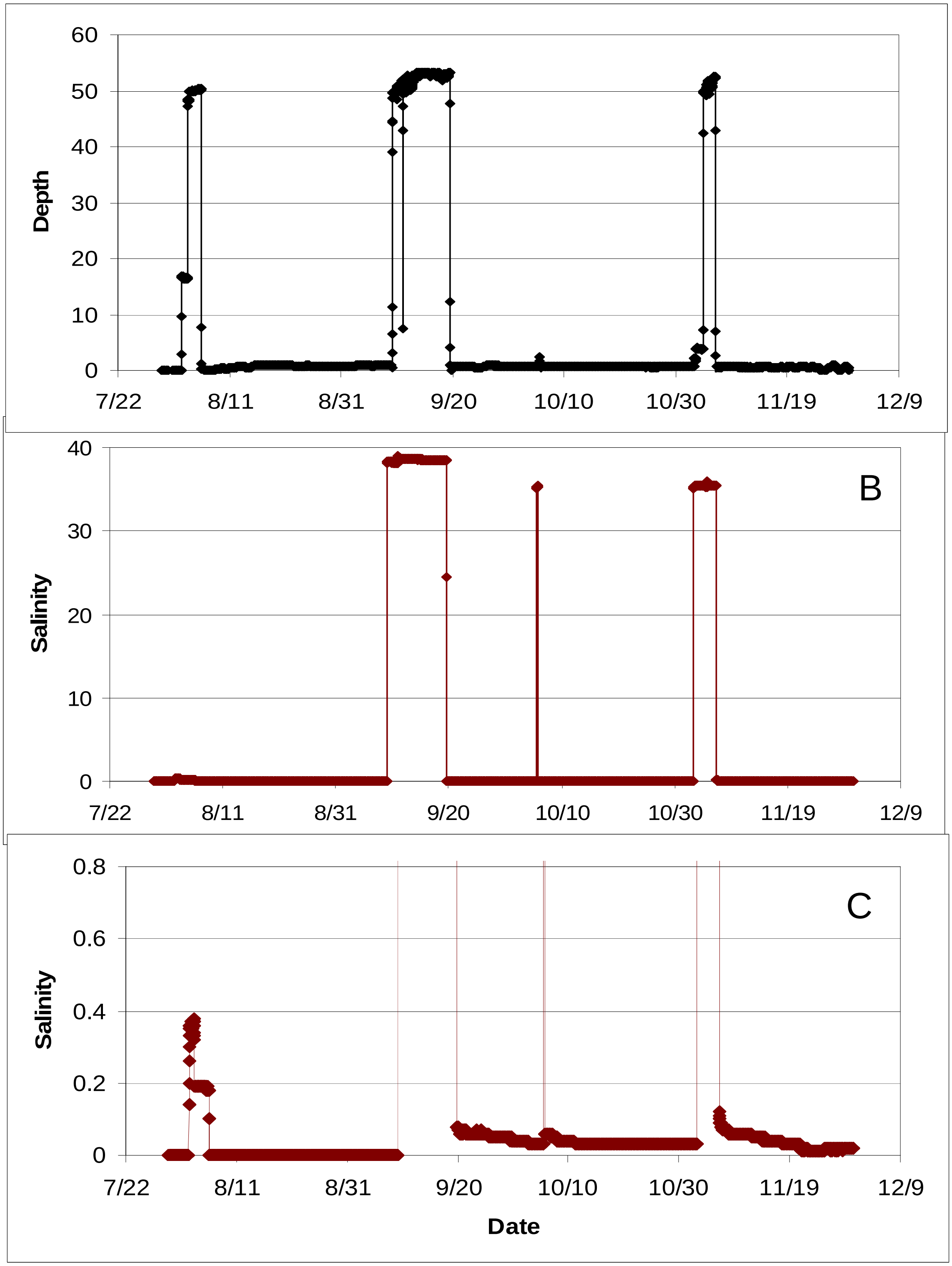


Fig.1.2. Time series of ballasting events recorded by a multi-parameter sonde moored within a ballast tank of the MV IRMA during a voyage from July to November 2004. Panel A; depth in feet. Panel B and C are both salinity time series but plotted at different scales to clearly show the initial ballasting event, which was in freshwater.

Table 1.2. Ballasting history summary for the MV/IRMA for one deployment period covering July through November 2004, comparing the ship's ballasting logs against our recorded instrument data.

Sonde Data				**Ship Ballast Logs**			
Date	**Time**	**Depth**	**Conductivity**	**Date**	**Local Time**	**Port**	**Ballast Activity**
7/29/04	Instruments Started			29-Jul		Cleveland, US	
8/2/04	10:31	33.4	1				
8/2/04	18:01	51.0	289	2-Aug	0805 - 0930	Burns Harbor	Ballast FW
8/3/04	9:01	51.0	294				
8/3/04	10:01	82.7	405			Burns Harbor	Ballast FW
8/5/04	15:31	82.4	340				
8/5/04	17:01	33.7	1	6-Aug	1530 - 1715	Thunder Bay	Deballast
8/26/04	7:31	34.2	0				
8/26/04	9:01	74.9	56040	26-Aug	1400 - 1530	Marseille, France	Ballast SW
8/26/04	10:01	47.8	56050			Adjusting trim at port	
8/31/04	5:31	52.0	56220				
8/31/04	8:01	33.2	30	31-Aug	1310 - 1440	Manfredonia, Italy	Deballast
9/8/04	7:01	34.4	11				
9/8/04	10:31	80.4	57050	8-Sep	0730 - 0850	Manfredonia, Italy	Ballast SW
9/10/04	22:01	84.5	57050				
9/10/04	23:01	40.2	57050	12-Sep	0205 - 0340	Mid Ocean	SW Exchange
9/11/04	0:31	86.6	58110				
9/13/04	20:01	87.0	57720				
9/13/04	22:31	34.2	217	14-Sep	0300- 0510	Ashdod, Israel	Deballast
10/6/04	2:01	34.1	63				
10/6/04	2:31	33.8	53930	6-Oct	0430 - 0800	Mid-ocean	SW Flushing
10/6/04	5:31	34.2	182				
10/30/04	16:31	34.2	62				
10/30/04	18:31	83.6	54830	30-Oct	1735 - 1920	Aratu, Brazil	Ballast SW
11/6/04	21:01	82.3	55290				
11/7/04	0:01	33.2	87	6-Nov	2210 - 0045	Maceo, Brazil	Deballast
11/30.04	End Deployment			30-Nov		Cleveland, US	

Water Quality Characterization

Instruments were also used to examine variations in water quality of the incoming ballast water, and to examine how sediments move within the tank during ballasting and deballasting operations and when underway. In general, ballast from freshwater sources had significantly higher turbidity, ranging from 10 – 150 NTU, with typical instrument readings of around 50 NTU during ballasting. Saltwater ballast sources had much lower turbidity levels, ranging from 2 – 12 NTU, with typical instruments readings around 7 NTU during ballasting. We did not differentiate these trends to specific locations of the ballast, but obviously freshwater sources represent ports located on river systems and saltwater sources represent coastal ports or open-ocean sources of ballast. The main point here is that ballasting in riverine ports will add a significant amount of sediment, as well as freshwater organisms, to the tank. It would therefore be beneficial to minimize the amount of ballast added at these riverine ports and to flush these tanks with clean offshore saltwater at the soonest opportunity.

In addition to bringing in sediments, incoming ballast water also carries significant quantities of phytoplankton, zooplankton, and microbial organisms. These findings are discussed in more detail in Task 2 sections, but instrument records corroborate the initial spike of phytoplankton (as measured by chlorophyll-a,) and also show the rapid decay in the phytoplankton community during the storage period of the ballast water within the tank (Fig. 1.3). The later result is not surprising given the total darkness of the tanks. Overall, differences in chlorophyll concentrations among ports and source water followed similar patterns to that for turbidity concentrations. Chlorophyll concentrations were generally much higher for freshwater ballast sources (2 – 6 µg/L) than for saltwater sources (typically 1-2 µg/L).

We also frequently observed a rapid decline in the dissolved oxygen concentration in the ballast water while in the tanks. The rapid rate of oxygen consumption presumably results from a high biochemical oxygen demand associated with the decay or organic material present in the incoming water and sediment, as well as that resident in the ballast tanks. However, the rate of oxygen depletion was also strongly temperature dependent and in the colder months almost no oxygen decline was observed. For example, figure 1.3 shows that almost 90% of the initial oxygen content was lost in 10 days. The water temperature during this period ranged from 22 – 28 ^{o}C. In contrast, no decline in dissolved oxygen concentration was observed during a ballasted event in December (Fig. 1.4) when temperatures were only between 3 - 5 ^{o}C.

The turbidity time-series in figure 1.3 shows that a significant amount of sediment can be resuspended inside the tank. The two small spikes, one on ~10/01 and one on ~10/03, likely are weather-related, while the large increase at the end of the deployment period coincides with deballasting. Such an increase in turbidity during deballasting events was fairly common in our instrument records.

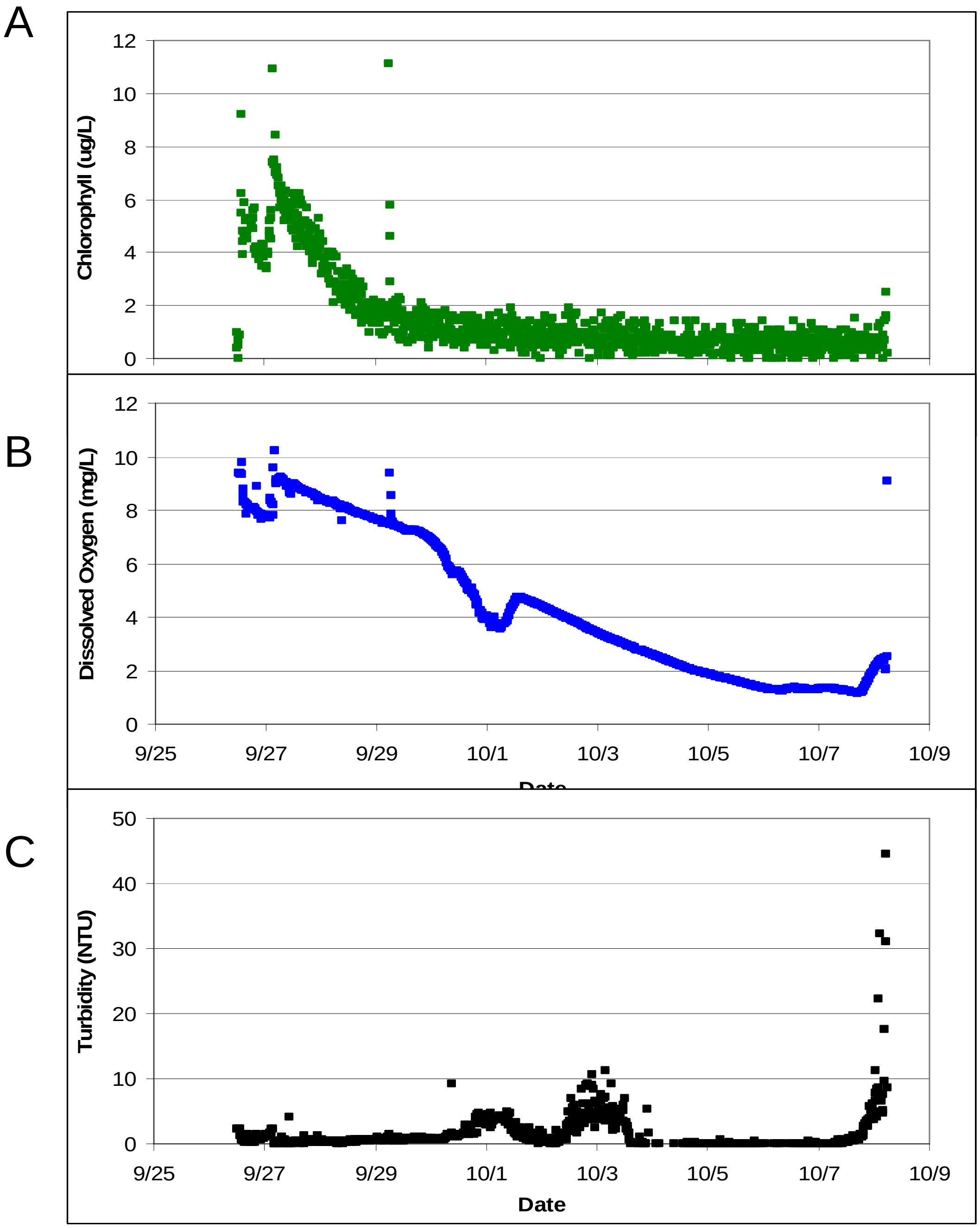


Fig. 1.3. Time series for water quality conditions of entrained ballast water over an eleven day period from September 26 – October 8, 2005. The ballast water source was from the Port of Tarragona, Spain.

.

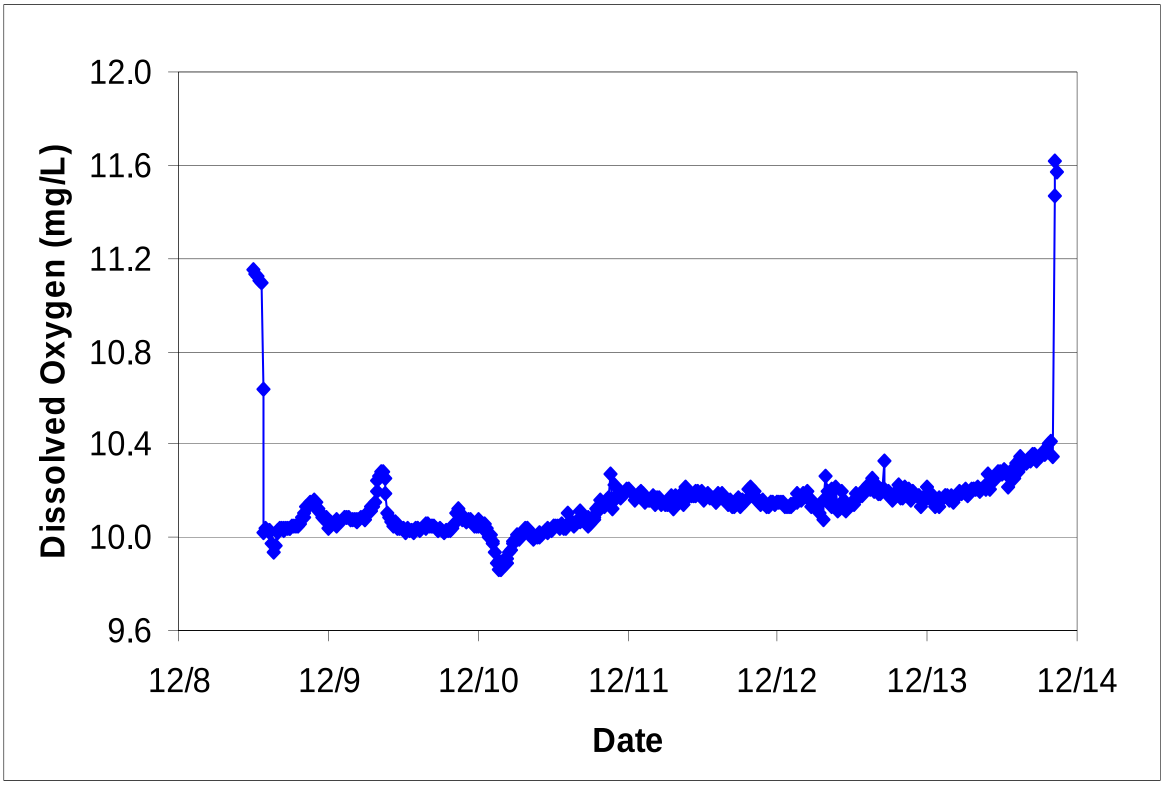


Fig. 1.4. Time series of in situ dissolved oxygen concentration for ballast water during a winter time ballasting event.

<u>Summary of Ballasting Activities for Participating Vessels</u>

In total, 40 ballasting operations where conducted by the two participating vessels during the course of this study (Table 1.3). Two of the operations were for required BWE and three were specifically for our proposed BMP enhancement of a saltwater flush while operating in a NOBOB condition. Of the remaining 36 port-based ballasting events, 13 of them occurred in saltwater ports, 6 in brackish water ports, and 17 in freshwater ports. Consequently, the source contributions of organisms to the ballast tanks was quite variable and as described in Task 2, each sample of ballast water and sediment residual represented a mixture of these ballast sources, thereby limiting the direct interpretation of specific BMP activities. In general, the number and frequency of saltwater flushing events was much lower than planned for the study.

Table 1.3. Summary of ballasting events for the MV Irma and MV Lady Hamilton throughout the study period from July 2004 – July 2006.

	Total # Ballasts	BWE Events	Flushing Events	Saltwater Ballasts	Brackish Ballasts	Freshwater Ballasts
IRMA	28	1	2	9	3	14
Lady Hamilton	12	1	1	4	3	3

Objective 1.2: Assess the ability of the ship to apply stated Best Management Practices under operational conditions and determine whether local sources of water quality information was sufficient to guide desired ballasting decisions. (Reid, Johengen and Jenkins)

Overview

To our knowledge, no one has attempted to verify how regularly the practices identified in the current Code can be implemented under actual operating conditions. Safety and cargo-operation constraints are key factors regulating ballasting decisions by operating vessels. Furthermore, a ship's master may have very little local knowledge of specific water quality conditions within the harbor or port where he is operating. Histories of ballast management activities were evaluated using data from the water quality sondes as well as the detailed ballast records provided by the ship. In addition, we proposed to assess to what extent knowledge regarding local water quality conditions, whether obtained by local Port authorities or directly from the use of a project-supplied water quality sonde, might affect ballast decisions. The overall purpose of Objective 1.2 was to assess the utility and practicality of the management practices identified in the Shipping Federation's "Code of Best Practices for Ballast Water Management ('the Code,' Shipping Federation of Canada 2000)."

Results and Discussion

<u>Assessment of Best Management Practices (BMPs)</u>

Original Experimental Plan

For Objective 1.2 we proposed to conduct directed experiments to test the following established and proposed enhancements to BMPs for ballast water management for their effectiveness in minimizing both sediment accumulation and abundance of live freshwater organisms and viable resting stages:

1. Avoid ballasting sediment-laden water when possible (avoid ballasting near dredging activity; avoid ballasting in shallow waters where the propellers may stir up sediment; avoid ballasting in areas with naturally high levels of suspended sediments, e.g. river mouths, and delta areas, or in locations that have been affected significantly by soil erosion from inland drainage), Code BMP #6b-6d.
2. Avoid ballasting in areas with algal blooms or where pathogens are known to occur in high abundance, such as near sewer outfalls; Code BMP # 6a and 6f.
3. If ballasting has to be undertaken under any of the aforementioned circumstances, limit the consequences by minimize the number of tanks and amount ballasted if this can be done safely, and replace the water in those tanks as soon as the ship enters cleaner water (Code BMP enhancement).
4. Conduct ballast water exchange of each ballasted tank, as required by United States Coast Guard (1993) Regulations (Code BMP #3) and conduct a saltwater flush on each empty tank during each transoceanic leg throughout the shipping season. (Code BMP enhancement). "Flushing" is accomplished by adding a small amount of water to the tank and allowing it to slosh around as a result of the ship's rolling and pitching motion, to agitate and resuspend trapped sediments during passage, then discharge the water in

the open ocean. The process can be repeated a number of times if circumstances permit. However, flushing is not a practice included in the Code.

We planned to use two sets of port-starboard paired tanks for this study. One tank in each pair was to be the control, and the other was to serve as the treatment tank to which BMPs were applied at every opportunity. All four tanks were to be ballasted and deballasted at the same time, except that the control tanks were not to be subjected to flushing.

Over the two year field period for this project we found it impossible to implement our experimental plans as designed. Although the ships with which we obtained agreements for participation were very accommodating in allowing us access to their tanks and agreeing to maintain tank specific ballast logs throughout the experimental period, their operating plans changed throughout the period of the proposed experimental work. As a result, their ballasting operations did not accommodate our experimental design or specific flushing experiments. In general, both ships that we attempted to use went off their expected European-Great Lakes rotation and spent 2-3 months between visits that afforded us sampling opportunity, during which time multiple ballasting events occurred in each of the targeted ballast tanks. Thus we could never access the tanks “before” and “after” any particular ballasting or ballast management event. It was usually not known until the very last minute what the specific loading and ballasting situation was going to be at any port or during any transit and thus it was not possible to sample effectively. We thus implemented an alternative assessment in which we examined and assessed the theory and practicability of the BMPs outlined in the Code. Although not what we had originally intended, it is informative and serves as a very useful “reality” check.

Alternative Approach: an Assessment of the Theory and Practicability of BMPs for Ballast Water Management on Transoceanic Ships

What is a “Best Management Practice?”

In general, “best management practices” are defined as “effective, feasible (including technological, economic, and institutional considerations) conservation practices and management measures that avoid or minimize adverse impacts to natural and cultural resources (National Park Service 2000).” In the case of ballast, the Code consists of ten operational principals specifically aimed at reducing the AIS risk associated with ballast tank operations. Two key changes in operating philosophy reflected in the Code are 1) that the focus is on total ballast management, which targets sediment reduction as well as ballast water, and 2) the ballast management activities outlined in the Code are to be conducted as often as practicable. This was a significant expansion of the philosophy that originated in the early 1990s that focused exclusively on ballast WATER.

Implementation of “the Code”

An agreement between the shipping industry, the Michigan legislature, and later the Seaway authorities, required a commitment by the owners and operators of ships entering the Seaway and the Great Lakes to conduct ballast management whenever practical and at every opportunity in order to:

- ensure that residual ballast on board will be subjected to practices in the Code
- minimize sediment accumulations in ballast tanks, and
- where mid-ocean exchange is practiced, subject fresh-water organisms to an extended exposure to salt water.

The principles outlined in the Code came from several sources, especially specific practices engendered in IMO resolutions A.774 (IMO 1993) and A.868 (IMO 1997), but also regulations implemented by the State of California and the State of Washington, and several other source materials (I. Lantz, pers. comm..).

Dissecting the Code

The Code (Appendix 1) can be divided into Regulated Actions (Item 2-5, 7), Cooperative Actions (Items 8-10), and Environmental Precautionary Actions (Items 6a-f):

Regulated Actions (Item 2-5, 7)

2. Regular inspection and removal of sediment from ballast tanks
3. Ballast water exchange
4. Record keeping and reporting
5. Provide information and logs to authorized inspectors and regulators for the purposes of verifying the vessel's compliance with this Code of Best Practices
7. Disposal of accumulated sediments

Since these are regulatory items, adherence by each individual ship is now mandatory. However their incorporation into the Code reflects acceptance by the industry of the seriousness of the ballast water invasive species problem and the importance of and reasons for the regulations.

Cooperative Actions (Item 1, 8-10)

1. Conduct ballast water management whenever practical and at every opportunity
8. Foster and support scientific research sampling programs and analysis
9. Cooperate and participate in standards development and treatment systems testing and approval
10. Strive toward global, integrated ballast water management strategies in conformity with internationally agreed principles that respect national and regional aquatic ecosystems.

Although these are statements of "cooperation" rather than specific practicable actions, they serve to engage the ships and their owners and operators as partners in achieving a mutually beneficial solution to the ballast tank AIS vector problem. Number 1 is critical to maximizing the effectiveness of BWE and ballast tank management and commits the industry to using ballast water management more often and more consistently than required by regulations, but not necessarily 100% of the time. Numbers 8 and 9 are critical to achieving the development of effective treatment systems and practical standards.

Environmental Precautionary Actions (Item 6)

Minimize ballasting operations under the following conditions:

a. In areas identified with toxic algal blooms, outbreaks of known populations of harmful aquatic organisms and pathogens, sewage outfalls and dredging activity.
b. In darkness
c. In very shallow water
d. Where a ship's propellers may stir up sediment.
e. In areas with naturally high levels of suspended sediments
f. In areas where harmful aquatic organisms or pathogens are known to occur.

It is these Environmental Precautionary Actions that are the most critical components of the Code relative to potential immediate reduction in the risk of nonindigenous species being transported in ballast tanks prior to future implementation of effective on-board treatment. CFR Title 33, PART 151, Subpart D, §151.2035, establishes these (Item 6) and other practices as part of the mandatory ballast management program required by U.S. law and enforced by the U.S. Coast Guard. However, it is not clear how the actual use of these practices can be monitored or enforced, versus simply confirming that they are incorporated into the ship ballast management plan and assessing the level of familiarity with them by the captain and crew.

Analyzing Code Item 6: Theory and Practicability

Item 6(a): Minimize ballasting in areas identified in connection with toxic algal blooms, outbreaks of known populations of harmful aquatic organisms and pathogens, sewage outfalls and dredging activity.

In theory this will reduce propagule pressure, risks to human health, and reduce the likelihood of entraining dredge material during ballast water intake. In practice, the information necessary to follow this guideline is not generally available to ships. Individual ports can probably supply ships with information about local sewage outfalls and dredging activities, and many experienced Masters may already be familiar with major port characteristics. Ports would likely issue notices of communicable disease risks, such as cholera. However, in general there is no widespread formal “alert” system that provides advisories or information related to coastal and port water quality for ballast water decisions.

In formulating their Guidelines and the International Convention for the Control and Management of Ships Ballast Water and Sediment, IMO has recognized that the development and propagation of such critical information is the responsibility of every Port State, which would include both Canada and the United States, and this is an area where clearly much work is needed.

Item 6(b): Minimize ballasting in darkness.

In theory this will reduce the likelihood of intake of aquatic organisms that migrate from deep to shallow waters at night (diel organisms). In practice most ballasting occurs at docks in ports where diel migration may not be significant, depending on habitat quality at the docks. In general, the ability of a Master to restrict ballasting during darkness is almost completely dependent on the commercial requirements for loading and unloading. If a ship is on a standard daytime loading/unloading schedule, this practice can be observed, but if it’s on a 24/7 loading/unloading schedule, it is unlikely (and unsafe) that ballasting could be delayed at night. In fact, most ballasting cannot be delayed no matter when it is needed because of safety considerations – a ship must usually ballast whenever cargo activities dictate the need to balance weight and adjust trim. However, strictly speaking, at least partial compliance with this provisions (i.e., to minimize ballast) can be achieved if ships can and do limit ballast intake at night to the minimum required for safety, and complete the operation during daylight hours.

Items 6(c, d, & e): Minimize ballasting (c) in very shallow water; (d) where a ship’s propellers may stir up sediment; (e) in areas with naturally high levels of suspended sediments.

In theory this will reduce intake of mud and benthic organisms, including resting stages (diapausing eggs and spores). Depth information is readily available for all ports, although many ports are not natural deep-water ports and are therefore dredged to a controlled depth that is often only a few feet deeper than the draft of the ship. Also, ships have a high and a low (or shallow and deep) ballast intake. In shallow water the high intakes can be used to avoid sucking up mud from the bottom.

In practice the ability of a ship to exercise this option is subject to the commercial dictates of the cargo and voyage. Even where operating imperative allow it, a ship can only perform these options when local environmental conditions and/or work rules permit it. If it's the rainy season and the port is at a river mouth, the river plume is likely loaded with sediment. But if cargo is being discharged, a ship doesn't have much choice about taking in ballast, except to limit how much dirty water is actually ballasted. If the ship's voyage path, cargo, and weather condition allows, dirty water could be discharged and replaced with cleaner offshore water after leaving the port.

During the field work for this program (NOBOB-BMP) we worked with a ship that had to ballast at a berth where the water and sediment were known to be particularly degraded – a sustained condition of that berth. The Master limited the number of tanks used for ballast and the amount of ballast loaded was restricted to minimum required for safety. However, the Master was unable to discharge and exchange that water immediately after leaving the port because of an immediate transit through a high ship-traffic area and operational priority had to be given to safe navigation. The ship discharged the remaining cargo in Antwerp, Belgium and loaded additional river ballast water into the already dirty tanks to facilitate a move to another dock within the port, where she gradually discharged all ballast as new cargo was loaded. When this ship arrived as a NOBOB in the Great Lakes there was thick coating (5-6 cm) of very foul smelling mud.

Item 6(f): Minimize ballasting in areas where harmful aquatic organisms or pathogens are known to occur.

In theory this will reduce risks to human health and reduce transport of invasive microorganisms. In practice it comes down to whether the ship's Master has access to appropriate information. Port or local health authorities may issue notices of outbreaks of communicable diseases, e.g. cholera, that may be available to ships. Also, an experienced Master will know when specific ports have a history of poor microbial water quality (for example, Inchon, South Korea or Calcutta, India). But there is no network or international framework to provide this information on a regular and sustained basis.

Conclusions

Clearly, implementation of the Code can be problematic, especially for the environmental precautionary actions. Regardless of regulations, observance and application of BMPs is dependent on local conditions – working rules of the dock (24/7 vs. daylight), rainy season vs. dry season, river berth vs. sheltered harbor or deep water harbor, and thus hard to require 100% compliance or enforce without severe impact on commercial shipping. Many of the recommendations put forth in Item 6 of the Code of Best Management Practices require information on local water quality conditions that is not generally available to the shipping industry, or are often not practical to conduct due to cargo loading and unloading requirements. Acceptance and implementation of the Code by the shipping industry must be understood primarily as a commitment to make a "good faith" effort.

Therefore, while BMPs, if consistently and repeatedly applied, can reduce the risk of introductions from NOBOB vessels by minimizing the amount of sediment and associated organisms that are transported within ballast tanks, the practical realities and limitations associated with vessel operations makes the existing BMPs inadequate as the lone strategy for reducing the risk of nonindigenous species introductions from NOBOB vessels. The designation and routine use of saltwater flushing as an official BMP would greatly improve the protection framework for the Great Lakes, <u>if aggressively implemented</u> by the shipping industry.

References

IMO (International Maritime Organization). 1993. Resolution A.774(18): "Guidelines for preventing the Introduction of Unwanted Organisms and Pathogens from Ships' Ballast Water and Sediment Discharges." November.

IMO (International Maritime Organization). 1997. Resolution A.868(20): "Guidelines for the Control and Management of Ships' Ballast Water to Minimize the Transfer of Harmful Aquatic Organisms and Pathogens." November.

Johengen, T. D.F. Reid, G.L. Fahnenstiel, H.J. MacIsaac, F.C. Dobbs, M. Doblin, G. Ruiz, and P. T Jenkins. 2005. A Final Report for the Project "Assessment of Transoceanic NOBOB Vessels and Low-Salinity Ballast Water as Vectors for Non-indigenous Species Introductions to the Great Lakes." National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, and University of Michigan, Cooperative Institute for Limnology and Ecosystems Research, Ann Arbor, 287 pp.

National Park Service. 2000. Yosemite Valley Plan, Supplemental Environmental Impact Statement, Volume Ib Part 2: Environmental Consequences.

Shipping Federation of Canada. 2000. Code of Best Practices for Ballast Water Management. September 28, 2000. URL:

(http://www.shipfed.ca/eng/library/other_subjects/ballats_water/BallastWaterBestPractices.html)

United States Coast Guard. 1993. Ballast water management for vessels entering the Great Lakes. Code of Federal Regulations 33-CFR Part 151.1510.

Objective 1.3: Assess the amount and characteristics of residual sediment in the ballast tanks after each round-trip voyage; identify changes in residual sediment accumulation, volume, and deposition within tanks.

Overview

We proposed to evaluate the effects of various management practices on the accumulation and retention of sediment in the tanks by combining careful records of ballast events provided by the ship, detailed records of the characteristics of ballast water intake and discharge obtained from the in situ instrumentation, and direct observations and measurements of changes in sediment accumulation and characteristics in each of the tanks. The goal was to compare control tanks (for which BMPs were NOT used) with BMP tanks, the latter being the tanks that have been managed to the greatest extent possible using the designated Best Management Practices. Time series of turbidity recorded by in situ instruments placed at different locations and heights within the tanks were also used to examine the dynamics of sediment resuspension and mixing during ballasting and de-ballasting events.

Results and Discussion

As discussed earlier, significant difficulties were encountered in trying to implement our original experimental design. These difficulties reflect the complexity of commercial ship operations and the difficulty of trying to conduct well planned scientific experiments and observations on board a commercial ship. Both of our participating ships had been committed to the project by the owners/operators, with stated expectation of regular voyages between the Great Lakes and Western Europe for the duration of the project. Under normal operating circumstances that would mean each vessel would conduct between 4 - 5 trips into the Great Lakes each year. It was our intention to board and enter the instrumented tanks each time a ship returned to the lakes to document any changes in condition with respect to sediment accumulation and distribution after orchestrating a specific management procedure with the Master on the transoceanic voyage. However commercial considerations came into play that frustrated this effort, and in fact took both ships away from the Great Lakes trade for protracted periods of time. During these extended voyages numerous ballast events occurred with little to no difference in the procedures applied to the designated control versus treatment tank.

<u>MV Irma</u>

Our initial collaboration was with the Polsteam vessel, MV Irma, during the 2004 season. We installed instruments in the #4 and #5 starboard ballast tanks (see Figure 1-5), with #4 tank intended to be a control tank, #5 the experimental tank. Both tanks were carefully mapped with respect to sediment accumulation at the beginning of our experimental run. Unfortunately her first voyage from the Great Lakes to Europe took her to Italy where she was diverted from her expected trading pattern. Consequently a period of almost four months elapsed between when she started the experiment and the next observation of the condition of the tanks by members of the research team, during which time there had been three separate ballasting and deballasting operations undertaken, a sequential exchange carried out in the Mediterranean Sea on route from Italy to Israel and a deep ocean flushing of the tanks performed in the South Atlantic en route to Brazil (Table 1.1).

We were able to confirm that there had been a redistribution of sediment within the tanks and an estimated overall reduction in the amount of accumulated sediment of between twenty five and thirty percent. However it was neither possible for us to confirm which particular management process had been the most effective in reducing the amount of sediment in the tank, or what fluctuations in accumulated volume occurred with individual ballasting events.

The instruments were removed at the end of 2004 prior to the ship proceeding to dry-dock for routine maintenance during the winter. This was only her second major dry-docking, so there was no requirement to clean the double bottom tanks for survey purposes. On her return to Cleveland in April 2005 it appeared that there was a more general distribution of sediment throughout the bottom of the tanks and on horizontal surfaces but no significant change in overall volume. Unfortunately, again in 2005 the ship diverted from her expected Great Lakes trade pattern and was gone more than four months. During that period seven significant ballast events occurred before her next call in Cleveland in September, and then she was gone again for three months and eight events before her last call in the Great Lakes December 2005. It was impossible for the team to relate the effectiveness of individual management practices or specific ballasting events to conditions in the tanks with respect to residuals. However, there appeared to be a further overall reduction in sediment accumulation during the year, probably as a result of most of her ballasting operations being conducted in deep water or in sheltered ports.

MV Lady Hamilton

During the course of the project it appeared from information provided to us by the operators that a second bulk carrier, MV Lady Hamilton, would be more consistently employed in the Great Lakes/Europe trade in 2005 than MV Irma. We therefore switched our focus to this ship and took an initial set of samples and tank surveys in June 2005. However on the next six consecutive passages she was fully loaded and unable to submerge her loadline, and thus, was not able to conduct any saltwater flushing experiments. Before her seventh passage we were advised by the operators that the ship would be returned to owners and we were consequently obliged to remove the instruments.

This is a ship that routinely carries more than one hundred tonnes of unpumpable ballast, partly as a result of her ballast system design and partly the result of some inexperience on the part of the officers at the time. It was nearly impossible to accurately assess the amount and distribution of sediment in the presence of several inches of water. Thus no attempt was made to quantitatively assess changes in sediment accumulation or distribution along the bottom of the tanks. However, some anecdotal descriptions of changes sediment accumulation as related to ballasting history are provided below.

Ballast logs provided from the ship indicate that in the five month period between December 2005 and April 2006 the ship had made a transoceanic voyage to Europe fully loaded, five short sea voyages on the North Sea and Baltic coasts, a transoceanic voyage from the Baltic to Colombia and then a voyage to the US Northeastern seaboard. In this time, there were nine significant ballasting events, and while there had been no opportunity for deep-ocean flushing during the voyages, one tank flushing was performed while at anchor awaiting the loading berth at Porto Pradeco, Columbia. This was done following a short voyage from Barranquilla, Columbia where there had been no option other than to take full ballast at a riverside berth in a river that was extremely muddy and swollen from very heavy rain. Although all the dirty ballast was replaced within 24 hours, it was evident during our next sampling survey of the tanks that a significant amount of mud/sediment had settled out, particularly in the forward and after bays of the hopper side tank, and to a lesser extent along the bilge area of the hopper side tank where the water column is tallest. Accumulated mud was also noticeable on all horizontal surfaces throughout the hopper side and double bottom areas, and evident that due to the glutinous nature of the mud that it had neither been dislodged by the exchange at the anchorage, or in a subsequent ballasting and deballasting event Fall River, Massachusetts. It appeared unlikely that these deposits would be removed through ballast management procedures and would eventually require some mechanical action such as the use of high-pressure hoses to dislodge and/or re-suspend the material to pump it out. It was estimated that the total sediment accumulation had increased by approximately ten percent in the period between observations, most of which can probably be attributed to the Barranquilla ballasting.

These observations provide a cautionary note to one of the proposed enhancements to the Code of Best Management Practices. Observations during the Great Lakes NOBOB Assessment (Johengen et al., 2005) suggested that less accumulation of mud would result if a ship replaced 'dirty ballast' at the earliest possible opportunity after intake. While this practice is certainly still logical and generally valid, its effectiveness is clearly related to, and dependent on the nature of the sediment. It may be difficult to remove a significant amount of newly deposited mud, even with only a 24-hour delay after intake. Of course in addition to the mud there may have also been a significant addition of organisms with this riverine ballast and thus the open-ocean exchange of this ballast may still have had a significant, but unmeasured beneficial effect.

Sediment Dynamics

In situ water quality sondes, equipped with turbidity sensors, were used to examine particle loading and re-distribution within the tanks. Comparisons of the time-series data for instruments placed at various locations throughout the tank (Fig 1.5) helped elucidate the causes of the sediment distribution patterns noted by visual inspections during our in-tank sampling. For example, a comparison of two sensors placed on the floor versus 2 m directly above (Fig. 1.6A) revealed that residual sediment present in the tank mixed with the incoming ballast water to cause extremely turbid conditions near the floor of the tank. This material did not, however, appear to mix vertically, as the upper sensor recorded maximum turbidity levels of 50 NTU versus 400 NTU for the floor-mounted sensor. It is also apparent that resuspended material during the ballast event quickly settled out over time-scales of just a few hours (Figure 1.6A). We also observed significant differences in the amount of sediment transport based on the horizontal position within the tank (Figure 1.6B). The sensor in the forward area of the tank show a much greater increase in turbidity as open-ocean water was added to the tank and the sensor closest to the bell mouth shows the least amount of suspended sediment. These differences are consistent with direct visual observations that sediment accumulation (retention) is much more significant in the forward sections of tanks, furthest from the bellmouth and in the outer bilge areas of the tanks. Particles that may have deposited in regions closer to the bellmouth appear to be scoured from the floor during ballasting events (ballast water flow) and either redistributed within the tank or discharged.

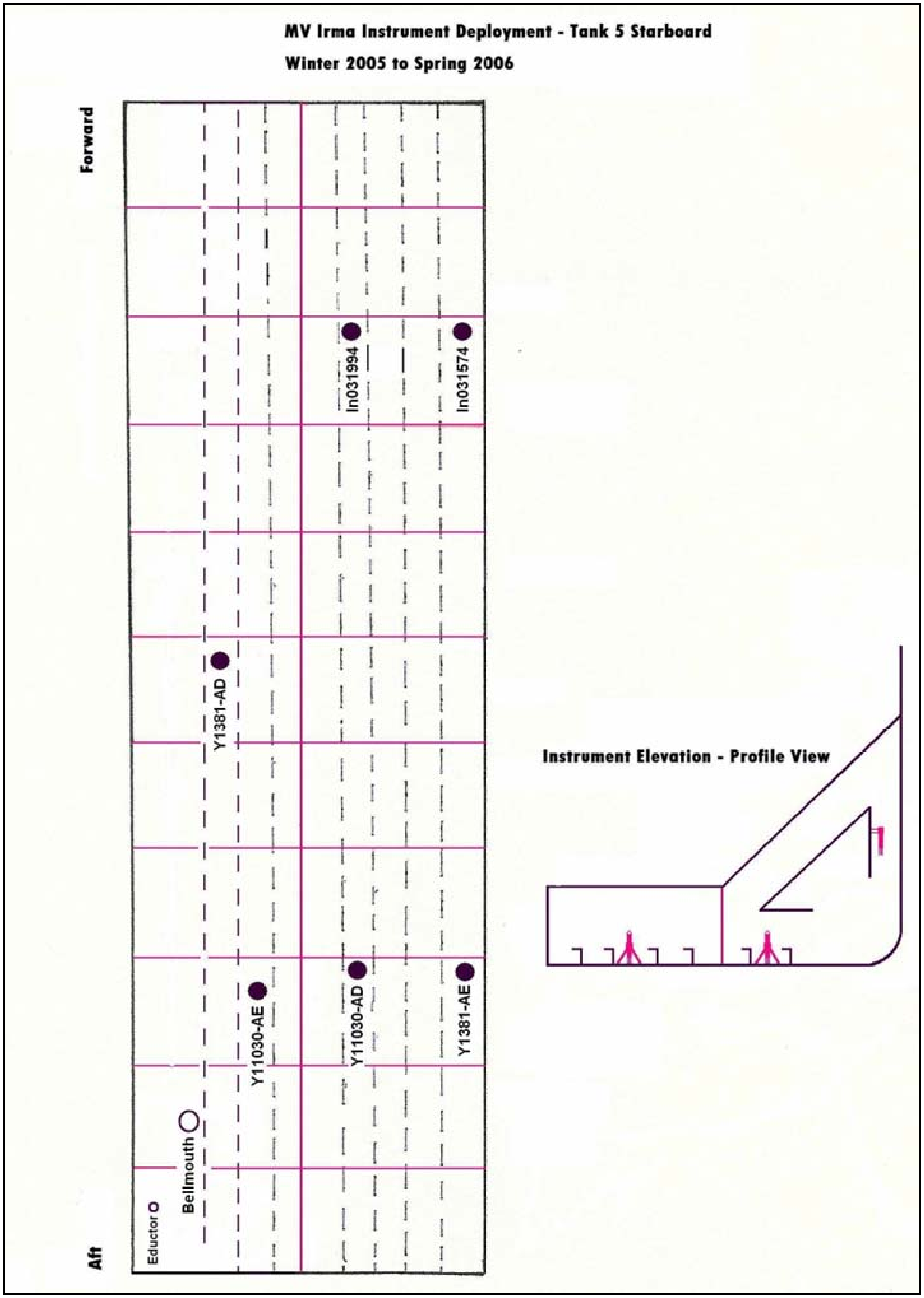


Fig. 1.5. Schematic diagram of the ballast configuration for the MV Irma and the locations of in situ water quality instruments used to examine sediment dynamics and water quality characteristics of ballast water during and between ballast intake and discharge events.

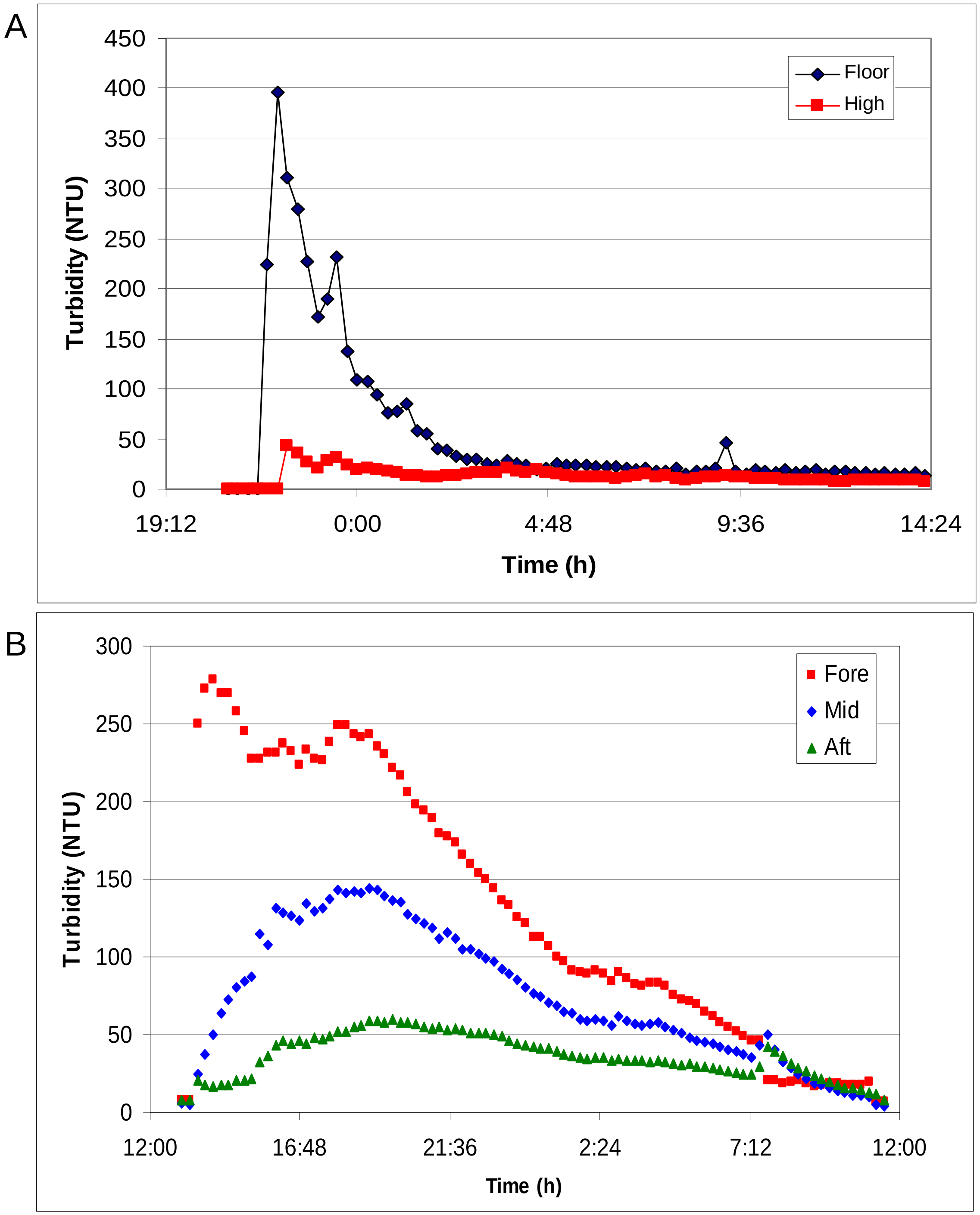


Fig. 1.6. Patterns of resuspension and particle movement within the tank as measured with turbidity sensors. (A) Comparison of turbidity levels near the tank floor and 2m off the floor during a typical ballasting event; and (B) comparison of turbidity levels at various floor-level locations within the ballast tank during a saltwater flushing event (Aft=closest to bellmouth; Fore = furthest from bellmouth).

We see evidence for resuspension of sediment in most of deballasting events. For example, in Figure 1.7 there was a spike in turbidity recorded by a sensor near the bellmouth, but not until the water depth dropped below ~2 ft. Based on observations of scouring and deposition patterns in many ballast tanks, we believe that as the water level drops below the top edge of the longitudinal stiffeners (structural members along the bottom that are 10-14 inches tall), the flow is forced into narrow high-speed jets that have sufficient energy to significantly resuspend accumulated sediment along the flow paths. Once deballasting ceased (pumps turned off), the resuspended sediment settled out over just a few hours (Figure 1.7).

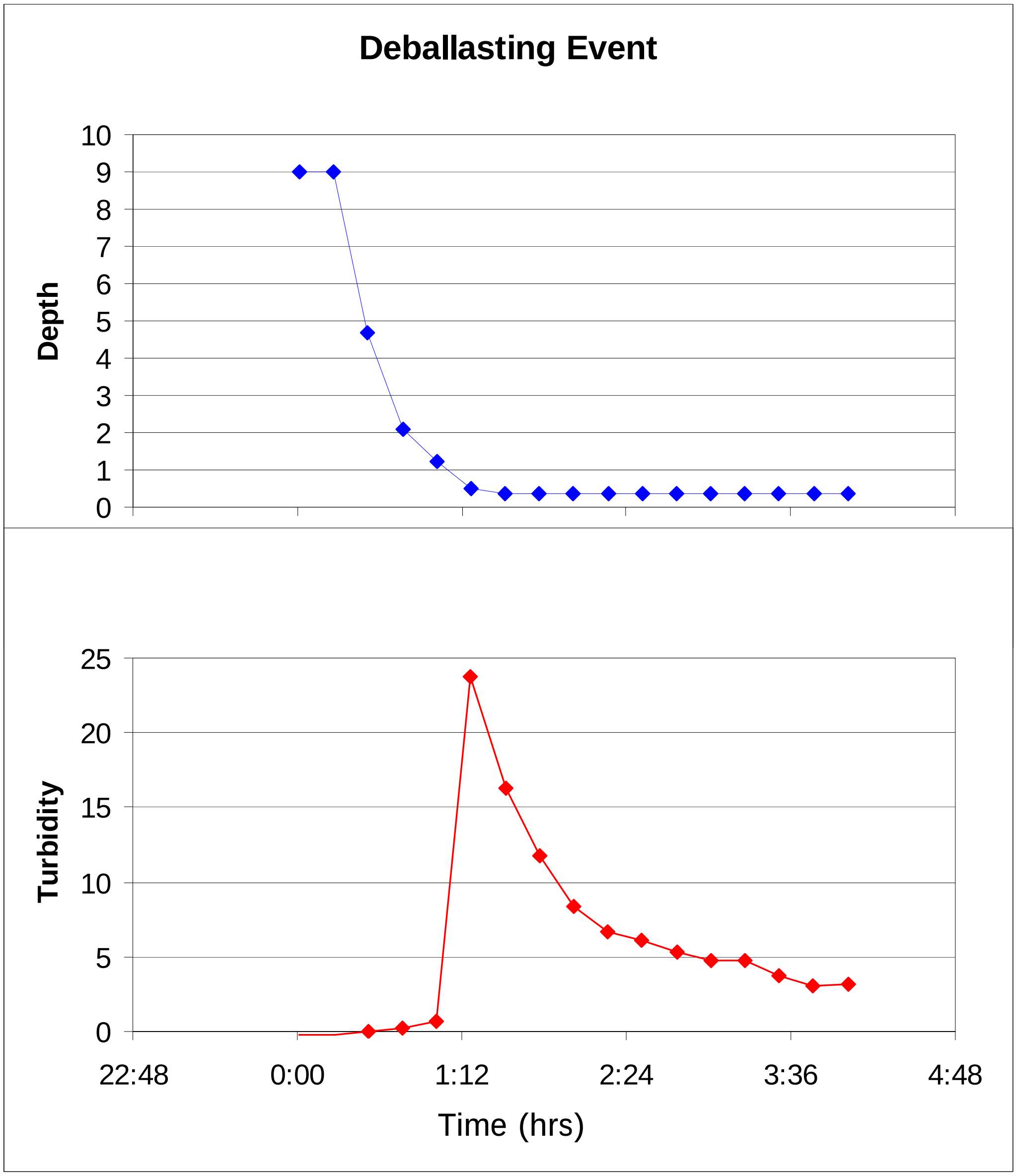


Fig. 1.7. Time series of turbidity during a ballast discharge event. Top panel is water depth (in feet) within the ballast tank. Bottom panel is recorded turbidity for a sensor placed near the bellmouth discharge point.

It is also interesting to note that the high turbidity levels generated during the saltwater flushing event depicted in Figure 1.6B, presumably due to the clean open-ocean water resuspending residual sediment, began to decrease after only six hours and by the time the water was discharged, turbidity at all locations had returned to near background levels. While this only represents a single experiment, it appears that the motion of the ship was inadequate to keep particles in suspension during the 24 hour interval. This may be an important consideration regarding the ultimate effectiveness of this practice for keeping the ballast tanks clean of sediment deposits.

Further evidence of the importance of ship motion for keeping particles in suspension was noted in another set of instrument records from a partially ballasted tank (Figure 1.8). Turbidity increased after the ship left the Seaway channel and moved into the Atlantic Ocean (Oct 3-5). There was a temporary decrease as cleaner offshore oceanic water was added to the tank on Oct 5 and perhaps a decrease in motion on the ship, with a return to higher turbidity as the ship continued it's Atlantic crossing, likely reflecting resuspension as the flush water sloshed around the tank due to ship motion. These results support our hypothesis that flushing empty ballast tanks with small volumes of clean off-shore water while the ship is in transit can scour and resuspend residual sediment.

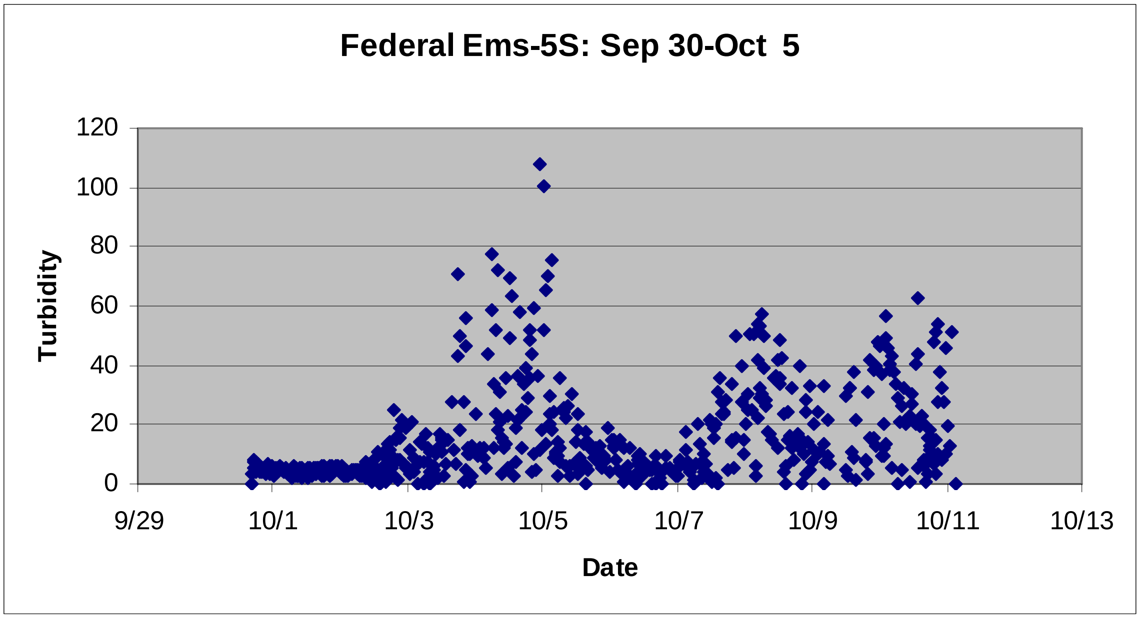


Fig. 1.8. Turbidity time-series for a partially filled ballast tank after initially taking ballast in Montreal and then heading across the Atlantic Ocean.

Conclusions

There are many, often uncontrollable factors that impact the effectiveness of BMPs to reduce the threat of transporting nonindigenous species posing a high risk to the Great Lakes, or to reduce the accumulation of sediment. Structural design of the tanks, the ballasting system, operational variables, and environmental variables all come into play. Much of the information needed by a ship to implement several key BMPs is not generally available, while specific actions required by other BMPs are simply not always practicable.

Placing instruments in ballast tanks can confirm ballast and deballast events and can provide useful information and insight into the ballast tank environment during and between ballast events.

Turbidity measurements provided evidence of resuspension during ballast flow events and supported conclusions based on visual observations. Instrument and observational data suggests that significant resuspension and removal of sediment occurs but in variable amounts affecting between 30% and 80% of the bottom area, depending on the previous ballasting and sediment management history of the tank. However, interpretation of turbidity measurements as a direct measure of the quality of the incoming ballast water must be approached cautiously. Sensors for that purpose should be placed fairly close to the bellmouth.

Instrument records confirm that saltwater flushing can promote sediment resuspension because of the scouring activity in a partially filled tank subjected to rolling action while on the open-ocean. Saltwater flushing should be considered a useful and advantageous practice when it can be performed safely and legally.

Task 2. Assess the effectiveness of specific ballast management practices to reduce the density and viability of organisms and resting stages.

The goal of this task was to compare results of detailed biological characterizations of the sediment and water residuals between BMP-treated and control ballast tanks on our participating NOBOB vessels. Biological characterization included: determination of the abundance of live invertebrates in sediment and water residuals; abundance and viability of invertebrate resting eggs; detection of harmful microalgae; detection of bacterial and protozoan pathogens likely to be transported and survive discharge in freshwater environments; and phytoplankton composition and viability in water and sediment residuals. Particular emphasis was placed on examination of the effects of seawater exposure (via open-ocean flushing) on resting egg density and viability.

Objective 2.1: To quantify the presence, abundance, and viability of pathogenic microbes in control versus BMP-treated NOBOB ballast tanks.

Overview

The original goal of ODU's portion of this project was to quantify the presence, abundance, and viability of pathogenic microbes in control versus BMP-treated NOBOB ballast tanks. The vagaries of the shipping trade, however, were such that the ships we engaged were unable to assist us as anticipated. Thus, in a reflection of the ships' altered schedules, the character of our experimental design, one predicated on sampling "paired-tanks", was altered completely. Instead of sampling "control" and "treatment" tanks, we collected a chronological series of samples to the extent we could. Our strategy for their analyses was to evaluate trends and investigate possible correlations between microbiological measures and the ships' ballasting history.

To that end, we screened residual water and sediment samples for the presence of selected indicator organisms and pathogens, including bacteria (enterococci, *E. coli*, *Vibrio cholerae*, intestinal protozoans (*Giardia lamblia*, *Cryptosporidium parvum*, *Enterocytozoon bieneusi,* and three *Encephalitozoon* spp.*)*, and the harmful algae *Pfiesteria piscicida* and *P. shumwayae*. These various measures and methods are listed in Table 2.1 and details are provided in the following section of text.

We were ably assisted in these analyses by colleagues whose collaboration we appreciate and acknowledge here. Dr. Thaddeus Graczyk (Johns Hopkins University) was subcontracted to analyze samples for intestinal pathogens. Analyses for *Pfiesteria* spp. were performed under subcontract by Dr. Parke Rublee (University of North Carolina Greensboro).

The project dealt with a major shift in personnel at the end of 2004. At that time, Principal Investigators Martina Doblin and Lisa Drake left Old Dominion University for other positions. Their departure greatly changed the abilities and expertise in Dobbs's lab. As discussed with Project Managers Johengen and Reid, however, and communicated to the Great Lakes Protection Fund, Dobbs rebudgeted and partially supported a post-doc to assume Doblin's and Drake's roles. Despite our best efforts not all the project's original objectives could be met. Those diminished portions of the project were: 1) some of the planned assays for harmful algae and 2) the "live-dead" analyses of bacteria.

Table 2.1. Summary of Microbial Metrics Determined in NOBOB BMP Study

Pathogen or indicator species	**Method(s)**
Enteric bacteria (enterococci, *E. coli*)	Culture
Vibrio cholerae	Culture, biochemical testing, immuno-fluorescent antibodies, and PCR
Pfiesteria piscicida and *P. shumwayae*	Real-time PCR
Giardia lamblia, Cryptosporidium parvum, Enterocytozoon bieneusi, and *Encephalitozoon* spp.	Immunoassays, fluorescence in-situ hybridization, and PCR

Methods

Replicate samples of residual ballast water (typically 6 liters per sample) and sediment (300-400 grams per sample) were collected aseptically using a hand-pump and spatulas, respectively. Samples were stored in the dark at 4°C and shipped by overnight delivery to ODU for processing and analysis.

Detection of enteric bacteria.

Water samples were tested for enterococci and *E. coli* using the Enterolert and the Colilert-18 Test Kits (IDEXX Laboratories). We minimized the potential for marine bacteria to cause false-positive results by using the Colilert-18 method (Pisciotta et al., 2002) and diluting samples as recommended by the kit's manufacturer. We used known commercially obtained bacteria (*Enterococcus faecalis* and *E. coli*) as positive controls. These kits provide only qualitative data (i.e., presence/absence), but for two sets of samples (B-05314 and B-05336), we were able to quantify the numbers of these enteric bacteria using IDEXX's Quanti-Tray. In essence, this method uses a most-probable-number approach.

Detection of *Vibrio cholerae*.

Water (or porewater extracted via centrifugation) was filtered (0.45 µm pore size), the filter placed on TCBS agar, and incubated overnight at 35°C. The following day, yellow colonies (sucrose-positive) were picked from each filter and streaked onto LB agar to confirm isolation and provide colonies for confirmatory analyses. Counts of these putative *V. cholerae* served as the basis for the data presented in this report. Subsequently, the biochemical method of Choopun et al. (2002) was used to identify *V. cholerae* and final confirmation was made using PCR-based analysis of 16S-23S rRNA intergenic spacer regions (Chun et al., 1999). As positive controls for these analyses, we maintain cryopreserved, reference cultures of non-toxic *V. cholerae.*

Assay for *Pfiesteria* spp.

Molecular probing of samples for members of the *Pfiesteria* species complex is described in detail in Bowers et al. (2000). Briefly, PCR primers were designed to unique regions of the small subunit ribosomal DNA of *P. piscicida* and *P. shumwayae*. Samples (100 ml of unpreserved water) were drawn onto 25-mm glass-fiber filters and immersed in a CTAB lysis buffer at room temperature. Sediment was simply placed into 6-ml plastic vials. On arrival in Dr. Rublee's laboratory, DNA was extracted from water samples with chloroform and purified; DNA from sediment samples was extracted and purified using a commercial kit. Aliquots of purified sample DNA were then assayed by PCR and reaction products were visualized by agarose-gel electrophoresis and ethidium-bromide staining. Both positive (DNA extracted from cultures) and negative (no template) controls were run in every PCR reaction and gel.

Assays for intestinal pathogens.

Assays for the protozoans *Cryptosporidium* and *Giardia* followed methods described in Graczyk et al. (1997, 2004, 2006, 2007). Briefly, water samples were filtered through 47-mm glass-fiber filters (GF/F) and stored at 4°C until processed. We tried to filter 1 liter of water, but this volume sometimes was reduced according to the sediment load of the sample. Sediment samples (ca. 50 ml) also were stored at 4°C until processed. On arrival in Dr. Graczyk's laboratory, immunofluorescence microscopy was used for direct enumeration of cells. Fluorescent identification of the oocytes was based upon comparison with fluorescent features of enumerated oocytes using standard criteria. A confirmation approach (PCR and/or bioinfectivity) was used to rule out presumptive oocytes of *Cryptosporidium* and *Giardia* as well as to test for viability. Similar assays were also performed for the microsporidians (a type of fungus), *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Encephalitozoon cuniculi*, and *Encephalitozoon hellem*.

Results and Discussion

Enteric bacteria.

Among the 13 tanks sampled, enterococci and *E. coli* were detected in 11 and 6 of them, respectively (see microbiological data in Appendix 2.1). In 5 of the 6 tanks where *E. coli* was present, so were enterococci. Thus, bacteria indicative of fecal contamination were found in most of the residual water samples. The source(s) of these bacteria is unknown; our sampling scheme did not allow us to distinguish when or where this contamination may have occurred.

In the case of two sets of water samples, those for B05314 and B05336, we quantified the number of enteric bacteria. The maximum value for *E. coli* was 41 colony-forming units per 100 ml and for enterococci, 175 colony-forming units per 100 ml. Compare these concentrations with the maxima permitted by the EPA for freshwater bathing-water standards: *E. coli* 126 per 100 ml; enterococci 33 per 100 ml (http://www.epa.gov/waterscience/beaches/local/statrept.pdf). We cannot assess the representative character of these few quantitative data, because the majority of our determinations for enteric bacteria are qualitative (i.e., presence/absence). Since the public-health issue with fecal contamination is not with these indicator bacteria, but instead the pathogens they potentially indicate, these data suggest the need for quantitative sampling in future studies. Of significance to ballast-water management, the fecal contamination was not predictably removed by at-sea exchange or other ballasting activities in both fresh and salt water. We suspect the bacteria, especially their resting stages, find "refuge" from saltwater flushing in the residual sediments, or simply are not easily flushed out from tanks once established.

<u>Vibrio cholerae.</u>

This bacterium was detected in 55 of a total of 67 (82%) samples analyzed (when all combinations of ships, tanks, and replicate water and sediment porewater samples were considered (see Appendix 2.1 for details). Of the 67 samples, 48 were from the Irma, of which 42 were positive for *V. cholerae*; 19 samples were from the Lady Hamilton, of which 13 were positive. Positive samples were collected on all sampling occasions (n=10), in all tanks, in all residual water samples, and in all but two residual porewater samples (B0511602ws from Irma and B0531402ws from Lady Hamilton). When all samples were considered, concentrations of putative *V. cholerae* (yellow, sucrose-positive colonies on TCBS agar) ranged from 0 to 96,000 colony-forming units (cfu) per liter (Figs. 2.1 and 2.2). The grand mean (standard deviation) was 8,689 (15,633) cfu/L and the median was 3,300 cfu/L. Porewater had roughly two-fold higher concentrations (average 9,933 *V. cholerae* cfu/L) compared to residual water (average 4,437 *V. cholerae* cfu/L). Values tended to be higher in samples from Irma's tanks compared to Lady Hamilton's and maximum values were much greater in samples from the Irma's.

From a standpoint of ballast-water management, it is instructive to consider their maximum and minimum values. In the former case, there were only 4 samples with concentrations greater than 30,000 cfu/L, and one of these was more than three-fold higher (Figs. 2.1 and 2.2). These were all porewater samples (B0619502ws, replicates 1 and 2; and B0611802ws replicates 1 and 2). At the other end of the distribution, *V. cholerae* was not detected in 12 of the total 67 analyses. These 12 samples partitioned into 6 from each ship, 3 water and 3 porewater samples from Lady Hamilton, and 2 water and 4 porewater samples from Irma.

When these end-member samples were evaluated in light of the ships' ballasting activities, there emerged no consistency, thus no predictability, with respect to the last ballasting activity and zero-levels of *V. cholerae*. Saltwater ballasting, mid-ocean exchange, and freshwater ballasting all preceded some samples having no cholera bacteria. Conversely, these same ballasting activities also yielded some samples that were positive for *V. cholerae*, sometimes at high levels.

Finally, what does the presence of *V. cholerae* in ships' ballast tanks portend for public health in the Great Lakes? While the risk from *V. cholerae* carried in ballast-tank residuals is not zero, we can muster no evidence that the risk is particularly high. First, our experience and that of others is that environmental strains of this bacterium usually do not carry the genes for toxicity. Second, even with that first argument aside, there is little likelihood for infection by direct contact with ballast-water residuals, since the "minimum infective dose" for cholera is approximately 10,000 to 100,000 cells for a healthy person. Although 18 of the 55 samples positive for *V. cholerae* showed concentrations in this range, at these concentrations one would need to ingest between 1 and 10 liters of undiluted ballast water to become ill. Thus, while we do not dismiss potential health concerns associated with this and other pathogens in ships arriving to the Great Lakes, it is relevant to consider that no outbreaks or epidemics of cholera, cryptosporidiosis, or giardiasis have been associated with ship traffic, or ballasting operations of ships in the Great Lakes.

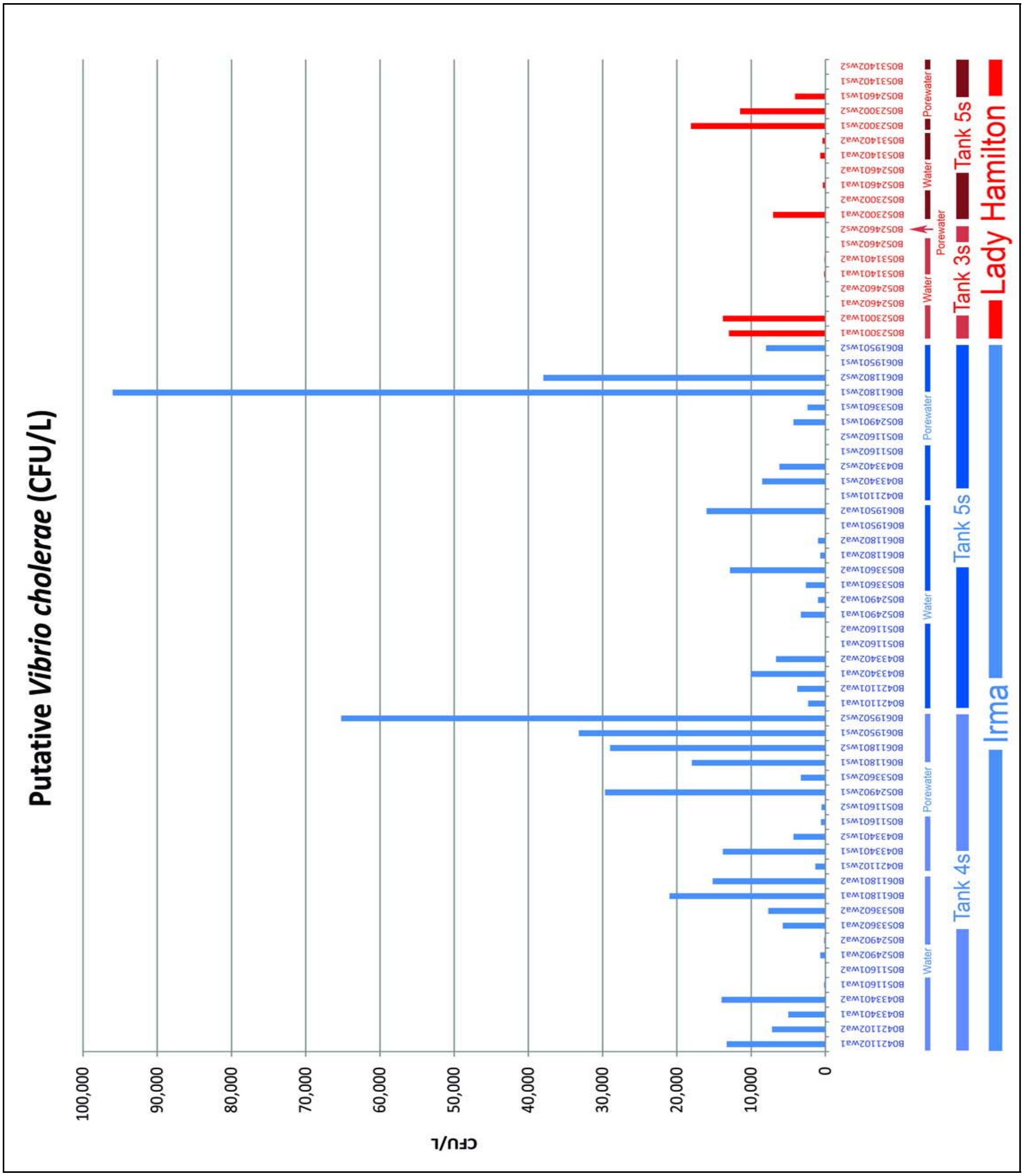


Fig. 2.1. Concentration of putative *V. cholerae* isolated from residual water and porewater in ballast tanks of NOBOB vessels. Values are reported as colony forming units (cfu) L^{-1} of water. Results are partitioned by ship (Irma data are in blue, Lady Hamilton data are in red), tank, and sample location ("water" indicates residual water; "porewater" signifies water extracted via centrifugation from sediments).

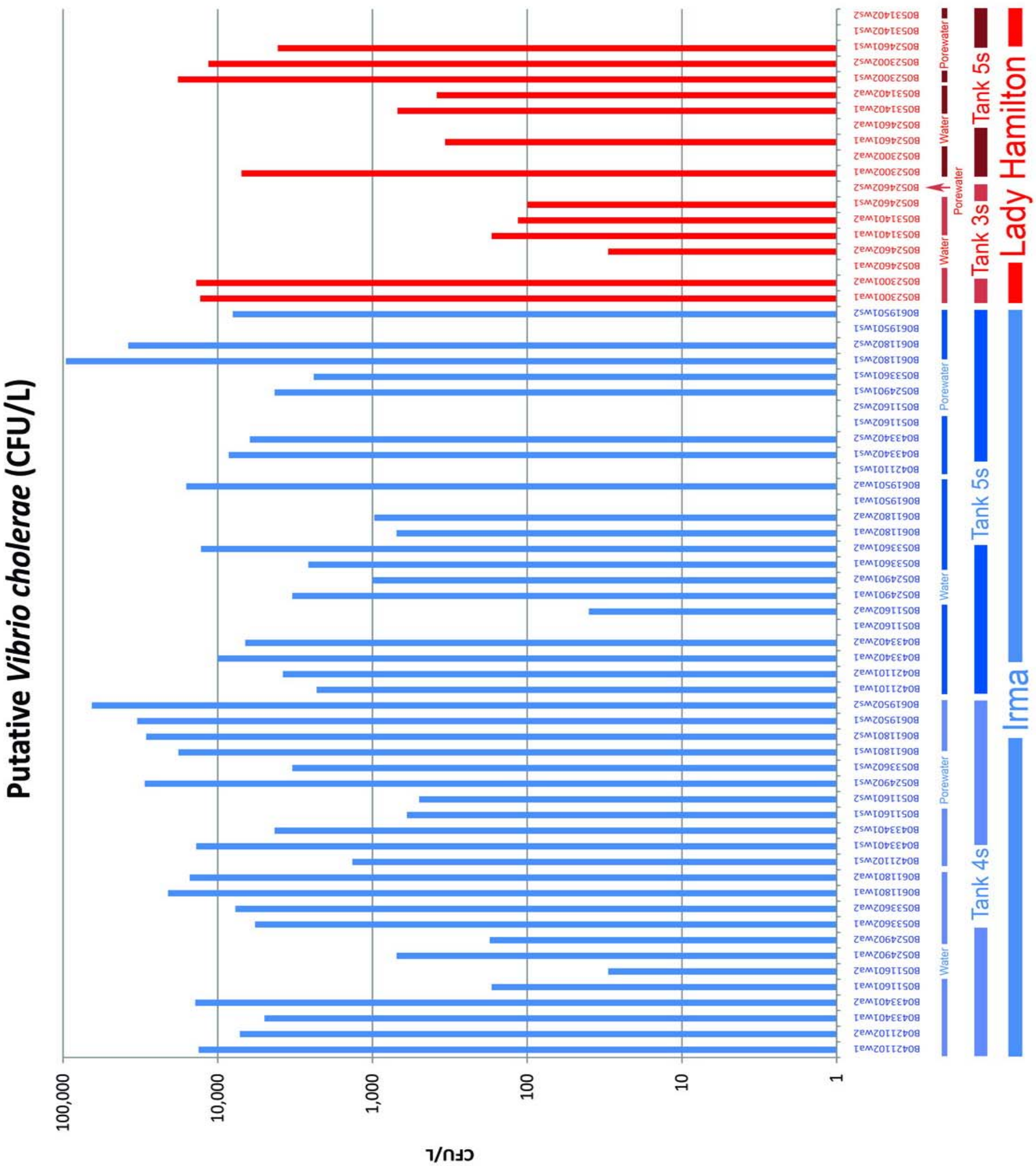


Fig. 2.2. Concentration of putative *V. cholerae* isolated from residual water and porewater in ballast tanks of NOBOB vessels. The data are the same as in the previous figure, but are shown here graphed on a log scale.

Pfiesteria spp.

One or both species of *Pfiesteria* was detected in 21 of a total of 96 analyses (when all combinations of ships, tanks, and replicate water and sediment samples were considered (see Appendix 2.1 for details). Seven of these "positives" were *P. piscicida* and the remaining ones (n=14) *P. shumwayae*.

Of the 10 sampling trips distributed between two ships, 7 yielded at least one sample positive for *Pfiesteria.* Positive samples for *P. piscicida* were collected on 4 trips, for *P. shumwayae* on 6 trips, and for both species on 3 trips.

When considering the 20 ballast tanks sampled in all, half (n=10) contained *Pfiesteria* (6 tanks contained *P. piscicida* and 9 tanks *P. shumwayae*). The replicated detection of *P. shumwayae* was higher than that for *P. piscicida*. Of 9 samples positive for *P. shumwayae*, in 5 (56%) of them both replicates were positive. In only 1 of 6 (17%) samples positive for *P. piscicida* were both replicates positive. These results suggest either a greater abundance of *P. shumwayae* in these samples or a greater sensitivity in its assay compared to that for *P. piscicida*.

The positive results were dominated by samples obtained from the water column, of which 27% (20 of 72) were positive for at least one species of *Pfiesteria*. In contrast, sediment samples comprised 24 of the 96 analyses, of which only 1 (4%) was positive for *Pfiesteria* (*P. piscicida*). Interestingly, neither species of *Pfiesteria* was detected in water samples from the ballast tank in which this positive sediment sample was collected. No clear patterns emerged relative to the salinity of the samples. Both species of *Pfiesteria* were present in samples having salinities ranging from 2 to 40 ppt.

In contrast, some interesting patterns emerge when considering ballasting histories. The Lady Hamilton's Tank 5S exhibited both species of *Pfiesteria* throughout the course of her sampling (17 August, 03 September and 10 November, 2005) which included three episodes of ballasting/deballasting and two open-ocean exchanges. In another interesting pattern, Tank 4S of the Lady Hamilton was initially negative for both species of *Pfiesteria*. After ballasting in Detroit, Michigan, however, both species were detected and persisted through three cycles of ballasting/deballasting and two open-ocean exchanges.

The sampling regime for the Irma was more extensive, yet fewer positive results were observed. Her Tank 5S had only one positive result (*P. shumwayae* in sample B-04334-02wa). At the beginning of the study, Tank 4S contained *P. piscicida* (sample B-04211-02ws), but following three ballasting/deballasting events and two open-ocean exchanges, the samples were positive for *P. shumwayae* and negative for *P. piscicida*. This pattern was maintained throughout the subsequent deployment of the ship (two samplings), and no *Pfiesteria* was detected in the tank's final sampling. Overall therefore, and with some exceptions, one or the other *Pfiesteria* species appeared in residual samples and persisted following a series of saltwater ballasting episodes. Conversely, sequences of mostly freshwater ballasting were associated with the absence of *Pfiesteria* and, in some cases, a shift from presence to absence.

Intestinal pathogens.

Samples positive for these pathogens were collected throughout the course of this study (see Appendix 2.1 for details). Of the six species, all but *Encephalitozoon cuniculi* were detected. *Cryptosporidium parvum* and *Giardia lamblia* were the most prevalent and were found 7 and 8 times, respectively, over the 10 times ships were sampled. When found on a ship, however, they often were not present in both of the ballast tanks sampled. *G. lamblia* was found in 65% of the 20 tanks sampled, *C. parvum* in only 40%. A factor driving the higher incidence of *G. lamblia* was its prevalence in the sediments; of 13 ballast tanks "positive" for this species, 11 had positive sediment samples, 3 had positive water samples, and 1 had both positive sediment and water samples. Conversely, the positive samples for *C. parvum* were principally from residual waters. Among 8 tanks in which this species was detected, 6 had positive water samples, 3 had positive sediment samples, and 1 had both positive sediment and water samples.

All three species of microsporidians detected (*Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, and *Encephalitozoon hellem*) were less prevalent than *C. parvum* and *G. lamblia*. *E. bieneusi* and *E. hellem* were found in 4 (20%) of the 20 ballast-tank samples and 4 (40%) of the 10 ships sampled, and *E. intestinalis* was found in only one of the 20 ballast-tank sampled. One sample from the Lady Hamilton (B-05246, Tank 5S), however, contained all three of these microsporidians. All samples "positive" for microsporidians were from residual water, a result that might be expected given the small size of their spores, which would be less likely to settle to the bottom of tanks compared to the relatively large cysts of *Cryptosporidium* and *Giardia*.

Overall, samples positive for these intestinal parasites were found in ballast residuals exhibiting a wide range of salinities. Furthermore, positive samples exhibited no obvious pattern with respect to ballasting activity. Therefore, unlike the pattern speculated for *Pfiesteria* spp. (see above), there was no consistency in "positive" samples with respect to ballasting and exchange. These results suggest that these organisms may be less likely than *Pfiesteria* to remain alive in ballast residuals between ballasting activities.

Recommendations

Given the dissolution of our intended sampling design, we cannot state much about the efficacy of "best-management practices" and indeed, we have tried to minimize our speculation in this regard. However, in every tank sampled over the course of this study (total of 20 tanks), at least one of the potential pathogens or indicator species for which we assayed was present (Fig. 2.3). In one case (B0524601), there were 8 such taxa present; most samples contained between 2 and 6. These may be regarded as "model" organisms; had we assayed for more such species, we believe we would have found them in some samples. We therefore reiterate a point expressed in our previous NOBOB study that, "it seems prudent to regard all NOBOB ships entering the Great Lakes as potential carriers of pathogens" (Johengen et al., 2005).

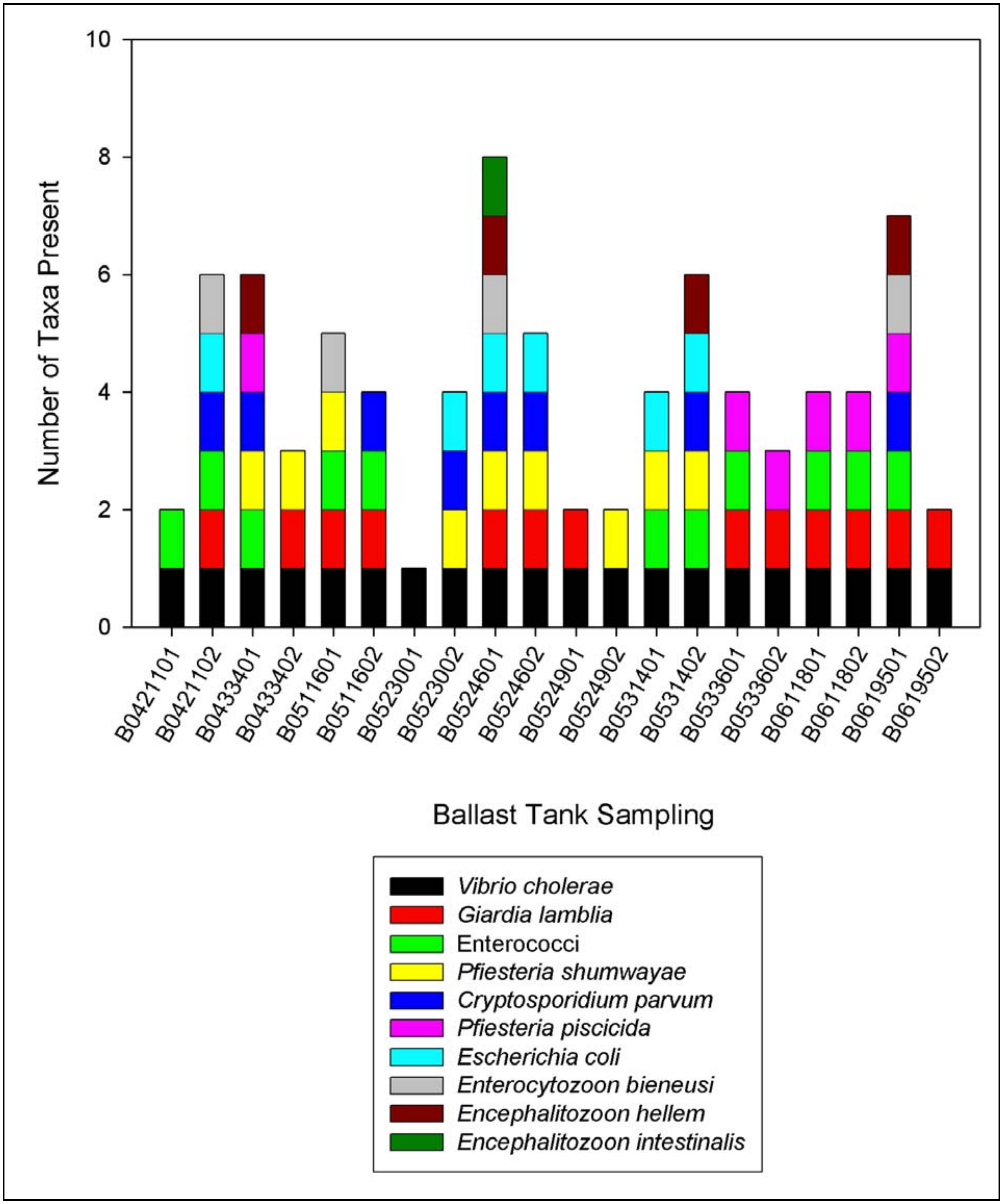


Fig. 2.3. Pathogens (and indicator bacteria) in ballast tanks of NOBOB ships sampled over the course of the "Best-Management Practice" study. The data have been collapsed to integrate across sample type (residual water, porewater, or sediment) and replicates. Thus, each entry on the abscissa represents a tank/time combination. Between two ships, a total of 10 sampling excursions—and 2 tanks sampled per ship per excursion—there were a total of 20 tanks sampled.

References

Bowers, H.A., T.Tengs, H.B. Glasgow, Jr., J.M. Burkholder, P.A. Rublee, and D.W. Oldach. 2000. Development of real-time PCR assays for rapid detection of *Pfiesteria piscicida* and related dinoflagellates. Appl. Environ. Microbiol. 66:4641-4648.

Choopun, N., V. Louis, A. Huq, and R.R. Colwell. 2002. Simple procedure for rapid identification of *Vibrio cholerae* from the aquatic environment. Appl. Environ. Microbiol. 68:995-998.

Chun, J., A. Huq, and R.R. Colwell. 1999. Analysis of 16S-23S rRNA intergenic spacer regions of *Vibrio cholerae* and *Vibrio mimicus.* Appl. Environ. Microbiol 65:2202-2208.

Graczyk, T. K., M. R. Cranfield, and R. Fayer. 1997. Recovery of waterborne oocysts of *Cryptosporidium parvum* from water samples by the membrane-filter dissolution method. Parasitol. Res. 83:121-125.

Graczyk, T. K., D. B. Conn, F. Lucy, D. Minchin, L. Tamang, L. N. S. Moura, and A. J. DaSilva. 2004. Human waterborne parasites in zebra mussels (*Dreissena polymorpha*) from the Shannon River drainage, Ireland. Parasitol. Res. 93:389-391.

Graczyk, T. K., A. S. Girouard, L. Tamang, S. P. Nappier, and K. J. Schwab. 2006. Recovery, bioaccumulation, and inactivation of human waterborne pathogens by the Chesapeake Bay non-native oyster, *Crassostrea ariakensis.* Appl. Environ. Microbiol. 72:3390-3395.

Graczyk, T. K., F. E. Lucy, L. Tamang, and A. Miraflor. 2007. Human enteropathogen load in activated sewage sludge and corresponding sewage sludge-end products. Appl. Environ. Microbiol. 73: in press.

Pisciotta, J.M. D.F. Rath, P.A. Stanek, D.M. Flanery, and V.J. Harwood. 2002. Marine bacteria cause false-positive results in the Colilert-18 rapid identification test for *Escherichia coli* in Florida waters. Appl. Environ. Microbiol. 68:539-544.

Objective 2.2: Assess the effectiveness of BMPs to reduce the amount and viability of phytoplankton cells in NOBOB ballast tanks.

Overview

The objective of this part of the study was to determine the growth and survival potential of phytoplankton resident in NOBOB ballast tanks of foreign vessels trading in the Great Lakes, and how that potential can be minimized through the use of best management practices. In order to accomplish this purpose we: 1) Conducted germination experiments with ballast tank sediment and water residual exposed to a selected suite of practices aimed at reducing the abundance of phytoplankton by flushing mud out of tanks and limiting the freshwater species invasion by replacing freshwater ballast water with saltwater; 2) Estimated the growth potential of phytoplankton in NOBOB tanks in Great Lakes' water after management practices were used; and 3) Determined the changes in the phytoplankton composition after a saltwater "swish and spit" rinsed the ballast tanks during transit. As stated earlier, we were unable to conduct our original sampling design and did not have the benefit of paired treatment versus control samples to analyze specific effects. Given these limitations we describe our results in the context of the confirmed ballasting activities that occurred surrounding our sampling points.

Methods

Ballast tanks on two ships were sampled during 2004 - 2006 at various ports in the Great Lakes. Deck hatches were used to gain access to ballast tanks. Once inside the tanks, 12L water samples were collected by hand pump from the bottom of the ballast tank and a 1L sub-sample provided for phytoplankton analysis. Approximately 10L of sediment were collected aseptically by using spatulas and a 500g sub-sample provided for phytoplankton analysis. The water and sediment samples were stored at 4^{o}C. In the lab, ballast samples were prepared for phytoplankton analysis and germination experiments. Germination experiments were designed to test the ability of phytoplankton in the ballast residual samples to survive and grow in the Great Lakes and to compare composition and viability as a function of the ballast management practices applied. Two growth media: 0.22 um-filtered Lake Michigan water (LW) and nutrient enhanced freshwater media (GL) (Guillard 1975) were used in the experiment. For water samples, five ml of residual water were added to 45 ml of culture media. For sediment samples, 2 ml of residual sediment were mixed with 30 ml of filtered residual tank-water to produce slurry; then, 2 ml of this slurry were added to 45 ml of culture media. All treatments were done in triplicate. For each treatment, in situ chlorophyll fluorescence was used as an indicator of phytoplankton abundance. When a significant increase in phytoplankton abundance was noted, the experiment was terminated. At this time phytoplankton cultures were preserved with Lugol's solution and then prepared according to Dozier and Richardson (1975). Phytoplankton identifications were base on morphological criteria. Statistical analysis of all data was performed with SYSTAT 8.0

Therefore, results from these germination experiments support the hypothesis that exposure to mid-ocean salinity can limit phytoplankton viability of freshwater species and reduce the risk of transferring species from outside low salinity source ports.

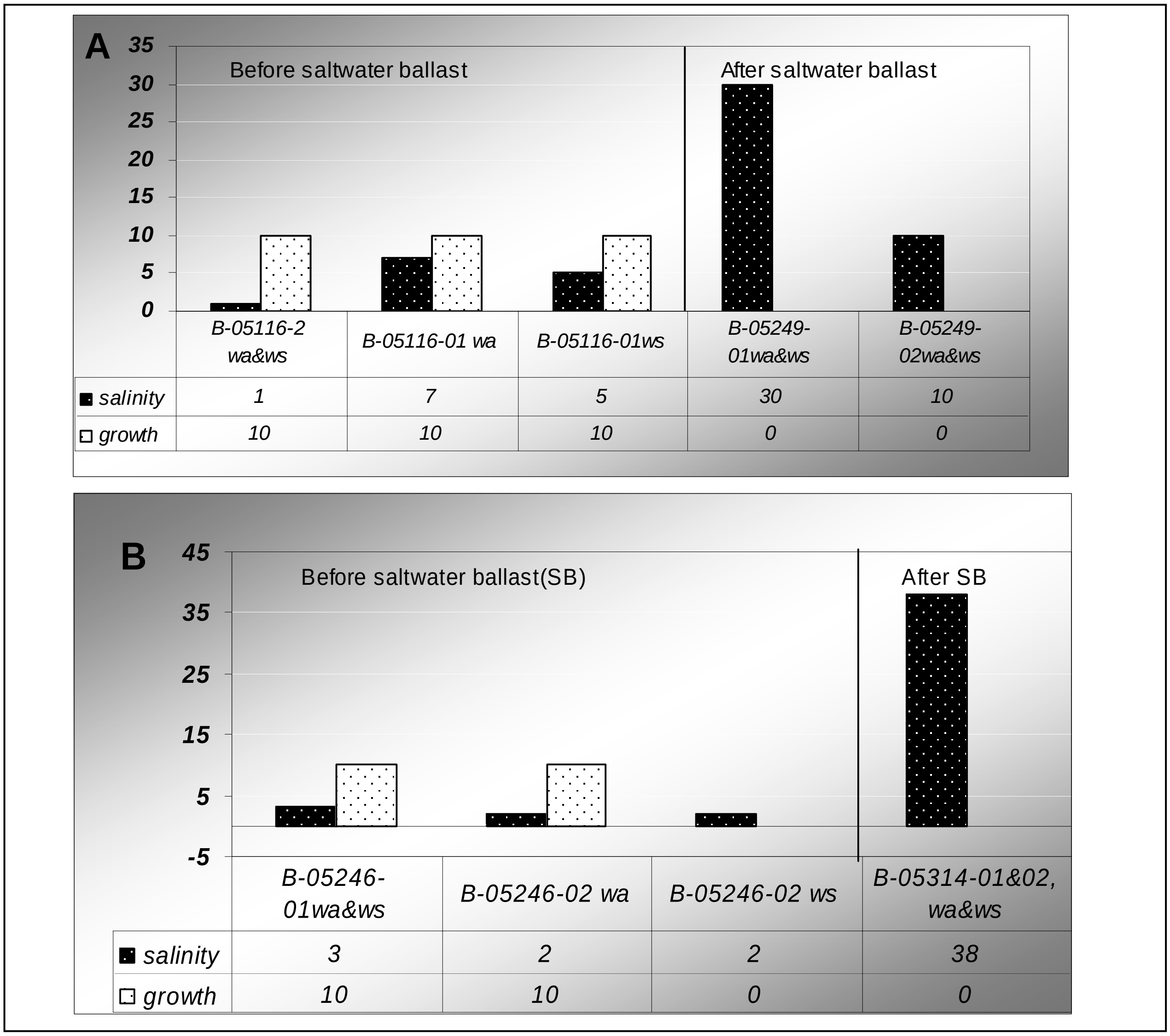


Fig. 2.4. Phytoplankton growth in germination experiments as a function of ballast history and salinity of the residual samples. Y-axis scale represents both salinity and growth. Phytoplankton significant growth = 10 and no significant growth = 0 A: ship Lady Hamilton; B: ship Irma

Results and Discussion

Phytoplankton viability

From 2004-2006, 32 residual ballast sediment and water samples were cultured in filtered Lake Michigan water (LW) and freshwater medium (GL). Phytoplankton viability of each sample was estimated by comparing its maximum versus initial chlorophyll-a fluorescence after culturing in these media. Freshwater (GL) media produced significant growth in 89% of the samples, whereas filtered Lake Michigan water produced germination and growth in only 32% of the samples. Ballast history had a significant effect on phytoplankton growth (Fig. 2.4). After ships ballasted with saltwater, most residual samples produced no significant growth in the filtered Lake Michigan water.

Therefore, results from these germination experiments support the hypothesis that exposure to mid-ocean salinity can limit phytoplankton viability of freshwater species and reduce the risk of transferring species from outside low salinity source ports.

Phytoplankton composition

From 2004-2006, 32 residual ballast sediment and water samples were cultured in filtered Lake Michigan water (LW) and freshwater medium (GL). The composition of the phytoplankton community from 20 LW cultures was analyzed. These 20 samples were collected from two ships, MV/Irma and MV/Lady Hamilton. Initial samples from each ship were of freshwater origin based on salinity measurements. After our initial collection of samples from each vessel, the tanks were either ballasted or flushed with saltwater on several occasions (see Appendix 2, also, Task 1). As a result we have initial samples with a freshwater source and then samples that represent residuals originating from or exposed to a variety of oceanic-salinity ballasting events including one saltwater flushing event, which was one of our proposed BMP enhancements.

A total of 53 phytoplankton species were identified from LW culture grow outs (Table 2.2). The composition of phytoplankton consisted of: 26% cyanobacteria, 34% diatom, 39% green algae and 1% micro-phytoflagellates.

Phytoplankton composition was also seasonally dependent, as residual water samples collected in the spring had more diatoms present in the germination cultures (Sample B-05116-01 and B-05116-02), while more cyanobacteria and green algae grew-out in summer samples (B-05229-01, B-05229-02) (Fig.2.5).

Fig. 2.5. Variabity of the dominant phytoplankton species among the sampels from different seasons

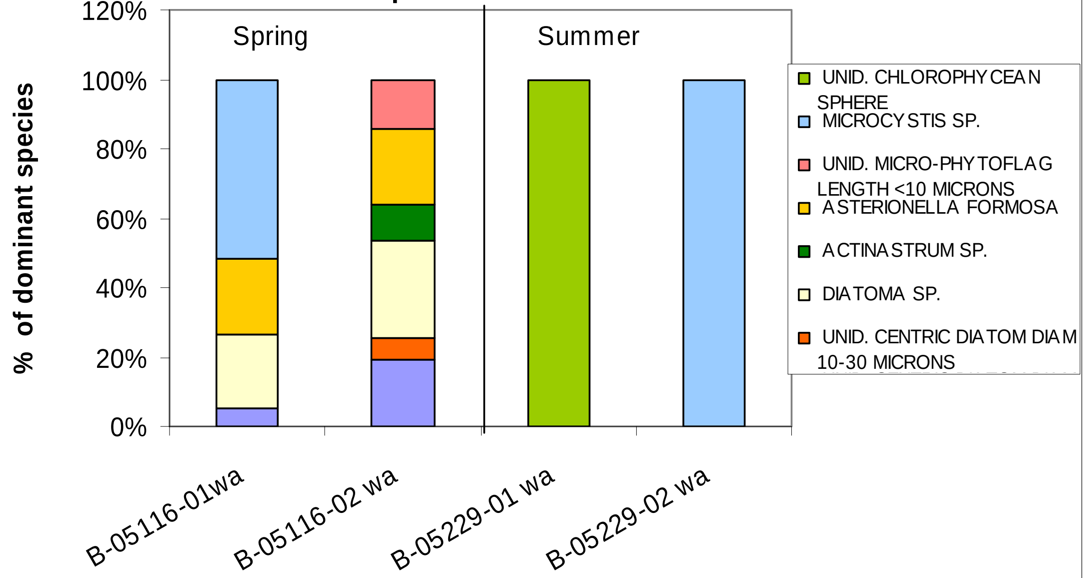


Table 2.2. Phytoplankton composition observed in germination experiments for filtered Lake Michigan cultures.

UNID. BLUE GREEN SINGLE SPHERE	SURIRELLA SP.
UNID. BLUE GREEN TRICHOME (CELL) SM BLUNT	CHLORELLA SP.
CYLINDROSPERMUM SP.	OOCYSTIS SP.
UNID. CENTRIC DIATOM DIAM <10 MICRONS	ANKISTRODESMUS SP.
UNID. PENNATE DIATOM <10 MICRONS LENGTH	KIRCHNERIELLA SP.
UNID. CHLOROPHYCEAN SPHERE	FRANCEIA SP.
CHROOCOCCUS SP.	LAGERHEIMIA SP. SYN. CHODATELLA SP.
COCCOCHLORIS SP.APHANOTHECE SP.	ACTINASTRUM SP.
MICROCYSTIS SP.	SCENEDESMUS SP.
OSCILLATORIA CELLS #1 DIAM <5UM	TETRADESMUS SP.
ANABAENA SP.	COELASTRUM SP.
NOSTOC SP.	GLOEOCYSTIS SP.
MALLOMONAS SP.	ELAKATOTHRIX SP.
MELOSIRA SP#1 DIAM <20 MICRONS	DIDYMOCYSTIS SP.
CYCLOTELLA SP#1 DIAM <10 MICRONS	PARALIA SULCATA
THALASSIOSIRA SP#1 DIAM <20 MICRONS	SKELETONEMA POTOMAS
COSCINODISCUS SP#1 DIAM <40 MICRONS	ASTERIONELLA FORMOSA
ACTINOPTYCHUS SP.	TETRAEDRON REGULARE
CHAETOCEROS SP#2 DIAM 10-30 MICRONS	SCENEDESMUS ACUMINATUS
FRAGILARIA SP#1 LENGTH <30 MICRONS	SCENEDESMUS QUADRICAUDA
RHAPHONEIS SP.	SCENEDESMUS DIMORPHUS
DIATOMA SP.	GOLENKINIA RADIATA
COCCONEIS SP.	MICRACTINIUM PUSILLUM
NITZSCHIA SP#2 LENGTH 30-70 MICRONS	SCENEDESMUS OBLIQUS
NITZSCHIA SP#3 LENGTH >70 MICRONS	NAVICULA SP#1 LENGTH <20 MICRONS
UNID. MICRO-PHYTOFLAG LENGTH <10 MICRONS	NAVICULA SP#2 LENGTH 20-60 MICRONS
	CYMBELLA SP.

Conclusions

As a part of best management practices for application to the management of NOBOB vessels, the regular use of saltwater flushing will minimize, but not eliminate, delivery of viable freshwater phytoplankton to the Great Lake. Our results showed that switching from freshwater to saltwater conditions restricted phytoplankton growth in LW media. Variations in phytoplankton composition and growth in response to the ballast water exchanges existed among ballast tanks in the same ship. Nothing in our data points to a possible explanation for these variations.

Objective 2.3. Assess the effectiveness of BMPs to reduce the abundance of live invertebrates and the density of resting eggs

Overview

The primary Best Management Practice that we wished to test under this objective was saltwater flushing and its effects on live invertebrates and resting stages present in ballast tank residuals of NOBOB vessels. As described earlier, operational constraints on the participating vessels prevented us from implementing our sampling and experimental protocols as originally proposed. Given these limitations we moved forward on two separate fronts to address this objective. First we completed residual sampling at each opportunity when ships re-entered the lakes and examined results against the entire ballast history that occurred between samples. Secondly, to allow a more quantitative, scientific evaluation of the potential effects of saltwater flushing, we solicited vessels originating in the Great Lakes that were willing to: designate a pair of experimental tanks, operate in a partial BOB status, conduct an open-ocean saltwater exchange, and allow us to secure Incubator-Emergence Traps (IETraps) within the tanks. By including live invertebrate controls and sediments with pre-characterized resting eggs we were able to directly test the effects of saltwater exposure on both planktonic and benthic invertebrates and resting egg viability.

2.3.1. Results from NOBOB Ballast Residual Sampling

Two vessels, M/V Lady Hamilton and M/V Irma, were characterized over the course of the study to examine the effects of BMP's on live invertebrates in residual water and sediment as well as density of resting eggs in the residual sediment. Multiple ballast events occurred between all sample dates, so it is not possible to associate specific changes of within-tank conditions with any particular event or practice, however, temporal patterns are discussed below. For each tank sampled, 10L of residual sediment and (if possible) 50L of residual water were collected. Residual sediment was homogenized and four 500 g sub-samples were preserved, following which, two 500 g samples were processed to identify resting eggs and animals in the sediment. Water was filtered through a 30-μm plankton net, preserved for enumeration, and salinity and temperature were recorded at time of collection. Since the number of replicate samples was low, only mean number of eggs/animals with standard deviation is presented.

Lady Hamilton

Lady Hamilton was followed over a four month period in 2005 and was sampled on three separate dates: 1) August 17, 2005 in Cleveland, Ohio; 2) September 3, 2005 in Sorel, Quebec; and 3) November 10, 2005 in Hamilton, Ontario. Two separate ballast tanks (#3 starboard (3S) and #5 starboard (5S)) were sampled on each occasion.

Figures 2.6 and 2.7 show the mean number of resting eggs detected in tanks 5S and 3S respectively from two replicate 500 g residual sediment samples. The total number of resting eggs found in the sediments increased between August 17 and September 3, 2005. Sediments from tank 5S (Figure 2.6) had an increase in the number of *Asplanchna*, *Bosmina* and copepod eggs, while sediments from tank 3S (Figure 2.7) showed a substantial increase in the number of *Bosmina*, loose cladoceran and copepod eggs. This increase in the number of resting eggs was associated with a transit of the Great Lakes after the vessel was sampled in Cleveland. Two explanations for the increased egg density are: 1) they simply came in with sediment during ballasting in Cleveland and Detroit, or 2) freshwater animals drawn into the tanks during these ballasting events deposited eggs into the sediment as water quality conditions within the tanks deteriorated during the transit.

Tank 5S displayed an overall decrease in the number of resting eggs in the sediment between the sampling dates of September 3 - November 10, 2005. In contrast, there was an increase in the number of *Brachionus* eggs during this time. The reason for this increase is not clear. This animal is generally a freshwater genus, yet only saltwater ballast intake and mid-ocean exchange and flushing occurred between sample collections. However, little is known about the uniformity of eggs distributions throughout a ballast tank, so this could be a reflection of in-tank sediment faunal heterogeneity.

Sediment was collected from tank 3S on November 10, but it could not be processed due to a high concentration of oils within the sediment. Therefore, it is not possible to determine if the concentration of eggs within this tank changed due to the flushing or exchange.

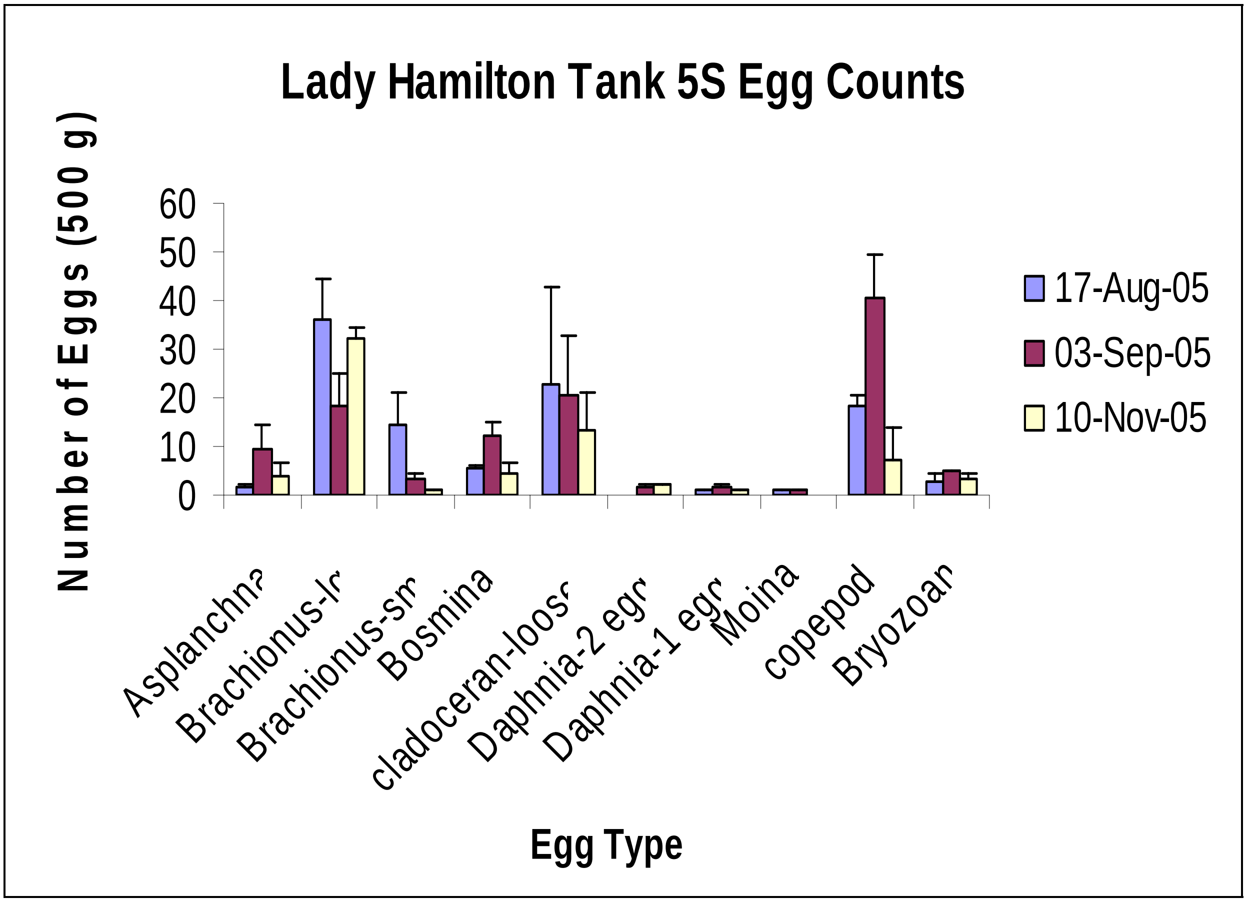


Fig. 2.6. Mean number (± S.D) of resting eggs detected in 500 g of residual sediment from ballast tank 5S on the Lady Hamilton.

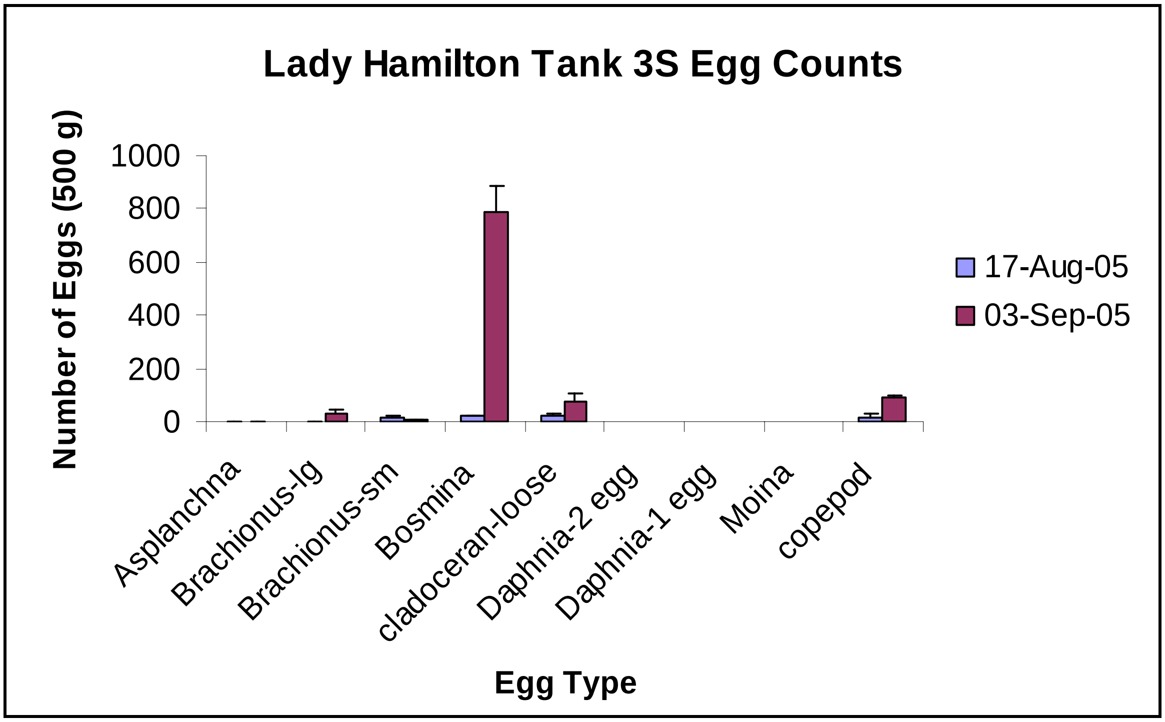


Fig. 2.7. Mean number (± S.D) of resting eggs detected in 500 g of residual sediment from ballast tank 3S on the Lady Hamilton.

Figures 2.8 and 2.9 show the number of animals collected within the sediment from tanks 5S and 3S respectively. In both tanks, only nematodes were found and their abundances decreased on each consecutive sampling date.

Only one 50 L sample was collected from tank 3S in November, due to the lack of residual water present within the tanks. This sample contained only 1 live harpacticoid copepod. It is not possible to draw any inferences about the effects of BMPs on live invertebrates in residual ballast water from this vessel.

M/V Irma (2004)

The M/V Irma was sampled repeatedly over a three-year span (2004-2006). Sampling dates included: 1) July 29 and November 29, 2004; 2) April 26, September 6 and December 2, 2005; and 3) April 28 and July 14, 2006. Two separate starboard ballast tanks, 4S and 5S, were sampled on each occasion.

Figures 2.10 and 2.11 show the mean number of resting eggs detected in tanks 4S and 5S respectively from two replicate 500 g residual sediment samples. A decrease in the overall number of resting eggs in the sediments occurred between July 29 and November 29, 2004. There were four different ballasting events with saltwater, one saltwater exchange and one saltwater flush completed during this time span.

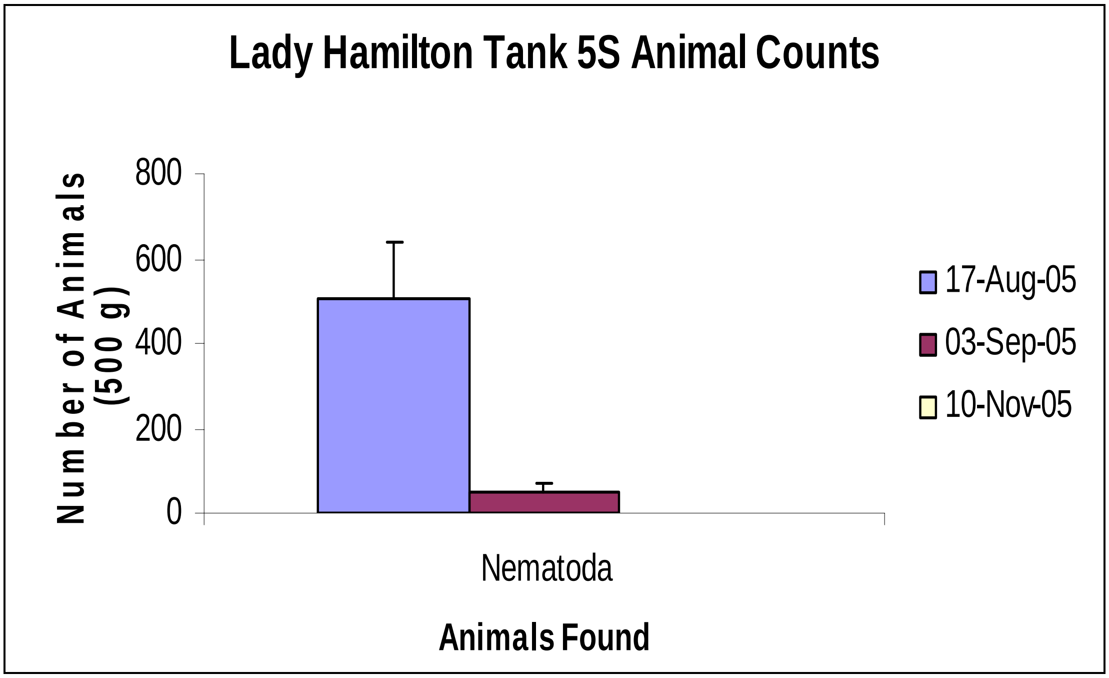


Fig. 2.8. Mean number (± S.D) of animals detected in 500 g of residual sediment from ballast tank 5S on the Lady Hamilton.

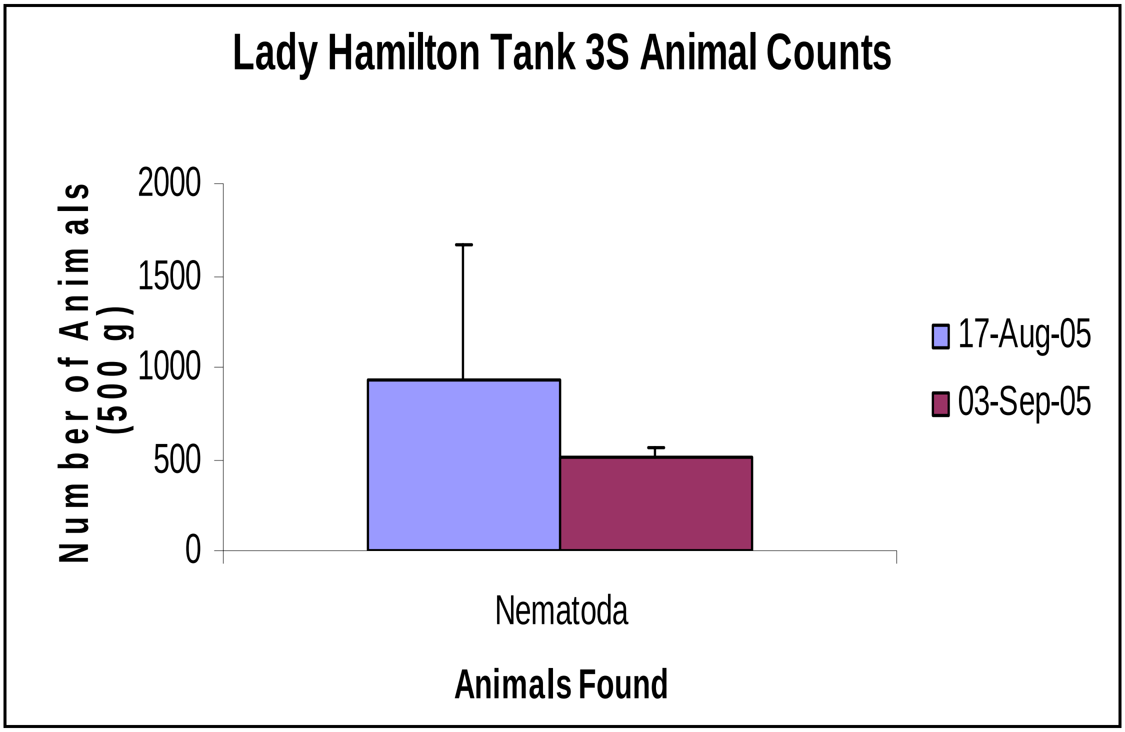


Fig. 2.9. Mean number (± S.D) of animals detected in 500 g of residual sediment from ballast tank 3S on the Lady Hamilton.

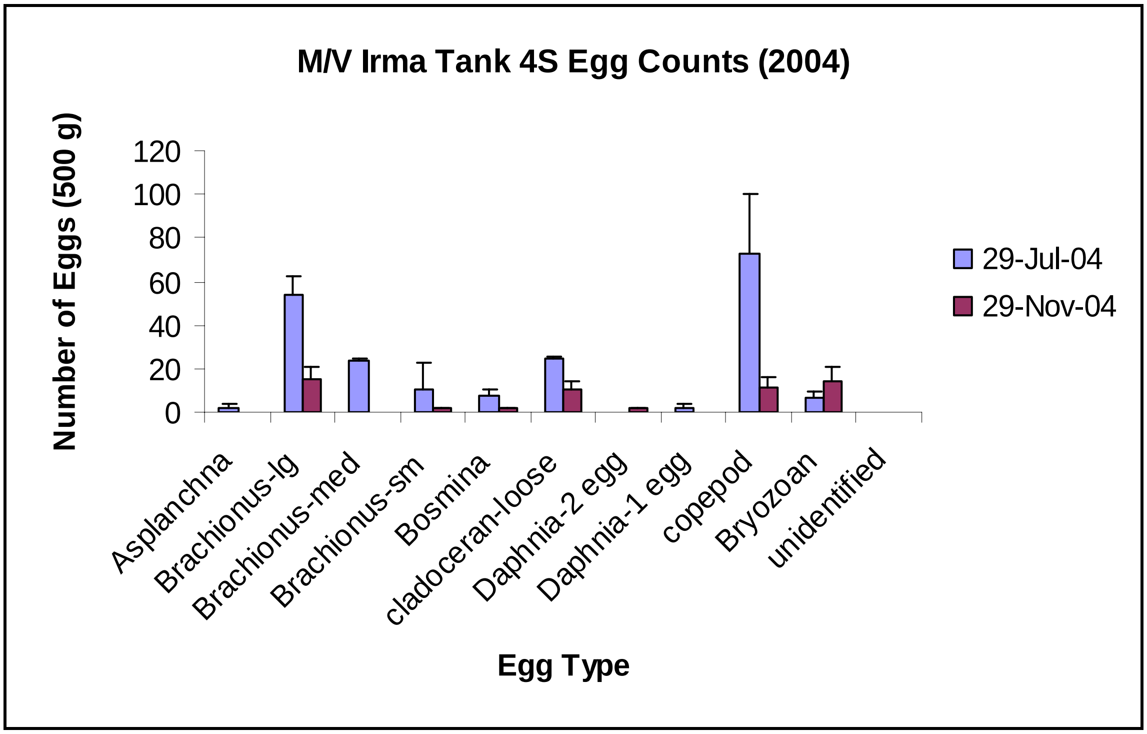


Fig. 2.10. Mean number (± S.D) of resting eggs detected in 500 g of residual sediment from ballast tank 4S on the M/V Irma.

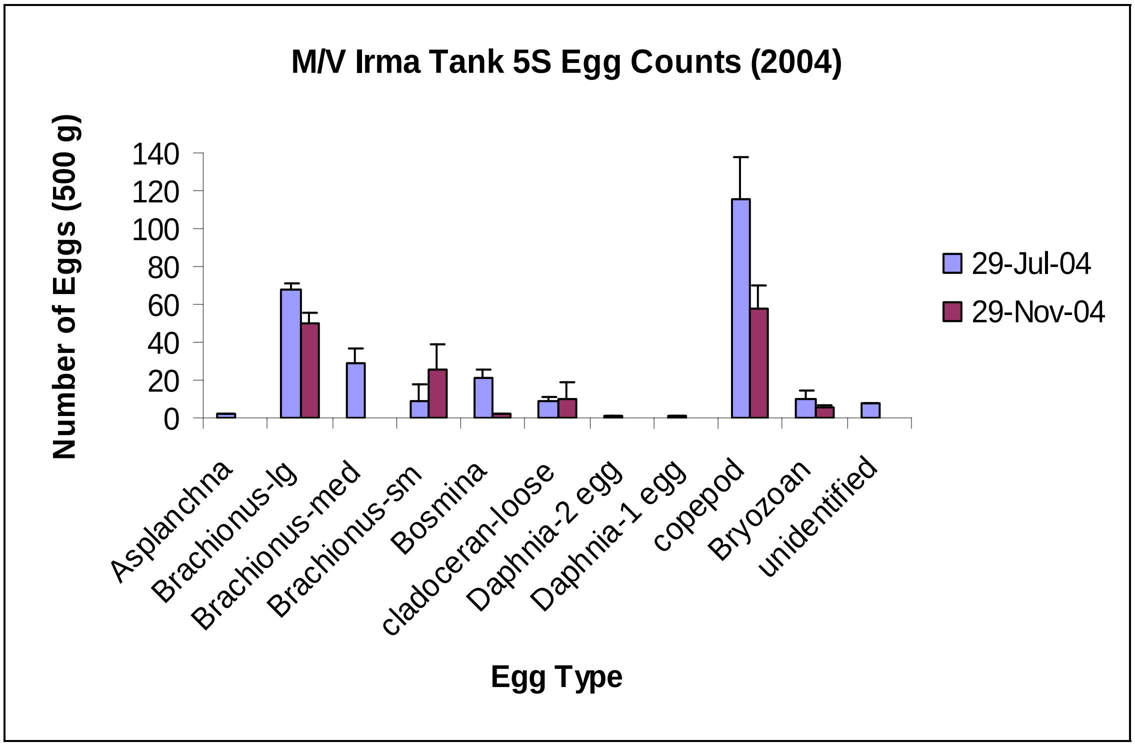


Fig. 2.11. Mean number (± S.D) of resting eggs detected in 500 g of residual sediment from ballast tank 5S on the M/V Irma.

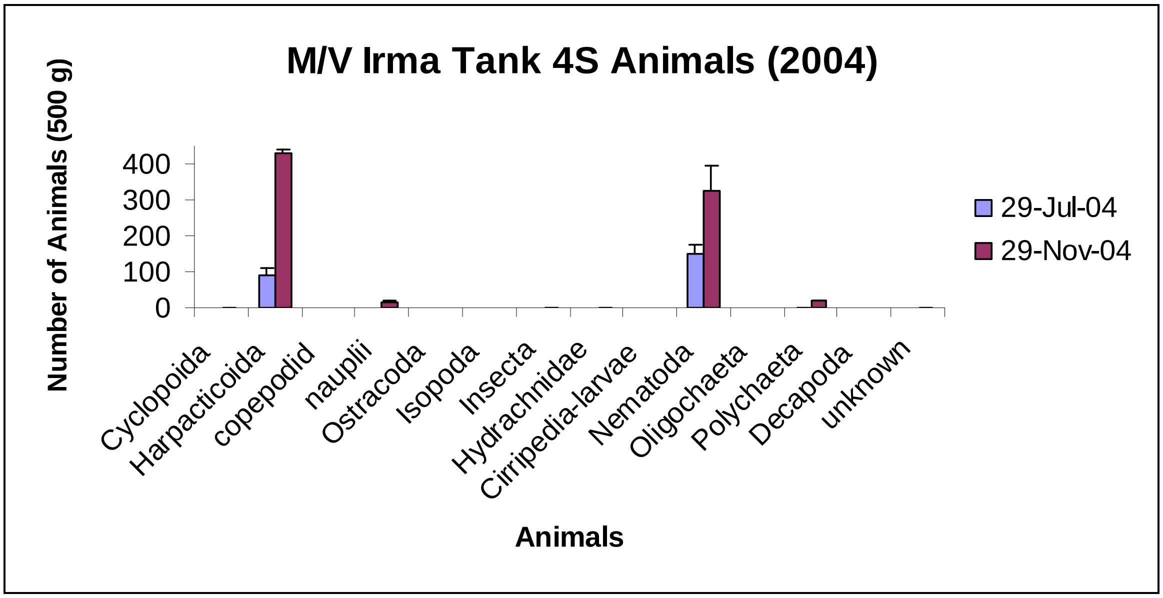


Fig. 2.12. Mean number (± S.D) of animals detected in 500 g of residual sediment from ballast tank 4S on the M/V Irma.

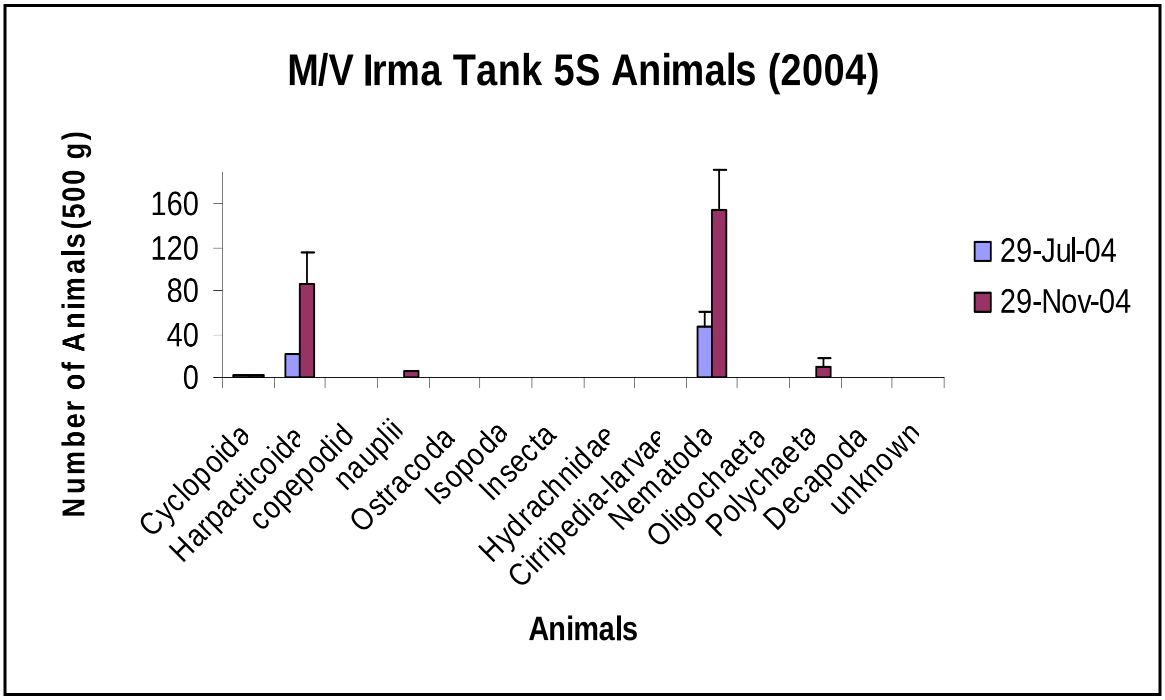


Fig. 2.13. Mean number (± S.D) of animals detected in 500 g of residual sediment from ballast tank 5S on the M/V Irma.

Figures 2.12 and 2.13 show the number of live animals present in sediment from tanks 4S and 5S respectively. Both tanks had an increase in the number of harpacticoid copepods, nematodes and polychaetes found in the sediments between July 29 and November 29, 2004. This increase is most likely due to the addition of animals during ballast intake events, the last of which was in Aratu, Brazil. These events could have added saltwater animals to tanks, thus increasing numbers, but which should have posed little risk to the Great Lakes.

Due to the lack of water in the tanks during 2004 no animals were collected from residual water.

M/V Irma (2005)

Figures 2.14 and 2.15 show the mean number of resting eggs detected in tanks number 4S and 5S respectively from two replicate 500 g residual sediment samples collected on three dates during 2005. A decrease in the number of resting eggs in the sediments occurred between April 26 and September 6, 2005, followed by an increase between September 6 and December 2, 2005. Four different ballasting events with saltwater occurred between the April 26 and September 6 samples, and four more, but primarily involving fresh and brackish water, occurred between the September 06 and December 02 samples.

Figures 2.16 and 2.17 show the number of animals collected from sediment for tanks 4S and 5S respectively. Tank 4S had a large decrease in the number of rotifera, and a large increase in the number of nematodes between samples taken on April 26 and September 06. There were large increases in the number of calanoid (4S), cyclopoid (4S & 5S), and harpacticoid copepods (4S), and nematodes (5S) in the sediments between samples of September 6-December 2, 2005. These increases are most likely due to the addition of animals during freshwater ballasting that occurred in Police, Poland or the Great Lakes.

Figures 2.18 and 2.19 show the number of animals detected in the residual water from tanks 4S and 5S respectively. The highest animal densities were found in freshwater samples collected on April 26. Several of the species present in the samples collected on April 26 were not present on September 06. What appeared as a slight reintroduction of cyclopoid copepods occurred in each of the tanks between the September 6 samples and the December 2nd samples. The latter could be a remnant of species introduced during the freshwater ballasting in Police, Poland.

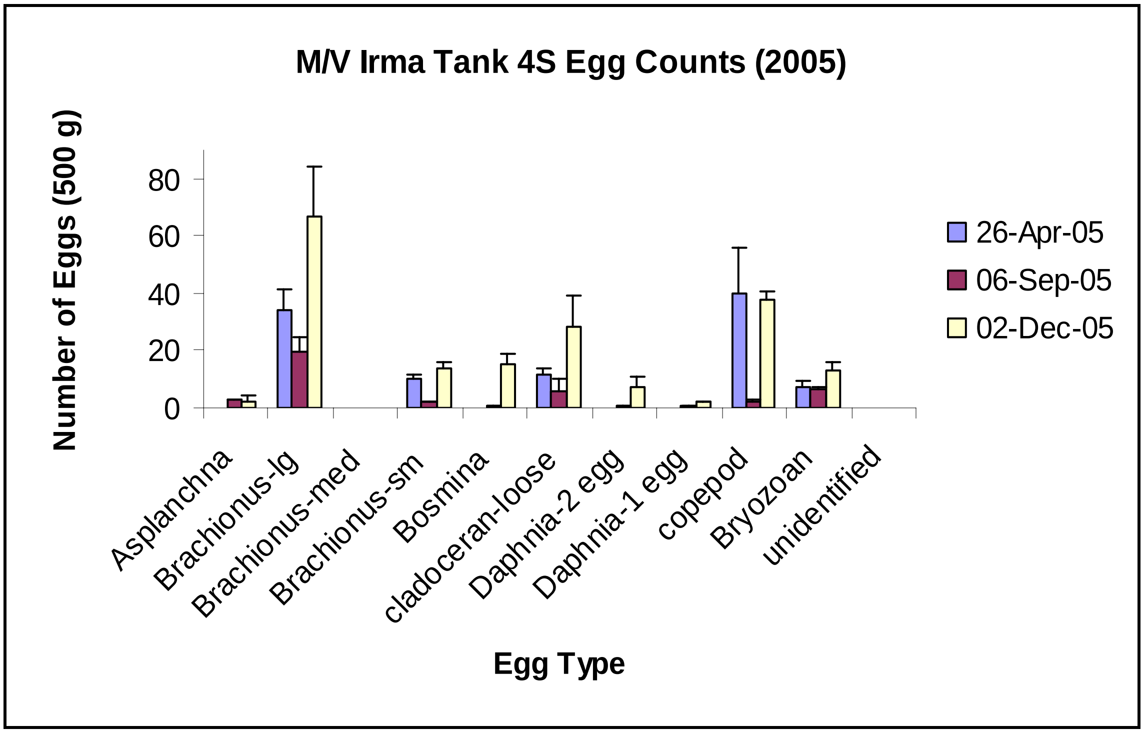


Fig. 2.14. Mean number (± S.D) of resting eggs detected in 500 g of residual sediment from ballast tank 4S on the M/V Irma.

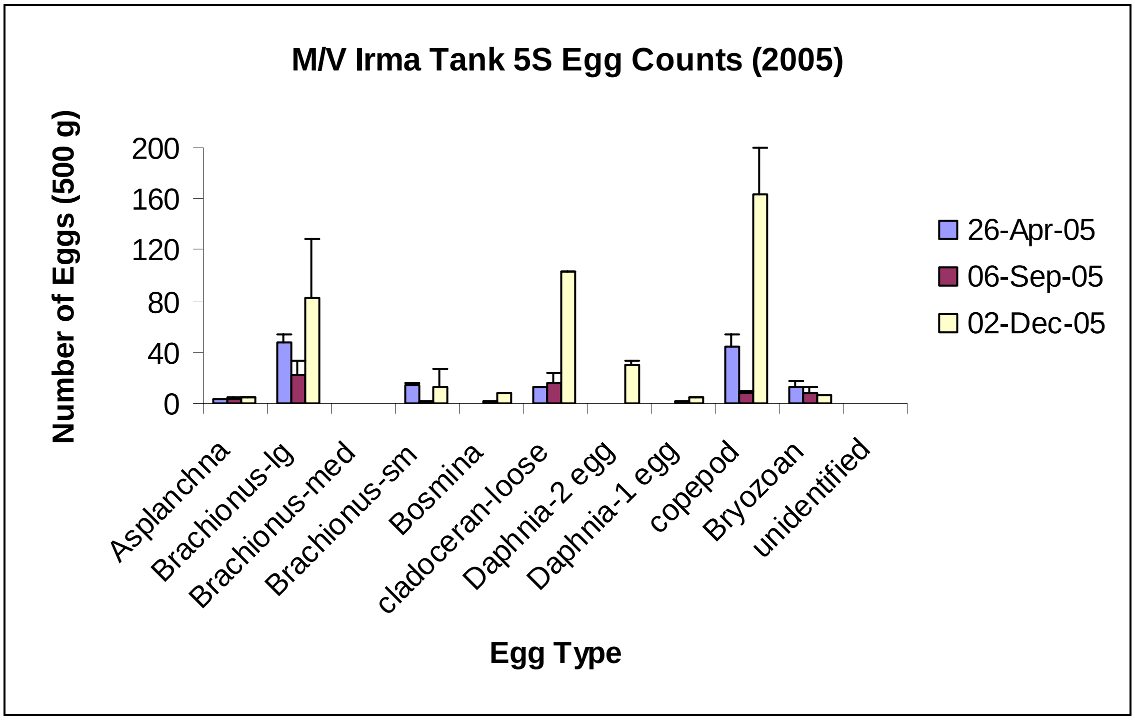


Fig. 2.15. Mean number (± S.D) of resting eggs detected in 500 g of residual sediment from ballast tank 5S on the M/V Irma.

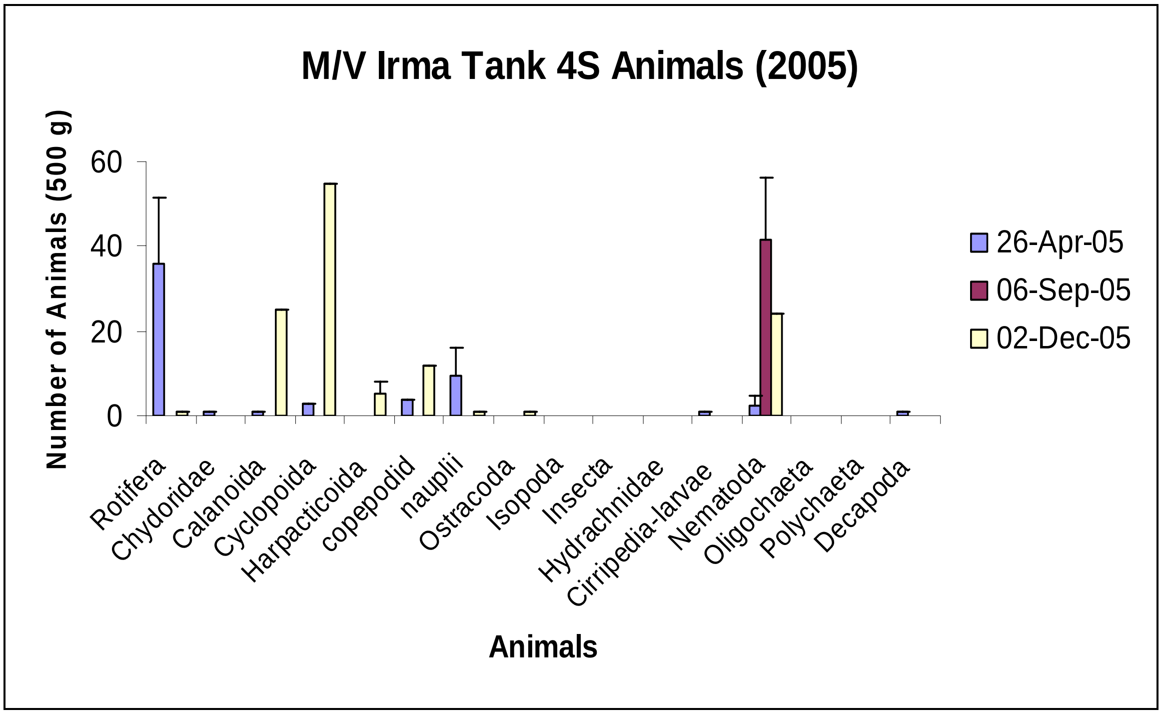


Fig. 2.16. Mean number (± S.D) of animals detected in 500 g of residual sediment from ballast tank 4S from the M/V Irma.

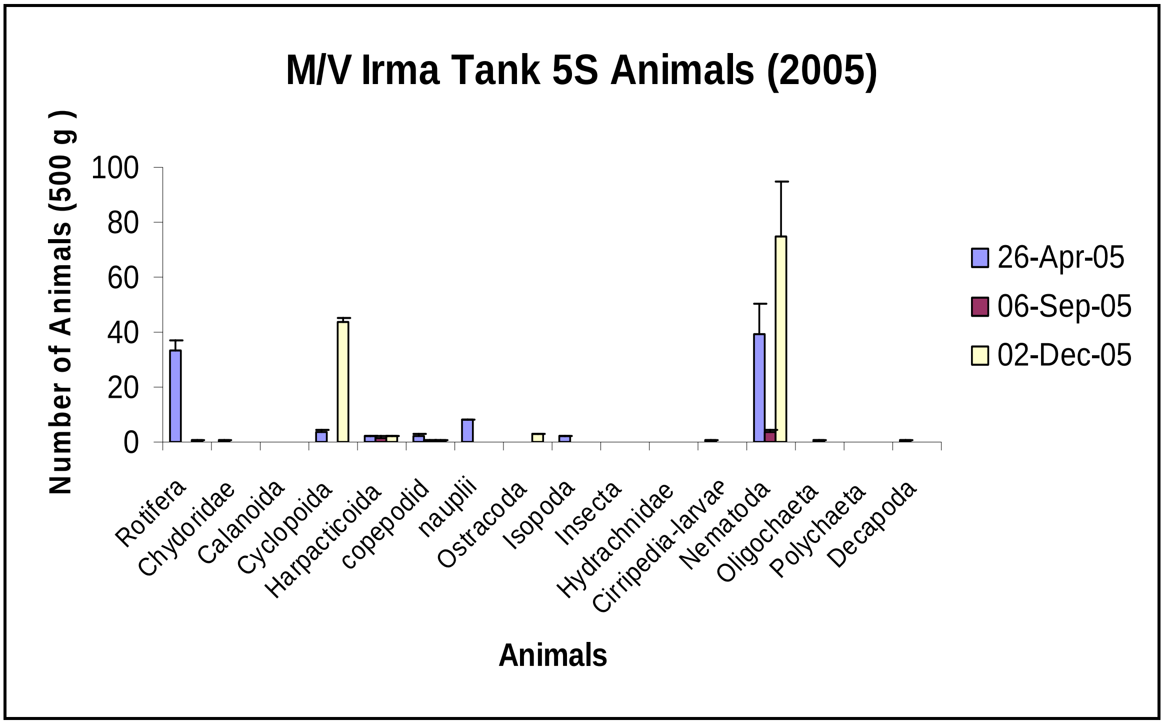


Fig. 2.17. Mean number (± S.D) of animals detected in 500 g of residual sediment from ballast tank 5S from the M/V Irma.

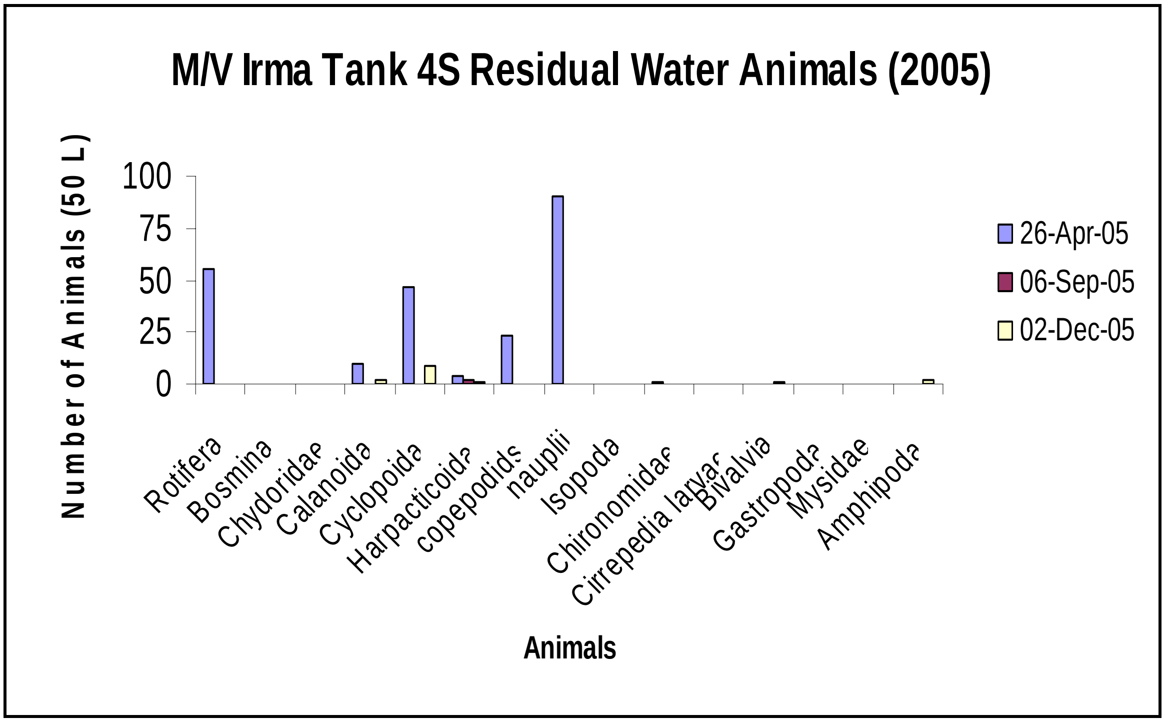


Fig. 2.18. Number of animals found in 50 litres of residual water from ballast tank 4S from the M/V Irma.

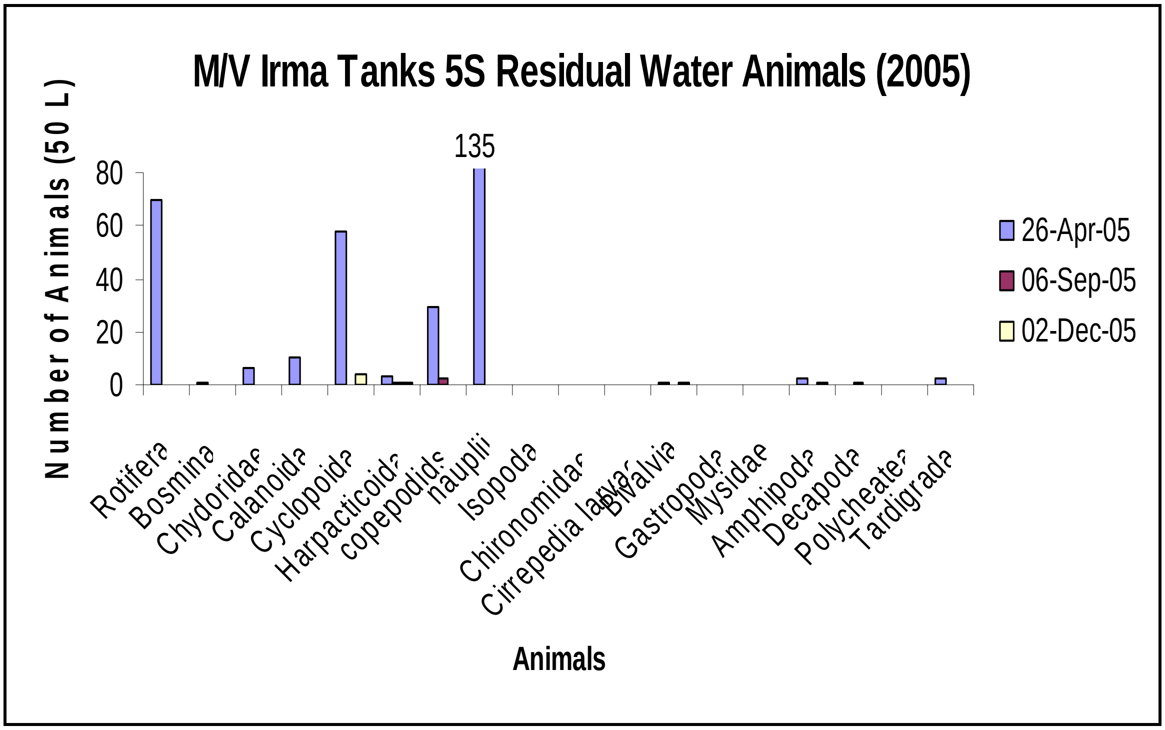


Fig. 2.19. Number of animals found in 50 litres of residual water from ballast tank 5S from the M/V Irma.

M/V Irma (2006)

Figures 2.20 and 2.21 show the mean number of resting eggs detected in tanks 4S and 5S respectively from two replicate 500 g residual sediment samples. An increase in the number of resting eggs found in the sediments occurred between April 28 and July 14, 2006.

Figures 2.22 and 2.23 show the number of animals collected from the sediment from tanks 4S and 5S respectively. Similarly as in 2004, both tanks had an increase in the number of harpacticoid copepods, nematodes and polychaetes between April 28 and July 14, 2006. This increase could be due to the addition of animals during ballasting/exchange events from Amsterdam/mid-ocean flushing. As previously noted, ballasting events have the potential to add saltwater animals to tanks, but they would pose a low risk to the Great Lakes.

No water samples were obtained in July 2006 so we cannot compare trends across ballasting events. However, invertebrate densities in the April 2006 water sample are dramatically higher than previously observed in 2005 water samples (Fig. 2.24). Several factors may be involved in explaining the extremely high number of organisms in this sample. First, the ship had last ballasted in Brayton Point only 6 days prior to sampling and the deballasting that emptied the tank to allow us to sample had occurred less than 20 hours prior. Consequently, there was not a significant amount of time for natural death to occur while the water was resident in the tank. Secondly, the source of this ballast water is a coastal port and invertebrate densities are typically much higher in coastal regions than in mid-ocean. This scenario also points to a serious gap with regard to the intended protection framework of current ballast water exchange regulations, namely that vessels conducting coastal trade are excluded from ballast exchange requirements.

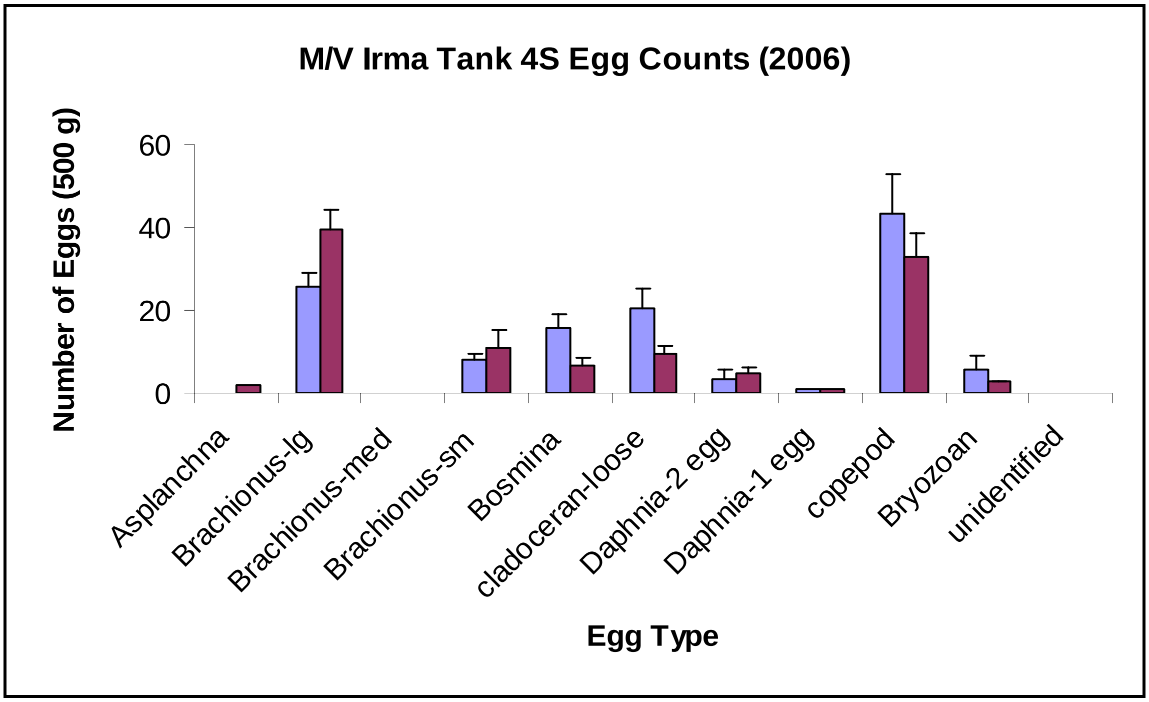


Fig. 2.20. Mean number (± S.D) of resting eggs detected in 500 g of residual sediment from ballast tank 4S of the M/V Irma.

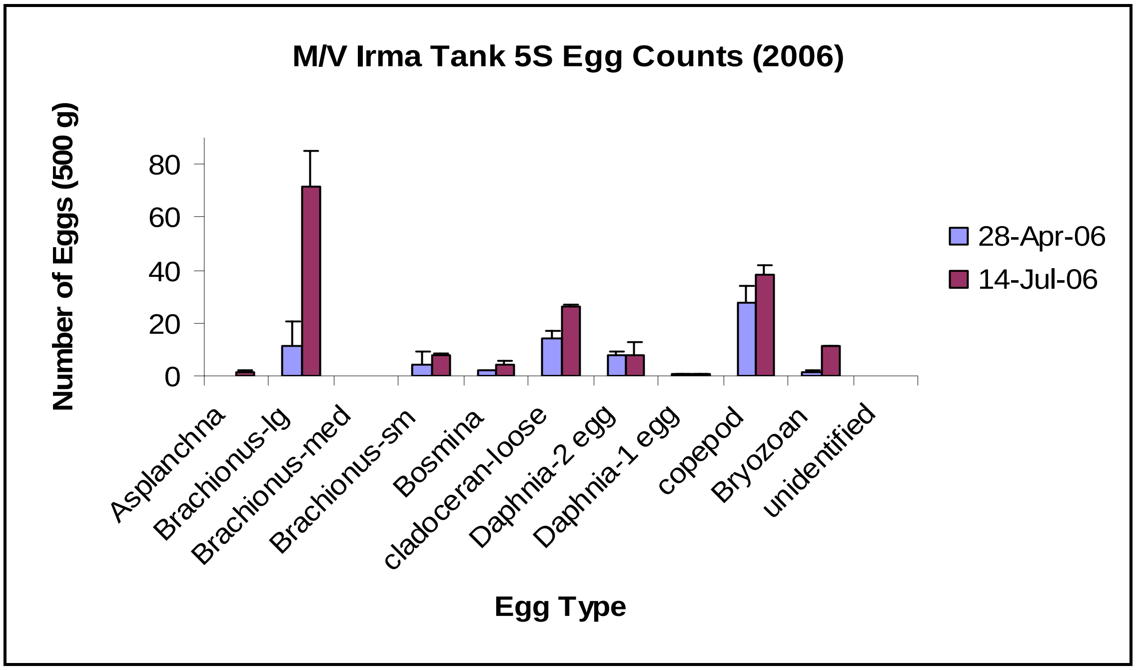


Fig. 2.21. Mean number (± S.D) of resting eggs detected in 500 g of residual sediment from ballast tank 5S of the M/V Irma.

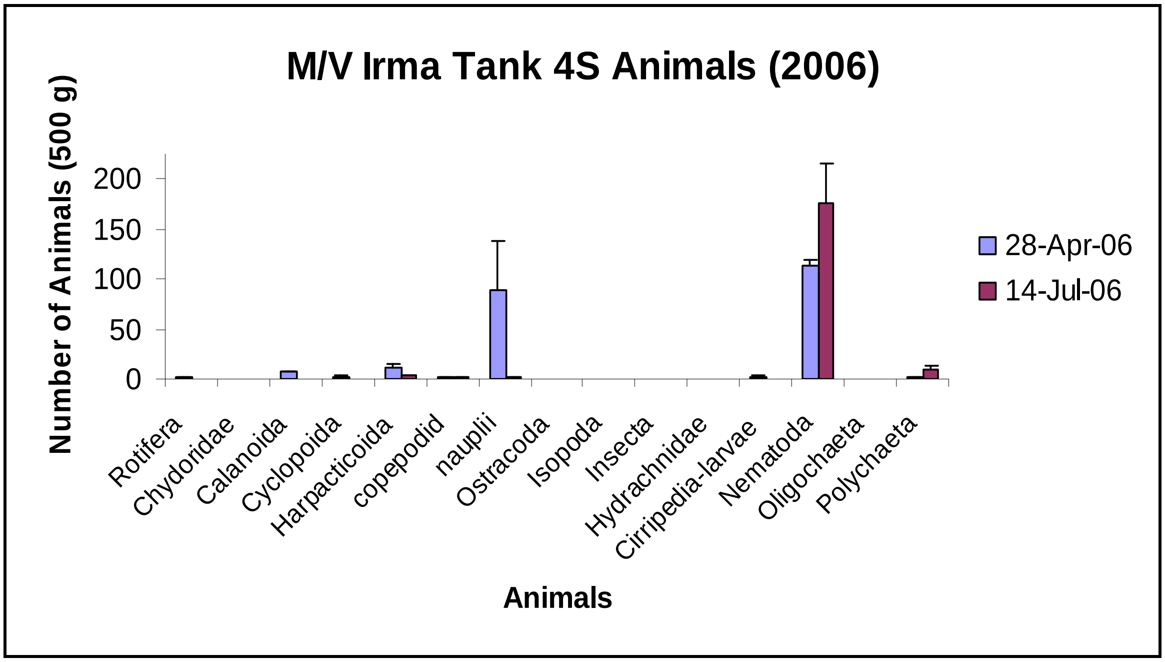


Fig. 2.22. Mean number (± S.D) of animals detected in 500 g of residual sediment from ballast tank 4S from the M/V Irma.

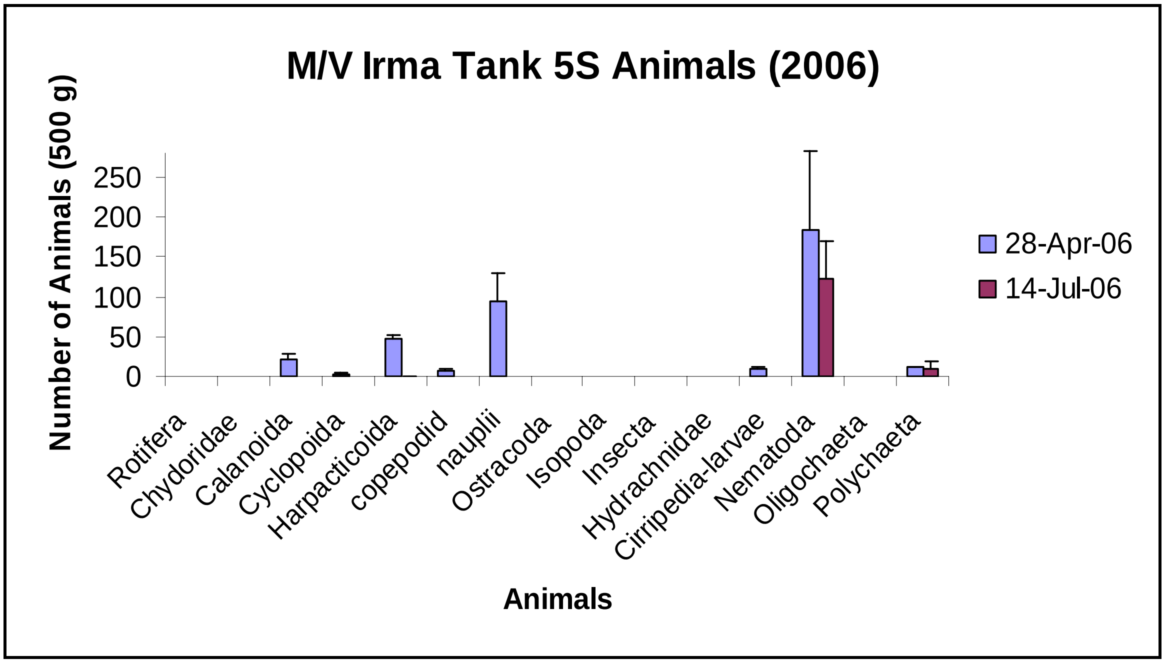


Fig. 2.23. Mean number (± S.D) of animals detected in 500 g of residual sediment from ballast tank 5S from the M/V Irma.

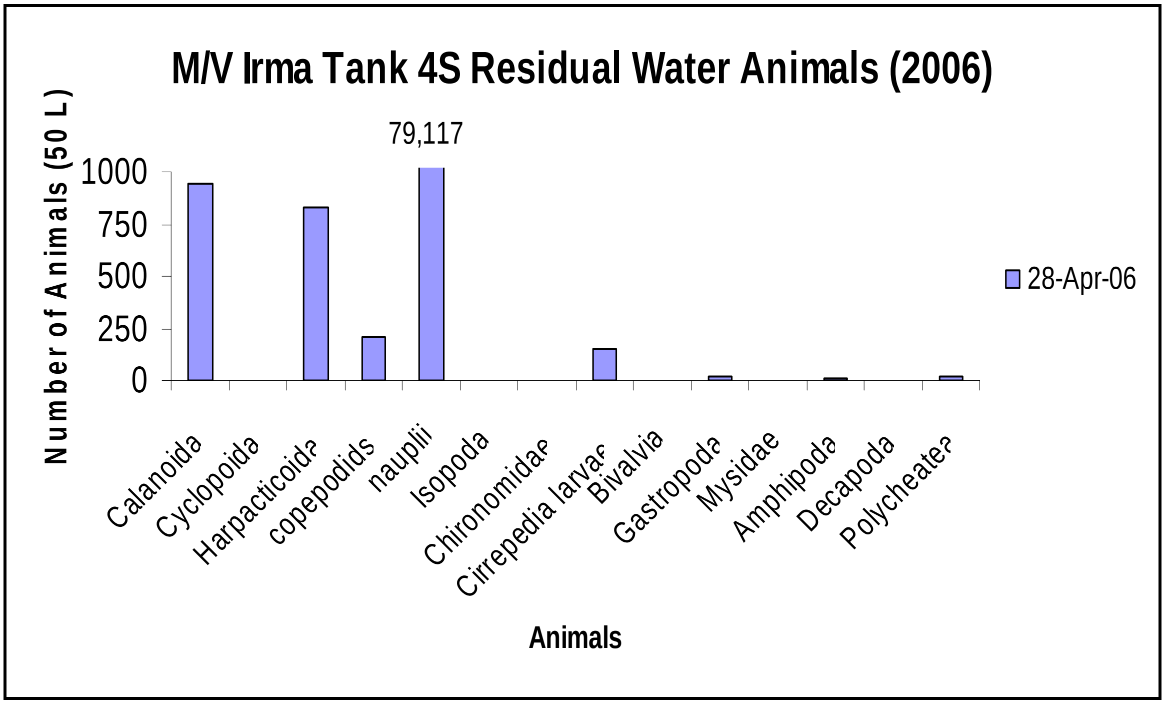


Fig. 2.24. Number of animals found in 50 liters of residual water from ballast tank 4S from the M/V Irma.

Conclusions

Ballasting events changed the number of organisms found in association with both water and sediment residuals, whether ballast was with fresh, brackish, or saltwater. However, there was no consistency to the changes, either in the types of organisms present, or the densities at which they were present. Due to the multiple ballast events between each sampling opportunity, we are unable to associate particular changes with specific ballast events or practices. High densities of animals were detected in residuals from both fresh- and salt-water sources. Therefore, it is recommended again that all ships complete a flushing/exchange in the mid-ocean during voyages to the Great Lakes to reduce or eliminate the viability of fresh- or low-salinity organisms, as the potential risk for introducing saltwater animals to the Great Lakes is much lower than those from potential freshwater sources.

2.3.2. Results from BWE Experiments

Overview

Ballast water exchange efficiency was assessed on cargo vessels traveling from the Great Lakes to European ports of call. In total, BWE experiments were conducted on six vessels transiting from North America to Europe between October 2004 and September 2006 (Table 2.3). Voyages ranged from 13 to 17 days depending on the travel distance, weather conditions, and port delays. Although these vessels traveled in the direction opposite to the original proposal, these experiments provided relevant information since they began in a freshwater system, allowing for an evaluation of the effects of BWE on freshwater organisms. Ballast water from the Great Lakes or St. Lawrence River was loaded into paired test and control tanks and immediately sampled at the beginning of each voyage. One of the paired tanks was randomly selected to undergo BWE during the transatlantic voyage (the treatment tank), while the other was designated as a control that would retain the freshwater for the entire voyage. While crossing the North Atlantic, BWE was conducted by the ship's crew >200 nautical miles from shore in water > 200m in depth using the empty-refill method. At the ships destination in Europe the tanks were again sampled prior to being emptied.

Table 2.3. Information on the experiments run in this study including the departure and destination ports, vessel type, the dates of the voyage and the type of ballast tank used for assessments. Ballast water exchange efficiency (objective 2.3) was assessed on vessels 1, 2, 4, and 5. The effect of exchange on resting stages (objective 2.4) was evaluated on all voyages. Vessel type: BC – bulk carrier; CT – chemical tanker. Ballast tank type: UW- upper wing; DB – double bottom.

Vessel	Departure port	Destination port	Vessel type	Date of voyage	Ballast tank type
1	Hamilton, Ontario	Cartagena, Spain	BC	04/10/01 – 04/10/18	UW
2	Hamilton, Ontario	Hamburg, Germany	CT	05/07/23 – 05/08/09	UW
3	Montreal, Quebec	Rotterdam, Holland	BC	05/09/29 – 05/10/11	DB
4	Hamilton, Ontario	Hamburg, Germany	CT	05/12/05 – 05/12/20	UW
5	Hamilton, Ontario	Hamburg, Germany	CT	06/04/25 – 06/05/09	UW
6	Hamilton, Ontario	Reyðarfjörður, Iceland	BC	06/09/01 – 06/09/14	DB

Methods

Planktonic Invertebrates

Three replicate zooplankton net (0.25 m diameter, 30-µm mesh) tows from each tank were obtained through deck access hatches, and the animals were preserved in 95 % ethanol. We were able to obtain plankton samples at both T_0 (immediately after addition of freshwater ballast) and T_1 (at the destination port in Europe) from four of the six voyages used in these experiments (Table 2.3). We were not able to collect T_0 samples from vessel 3 due to draft requirements that prevented the uptake of water in port, while equipment failure prevented the collection of T_0 samples from vessel 6. We calculated the percent change in zooplankton concentration in each tank as:

$$\% \, r = (T_1/T_0) \mathrm{x} 100;$$

Where % r represents the percent of target taxa density remaining in tank at T_1, T_0 is the initial concentration, and T_1 is the concentration following exchange. Using these values we calculated the exchange efficiency as:

$$\mathrm{Ex}_{\mathrm{Effic}} = [(C_{\% \, r} - X_{\% \, r})/(C_{\% \, r})] \mathrm{x} 100;$$

Where $X_{\% \, r}$ is the fraction remaining in the exchange tank and C $_{\% \, r}$ is the fraction remaining in the companion control tank. Exchange efficiencies were calculated for copepods, cladocerans and rotifers, as well as for the most abundant species of each group.

Benthic invertebrates

To evaluate the impact of BWE on benthic invertebrates, we used Incubator-Emergence Traps (IETraps, Fig.2.25, see Bailey et al., 2005). Each chamber was constructed from a 15 cm (inside diameter) PVC pipe cap with a threaded, sealable lid. The chambers were bolted to a rectangular PVC platform and the bolt holes were sealed with silicone. A total of twelve holes of 2.5-4 cm diameter were drilled through the lid (4 holes) and approximately half way up the wall (8 holes) of each chamber to allow for the exchange of water between the inside of the chamber and the ballast tank. 60µm nitex mesh was affixed to the exterior surface of each chamber body and interior surface of each top to completely cover all holes, and was secured with PVC cement and 18 cm diameter hose clamps.

To evaluate the effect of BWE on benthic invertebrates, 30 *Echinogammarus ischnus* amphipods and 30 *Brachiura sowerbyi* oligochaetes collected from the Great Lakes were placed with sediments inside one incubation chamber in control and experimental tanks of vessels 4, 5, and 6 at T0. Incubation chambers used for benthic invertebrates were not used for hatching experiments with diapausing eggs. We considered *E. ischnus* an ideal model species for these experiments since it is euryhaline, is introduced to the Great Lakes, and has a history of transport in ballast (Witt et al., 1997). Great Lakes' oligochaetes were included to test if saltwater would penetrate through residual ballast sediment during exchange and cause mortality of animals below the sediment:water interface. At the conclusion of the voyage, sediment in the live animal chambers was collected and passed sequentially through 4 and 1 mm sieves to isolate animals and determine if they survived the voyage.

Results and Discussion

Open ocean ballast exchange proved to be a highly effective method to reduce the concentration of zooplankton in the ballast tanks studied. Freshwater animals were completely absent from the exchanged ballast tanks of vessels 1 and 4, while low concentrations remained in the exchanged tanks of vessels 2 and 5 (Table 2.4). The abundance of organisms in the control tanks (not exchanged) remained high at the conclusion of the voyage for vessels 1, 4, and 5. Significant mortality occurred in the control tank of Vessel 2, however, live copepods, cladocerans, and rotifers remained in the tank at a density of 0.47, 0.04, and 0.17 individuals / L, respectively.

The high survivorship of organisms in the control tanks of vessels 1, 4, and 5 contrasts with the results of other studies that observed a sharp decline in abundance and species richness of plankton in ballast tanks within the first few days of the voyage (Gollasch et al., 2000a, b; Olenin et al., 2000; Rigby and Hallegraeff 1993, 1994). Unfortunately, *in situ* measurements of water quality were not performed during these experiments. However, the increase in the abundance of copepods and rotifers in the control tank of vessel 5 during the voyage suggests that physical conditions must have been favorable for reproduction. A possible factor is these voyages occurred when water temperatures were relatively cool and based on instrument data higher levels of dissolved oxygen may have been maintained in the ballast water.

To prevent ballast-mediated introductions, the International Maritime Organization (IMO) has adopted the International Convention for the Control and Management of Ships Ballast Water & Sediments, which requires vessels to meet either a “ballast water exchange standard” or a “ballast water performance standard.” To comply with the ballast water exchange standard, vessels must “[w]henever possible, conduct ballast water exchange at least 200 nautical miles from the nearest land and in water at least 2000 meters in depth.” At least 95% volumetric exchange is required. Alternatively, ships may meet the performance standard by conducting ballast management in a manner that results in the release of less than 10 viable organisms/m^3 ≥ 50µm in minimum dimension and less than 10 viable organisms/mL < 50µm in minimum dimension and ≥ 10µm in minimum dimension.

Zooplankton exchange efficiencies demonstrated in this study are higher than, or equivalent to those on ships transiting between marine ports. In this study, sequential (empty-refill) exchange resulted in a decrease in total zooplankton abundance by >99% for all four ships for which we were able to assess exchange efficiency (vessels 1, 2, 4, 5). Studies of sequential exchange between marine ports include Wonham et al. (2001) and Ruiz and Smith (2005). Wonham et al. (2001) measured reductions in zooplankton density >98% in their assessment of three ballast tanks and a cargo hold on one ship, while Ruiz and Smith (2005) found reductions in total zooplankton that varied between 51% and 99% for tanks on seven different vessels. The results from our study suggest that the effectiveness of BWE for freshwater organisms is less variable than that for marine organisms (Ruiz and Smith 2005). The reduced variability of BWE effectiveness in our study may result from pronounced osmotic shock experienced by freshwater animals remaining in ballast tanks after BWE. Vessels transiting between marine ports must rely on purging and dilution of ballast water to eliminate coastal organisms. Vessels transiting between freshwater ports can expect decreases in zooplankton density due both to purging of organisms and to salinity effects. Lastly, it should be noted that this subset of experiments (vessels 1, 2, 4, 5) was performed in upper wing tanks and the efficiency of water exchange in these tanks may be greater owing to their structural design and location.

Table 2.4. Treatment efficiency of open-ocean exchange for the ballast tanks assessed in this study. Included are the totals for copepods, cladocerans and rotifers, as well as for the most abundant species in each group. * Copepods and rotifers were found in the exchanged tank at the end of the voyage. However, calculated treatment efficiency was 100% due to a large increase in the abundance (reproduction) of animals in the control tank during the voyage.

Vessel	Taxon	Exchange efficiency (%)	Post-exchange density (Ind./L)
1	**Copepoda**	100.0	-
	Mesocyclops edax	100.0	-
	Cladocera	100.0	-
	Daphnia mendotae	100.0	-
	Rotifera	100.0	-
	Keratella cochlearis	100.0	-
	All zooplankton	100.0	-
2	**Copepoda**	100.0	-
	Mesocyclops edax	100.0	-
	Cladocera	97.8	0.0008
	Daphnia mendotae	95.1	0.0008
	Rotifera	97.9	0.0026
	Keratella cochlearis	99.3	0.0008
	All zooplankton	99.4	0.0034
4	**Copepoda**	100.0	-
	Diacyclops thomasi	100.0	-
	Cladocera	100.0	-
	Bosmina coregoni	100.0	-
	Rotifera	100.0	-
	Synchaeta kitina	100.0	-
	All zooplankton	100.0	-
5	**Copepoda**	100.0*	0.0063
	Diacyclops thomasi	100.0*	0.0063
	Cladocera	100.0	-
	Bosmina coregoni	100.0	-
	Rotifera	100.0*	0.0010
	Polyarthra vulgaris	100.0*	0.0010
	All zooplankton	100.0*	0.0073

2.3.3. Benthic invertebrates results from IETrap experiments

Most oligochaetes in the control tanks survived their intercontinental voyages, with mortalities of 16.6%, 0%, and 20%, for vessels 4, 5, and 6, respectively. However, nearly all individuals perished in the exchanged ballast tanks, with mortalities of 100%, 100%, and 96.6% (one live individual out of 30), for vessels 4, 5, and 6, respectively. *Echinogammarus ischnus* mortality in the control tanks was higher than that for the oligochaetes at 40%, 60%, and 53.3% for vessels 4, 5, and 6, respectively. In the treatment tanks that had undergone exchange, 100%

of *E. ischnus* individuals were deceased at the end of each experiment. These results suggest that saltwater exposure during BWE is likely to be lethal for many species found above the sediment:water interface. *Echinogammarus ischnus* is considered a euryhaline species (Witt et al., 1997), and can survive in laboratory experiments at salinities of up to 22 ‰ for 48 hours (S. Ellis, Great Lakes Institute for Environmental Research, Windsor, ON, *personal communication*). However, in these experiments *E. ischnus* were exposed to salinities >35 ‰ for five to eight days, depending on the length of the voyage and the date that BWE occurred. There were no survivors. The survival of one of the oligochaetes in the hatch-out chambers in the exchanged tank highlights one of the potential problems with BWE. The lone live individual was found at the very bottom of the sediment layer, suggesting that saline water may not have been able to penetrate through the sediment. If individuals can survive below the sediment:water interface then they could represent an invasion risk if sediments are disturbed during subsequent ballasting activities. Future experiments assessing the penetration of saline water through ballast sediments, and the survival of a variety of benthic invertebrates in these sediments, is needed to assess the risk this problem poses.

Objective 2.4. Experimentally assess the reduction in viability of resting stages contained within ballast sediments using our project-based hatch out chambers.

Overview

The effect of saltwater exposure on diapausing invertebrate eggs was evaluated both directly in the tank and in follow-up laboratory-based hatching experiments. Six IETraps covered with 60µm nitex mesh (Figure 2.25) were placed in both the treatment and control ballast tanks at the beginning of the voyage. The Nitex mesh affixed to the hatch-out chambers allowed the sediment inside to be exposed to saltwater during ballast water exchange (the exchanged tank) or to remain in freshwater (the control tank). Four of the IETraps installed in each tank were used for the salinity exposure experiments, one trap contained autoclaved sediment and served as a control for live external zooplankton entering the traps, and the last trap was used to incubate live benthic invertebrates as part of the experiments described above for objective 2.3.

Fig. 2.25. Seeding IETraps with previously collected ballast sediments to examine effects of BWE on hatching from diapausing eggs.

Ballast sediment (300g) previously collected from vessels operating on the Great Lakes was placed inside each of the chambers. The density of eggs in the sediment used for experimentation was supplemented by 100% to maximize the probability of hatching occurring during the course of the voyage. The diversity and abundance of diapausing eggs present in the supplemented sediments was characterized prior to their use in experiments (Table 2.5). At the end of the voyage we collected the sediment from the hatch-out chambers using sterile scoops and spatulas and shipped it back to the laboratory on ice for use in viability experiments.

Following incubation within the tank and exposure to BWE, sediments were retrieved from the traps and returned to the laboratory to conduct a follow-up hatching viability experiment. Experiments were conducted following the methodology presented in Gray et al. (2005) and designed to mimic the exposure of eggs to conditions similar to those in the Great Lakes. Sediment collected from each trap was thoroughly rinsed, inoculated in synthetic pond water (150mL; Hebert and Crease 1980) and held in an environmental chamber at 20ºC with a 16:8

light:dark cycle. We checked for hatched organisms every 48 hours for 10–20 days, with the experiment terminated when no hatching was observed on any day after the first 10 days.

Results and discussion

In-tank Hatching Experiments

Egg densities for the sediments used for these experiments ranged from 661.2 - 4045.2 eggs / 300 g (Table 2.5). Rotifer eggs were numerically dominant in all sediments representing between 64 % and 97 % of eggs. Cladoceran eggs were present in low numbers in all sediments. Nine rotifer species and one cladoceran species were recovered from hatch-out chambers at the conclusion of the ships' voyages. Rotifers larger than 60µm were not found in the traps containing autoclaved sediment, suggesting that contamination did not influence the results. The number of animals recovered from hatch-out chambers in control tanks was significantly higher than that from chambers in the exchanged tanks (Table 2.6; Paired *t*-test; t=3.45, df = 5, p=0.018). Between 0.5 and 3.25 individuals per trap were recovered from chambers in the control tanks, while 0 to 0.25 were recovered from chambers in the exchanged tanks (Table 2.6). More species were recovered from hatch-out chambers in control tanks versus treatment tanks; however this may simply be a function of the total number of individuals collected.

Table 2.5. Mean diapause egg density per 300g of supplemented ballast sediment placed in hatch-out chambers. Numbers (1-6) refer to vessels listed in Table 2.4.

Egg Type	**1**	**2**	**3**	**4**	**5**	**6**
Asplanchna	10.6	--	7.4	4.4	--	--
Brachionus	378	2958	334.4	784.4	2253	1801
Filinia	31.4	7.4	93	48	19.4	10.8
Synchaeta	4.4	604.4	982.4	--	859.4	711.2
Unidentified Rotifera	52.4	336	243	19.4	273	205
Bosmina	--	21	12	22.4	31.4	32
Daphnia	--	--	78	37.4	--	--
Unidentified Cladocera	135	34.4	114	79.4	63	51.2
Copepoda	49.4	84	724.4	4.4	54	24.4
Total	661.2	4045.2	2588.6	999.8	3553.2	2835.6

Table 2.6. Mean number of individuals recovered from hatch-out chambers (± standard deviation) in the control and open=ocean exchanged tanks. Vessel numbers refer to those listed in Table 2.4.

Vessel	**Mean hatching control (±SD)**		**Mean hatching exchanged (±SD)**	
1	3.25	± 0.63	0.25	± 0.25
2	1.80	± 0.58	0.00	± 0.00
3	1.40	± 0.40	0.20	± 0.20
4	0.75	± 0.48	0.00	± 0.00
5	0.71	± 0.24	0.00	± 0.00
6	0.50	± 0.29	0.00	± 0.00

The number of animals recovered from chambers in exchanged tanks was significantly lower than from chambers in control tanks. There are three possible explanations for the lower abundance of rotifers and cladocerans in chambers from exchanged tanks:

- First, saltwater exposure may have killed animals that hatched during the pre-exchange period. The pre-exchange period, during which both the control and treatment tanks contained freshwater, ranged from 6 to12 days, which was more than sufficient time for species to hatch from diapausing eggs (Bailey et al., 2004). Since the salinity of the water in the incubation chambers in exchanged tanks was measured at >26 at the end of the voyages, many freshwater animals that hatched during the pre-exchange period would presumably have perished owing to osmotic shock.
- Second, the presence of saltwater in the chambers could have prevented further recruitment from diapausing eggs in the sediment since environmental conditions would not cue hatching. Diapausing eggs often require specific environmental cues to encourage hatching (e.g., Schwartz and Hebert 1987), and the presence of saline conditions may have discouraged development of the eggs in the exchanged tanks. Previous work has indicated that diapausing eggs of freshwater species will not hatch when exposed to saline conditions, though viability of these eggs would not be adversely affected by such exposure (Bailey et al., 2004).
- Third, environmental conditions inside the incubation chambers deteriorated to conditions unsuitable for hatching. We conducted experiments in which instrument sondes were embedded inside separate incubation chambers of the same design used here. Results showed that exchange between ambient water and water trapped in the chamber can be limited, depending on ship motion, and hence biochemical oxygen demand from sediment can lead to hypoxic or anoxic conditions inside the chambers. Such conditions would prevent most diapausing eggs from hatching (Raikow et al., 2006), and could also explain the high mortality of the sentinel invertebrates in the

> exchange tank chambers. However, since the control tank chambers were set-up the same as their companion exchange tank chambers yet some had significantly higher hatching and survivorship of sentinel invertebrates, it would appear that the potential decline of oxygen inside the chambers cannot explain all the results. Still, the diapausing egg hatch rates observed in our exchange-tank chamber experiments must be considered as minima due to possible hypoxia or anoxia inside the chambers.

Hatching success in unexchanged tanks in this study was higher than in our previous NOBOB study using IETraps of almost identical design. Bailey et al. (2005) conducted *in-situ* hatching experiments with vessels transiting between Great Lakes' ports and found hatching of approximately 0.5 individuals per 500g of sediment. The average hatching in this experiment was 1.4 individuals per 300g of sediment in the control tanks. However, the average length of the ships' voyages in Bailey et al. (2005) was only 9.5 days compared to 15.3 for this study. This extra voyage time could have provided an opportunity for more animals to hatch. The density of diapausing eggs was also augmented in the sediments used for our experiments by approximately 100%, while those used for Bailey et al. (2005) were not. A higher density of eggs present in the sediment translates into a larger number of eggs within the top few millimeters of sediment. This could mean that more eggs would receive the cues necessary for hatching (e.g. light, oxygen; see Kearns et al., 1996), increasing the total number of animals that hatched.

Post-BWE Laboratory Viability Experiments

Twenty-four rotifer species, three cladoceran species, and unidentified nauplii hatched during the post-BWE laboratory viability experiments. Neither the total abundance of hatched individuals nor the species richness of hatched individuals differed significantly between sediments collected from hatch-out chambers in the exchanged versus control ballast tanks (Table 2.7). These results suggest that diapausing invertebrate eggs may be largely resistant to saltwater exposure, and that BWE may not mitigate the threat of species introductions posed by this life stage. Similar results were published by Gray et al. (2005), although their experiments simulated BWE in the lab, rather than performing the saltwater exposure under operational conditions in ballast tanks. In their experiments with both natural and ballast sediment, they found no significant differences in the abundance of hatched organisms or the species richness of organisms hatched between sediments that had been exposed to saltwater for ten days, versus those that were not. However, Bailey et al. (2004) performed experiments on diapausing eggs that were isolated from sediment and found significant differences in viability after exposure to saline water. This suggests that sediment may somehow protect the eggs from saltwater exposure. Sediment may also offer a refuge for benthic invertebrates, as discussed below.

Overall, our results suggest that BWE may reduce the recruitment of animals from diapausing eggs present in ballast sediments. However, our laboratory viability experiments and those of Gray et al. (2005), show that actual egg viability is generally not changed by exposure to saline water. Other treatment options, such as strong oxidizing biocides, may be needed to eliminate the risk of species introductions from diapausing eggs (Gray et al., 2006).

Table 2.7. Mean values with standard deviations for the total number of individuals that hatched during incubation experiments for sediments collected from IETraps in the tank that underwent ballast water exchange (exchanged tank) and the un-exchange tank (control tank). *P*-values display the results of *t*-tests performed to test for a difference in hatching between exchanged and control tanks.

Experiment	**Data Category**	**Exchanged tank**		**Control tank**		***P*-value**
		n	**Mean (SD)**	***n***	**Mean (SD)**	
1	Total hatching	4	1.50 (0.58)	4	2.00 (1.41)	0.549
	Species richness	4	1.75 (0.96)	4	1.75 (0.96)	1.000
2	Total hatching	4	8.00 (0.84)	4	4.40 (1.2)	0.033
	Species richness	4	3.00 (1.22)	4	2.60 (1.95)	0.708
3	Total hatching	4	19.40 (10.26)	4	14.60 (7.44)	0.422
	Species richness	4	6.40 (2.51)	4	6.60 (1.67)	0.866
4	Total hatching	4	3.50 (0.58)	4	2.00 (0.82)	0.024
	Species richness	4	2.00 (0.86)	4	1.25 (0.50)	0.168
5	Total hatching	4	12.50 (5.51)	4	19.25 (4.50)	0.106
	Species richness	4	5.40 (3.58)	4	6.40 (3.65)	0.673
6	Total hatching	4	2.00 (0.82)	4	2.50 (0.58)	0.356
	Species richness	4	1.25 (0.50)	4	1.25 (0.50)	1.000

References

Bailey, S.A., Nandakumar, K., Duggan, I.C., van Overdijk, C.D.A., Johengen, T.H., Reid, D.F. and MacIsaac, H.J. 2005. *In situ* hatching of invertebrate diapausing eggs from ships' ballast sediment. Diversity and Distributions 11:453-460

Balcer M.D., Korda N.L. and Dodson S.I. 1984. *Zooplankton of the Great Lakes*. Madison: University of Wisconsin Press

Burgess R. 2001. An improved protocol for separating meiofauna from sediments using colloidal silica sols. Marine Ecology Progress Series 214:161-165

Gollasch, S., Lenz, J., Dammer, M. and Andres, H-G. 2000a. Survival of tropical ballast water organisms during a cruise from the Indian Ocean to the North Sea. Journal of Plankton Research 22: 923-937

Gollasch, S., Rosenthal, H., Botnen, H., Hamer, J., Laing, I., Leppakoski, E., MacDonald, E., Minchin, D., Nauke, M., Olenin, S., Utting, S., Voigt, M. and Wallentinus, I. 2000b. Fluctuations of zooplankton taxa in ballast water during short-term and long-term ocean-going voyages. Internationale Revue der Hydrobiologie 85: 597-608

Gray, D.K., Bailey, S.A., Duggan, I.C. and MacIsaac, H.J. 2005. Viability of invertebrate diapausing eggs exposed to saltwater: implications for Great Lakes' ship ballast management. Biological Invasions 7: 531-539

Gray, D.K. I.C. Duggan & H.J. MacIsaac. 2006. Can sodium hypochlorite reduce the risk of species introductions from diapausing invertebrate eggs in non-ballasted ships? Marine Pollution Bulletin 52: 689-695.

Hebert P.D.N. and Crease T.J. 1980. Clonal existence in Daphnia pulex (Leydig): another planktonic paradox. Science 207: 1363-1365

Hudson, P. L., Reid, J.W., Lesko, L. T. and Selgeby, J.H. 1998. Cyclopoid and Harpacticoid Copepods of the Laurentian Great Lakes. Bulletin of the Ohio Biological Survey New Series. 50p.

Kearns, C.M., Hairston, N.G., Jr. and Kesler, D.H. 1996. Particle transport by benthic invertebrates: its role in egg bank dynamics. Hydrobiologia 332: 63-70.

Niimi, AJ and Reid, DM. 2003. Low salinity residual ballast discharge and exotic species introductions to the North American Great Lakes. Marine Pollution Bulletin 46: 1334-1340

Olenin, S., Gollasch, S., Jonusas, S. and Rimkutf, I. 2000. En-route investigations of plankton in ballast water on a ship's voyage from the Baltic Sea to the open Atlantic coast of Europe. International. Internationale Revue der Hydrobiologie 85: 577-596

Raikow, D. F., D. F. Reid, E. R. Blatchley III, G. Jacobs and P. F. Landrum. 2007. Effects of proposed physical ballast tank treatments on aquatic invertebrate resting eggs. Environ. Toxicol. Chem. 26: 717-725.

Rigby, G. and Hallegraeff, G.M. 1993. Ballast water exchange trials and marine plankton distribution on the MV "Iron Whyalla". Vol. 2, Australian Government Publishing Service, Canberra, 123 pp.

Rigby, G. and Hallegraeff, G.M. 1994. The transfer and control of harmful marine organisms in shipping ballast water: behaviour of marine plankton and ballast water exchange trials on the MV "Iron Whyalla". Journal of Marine Environmental Engineering 1: 91-110

Ruiz G. M., and G. Smith. 2005. Biological study of container ships arriving to the Port of Oakland. Part b - ballast water exchange efficacy: results of tests on eight container ships. A pilot study. Available online at:

http://www.serc.si.edu/labs/marine_invasions/publications/PortOakfinalrep.pdf

Schwartz S.S. and Hebert P.D. 1987. Methods for the activation of the resting eggs of Daphnia. Freshwater Biology 17: 373–379

Stemberger R.S. 1979. *A* guide to rotifers of the Laurentian Great Lakes. Report no. EPA-600/4-79-021. Environmental Monitoring and Support Laboratory, Office of Research and Development. U.S. Environmental Protection Agency, Cincinnati, Ohio.

Witt, J.D.S., Hebert, P.D.N., and Morton, W.B. 1997. *Echinogammarus ischnus*: another crustacean invader in the Laurentian Great Lakes basin. Canadian Journal of Fisheries and Aquatic Sciences 54: 264-268

Wonham, M. J., W. C. Walton, G. M. Ruiz, A. M. Frese, and B. S. Galil. 2001. Going to the source: role of the invasion pathway in determining potential invaders. Mar. Ecol. Prog. Ser. 215: 1-12

Task 3: Characterize source invertebrate populations and assess salinity toxicity as a barrier to prevent transfers of high-risk species to the Great Lakes in ballast tanks.

Objective 3.1: To characterize zooplankton communities and environmental conditions at several northern European source ports that have significant shipping traffic to the Great Lakes.

Port Traffic

Arguably the most important freshwater port system in the North America is located in the Great Lakes region. It has been estimated that 185 exotic species have invaded the Great Lakes region between 1840 and 2006 (Ricciardi, 2006; A. Ricciardi, McGill University, *personal communication*). Commercial shipping among the ports of the Great Lakes, North-West Atlantic Ocean, North Sea, Baltic Sea, Mediterranean Sea, and the Black-Azov Sea region has accounted for the majority of invasive species in these areas (Leppakoski et al., 2002; MacIssac et al., 2002; Reid and Orolva, 2002). Due to the greater proportion of ship traffic to the Great Lakes from low salinity ports of the North Sea and Baltic Sea, species from these ports are often classified as 'high invasion risk' taxa (See Figure 3.1)**.**

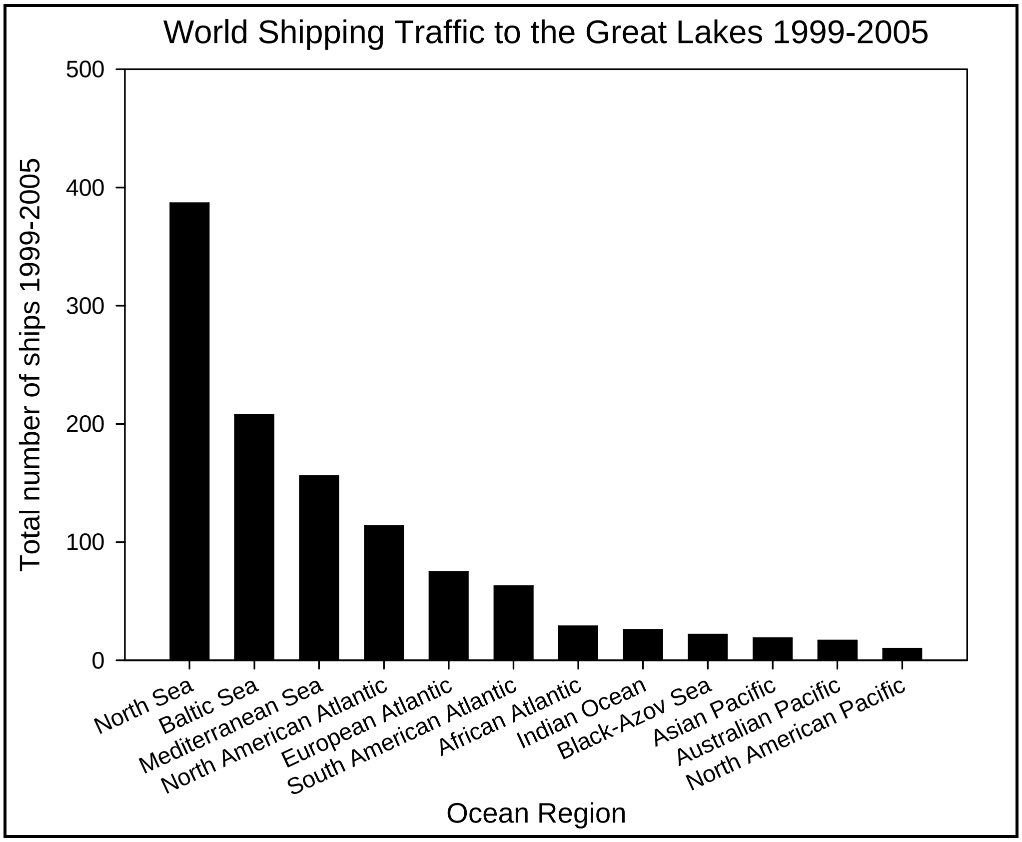


Figure 3.1: World shipping traffic to the Great Lakes 1999-2005

Tracking the number of arrivals from the last port visited is one measure of the species pools a ship may be carrying into the Great Lakes region. Information provided by the U. S. Maritime Administration (MARAD) indicates over 1100 ships entered the Great Lakes region from ports outside the St. Lawrence Seaway between 1999 through 2005. The majority of traffic originated from European ports particularly from The North Sea and Baltic Sea. Approximately 50% of the total ship traffic from the North Sea and Baltic Sea entering the Great Lakes during this six year period originated from ports in The Netherlands and Belgium (Figure 3.2). Overall, ship traffic from Baltic Sea ports accounted for approximately 38% of the total traffic into the Great Lakes. Among Baltic Sea ports, St. Petersburg, Kokta, and Oxelosund comprise the majority of these arrivals (27%).

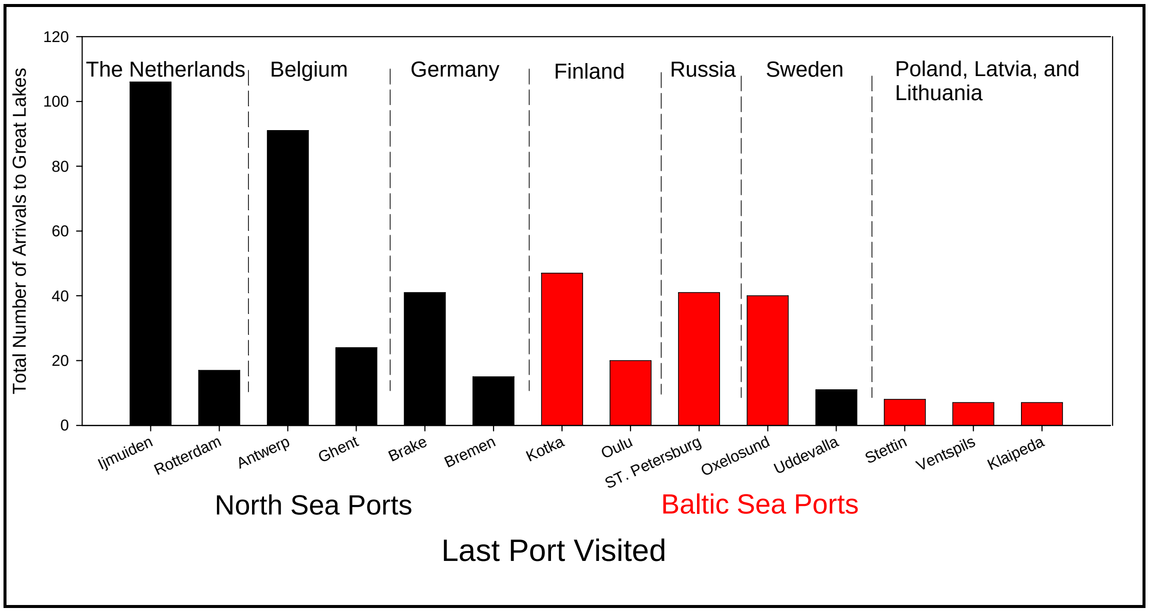


Figure 3.2: Total number of arrivals to US Great Lakes Ports from the Baltic Sea and the North Sea region 08/01/99 to 12/31/05 MARAD data.

Salinity and Temperature Ranges among the Ports of the North Sea and Baltic Sea

While there are some small differences in minima and maxima along their latitudinal positions, ports within either the North Sea or Baltic Sea exhibit a similar temperature range (0 - 25 ^{o}C) and seasonal shifts as for the Great Lakes (see Table 3.1). Consequently, all species from these port systems experience a temperature range that makes them a match for the Great Lakes and water temperature is not likely to be a good discriminator for selecting or ignoring fauna as potential Great Lakes invaders from any ports of the North Sea or Baltic Sea.

Patterns for salinity are much more diverse and ports of the North Sea and Baltic differ significantly in their average salinities and exhibit significant daily and seasonal fluctuations. Of the 14 ports listed in Table 1 all are located within an estuary except for Ijmuiden and Uddevala. Estuaries of the North Sea and Baltic Sea are documented areas of invasion success for several reasons such as their proximity to commercial shipping, minimum native species richness, and the physiological characteristics of estuarine fauna (Paavola et al., 2005; Nehring, 2006). Based on the specific salinity regime of individual ports there are clear seasonal trends in zooplankton composition and abundance as these low-salinity ports shift among freshwater, oligohaline, and mesohaline environments. Therefore overall salinity range, daily fluctuations, and seasonal shifts serve as good indicators of (1) what species are likely to be transferred among connected ports at different times of the year, and (2) the invasion risk for which potentially exotic species can survive in a distant habitat or an extended portion of their previous range.

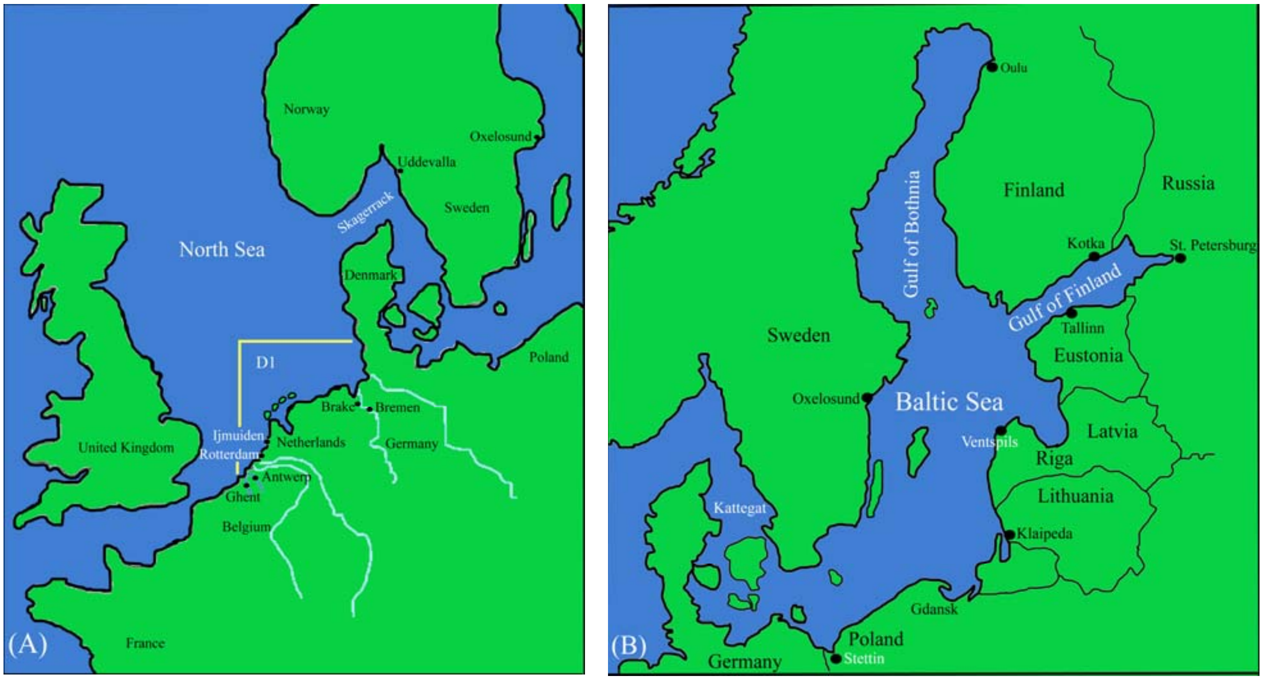


Figure 3.3: Geography of the ports of the North Sea and Baltic Sea with significant traffic to the Great Lakes. (A) The North Sea region. (B) The Baltic Sea region.

The Central North Sea exhibits an average salinity of approximately 34 ppt. Coastal regions of Belgium, The Netherlands, and Germany are influenced by the outflow of the major rivers of the region including; The Scheldt, Meuse, Rhine, Weser and Elbe. Many ports are located within these river systems in areas of low salinity and are potential source ports for high invasion risk taxa (see Table 3.1). In contrast, coastal ports of the North Sea such as Ijmuiden (The Netherlands) usually exhibit average salinities between 28-32 ppt. While the zooplankton species present within this salinity range are not generally considered to pose a high risk for invasion to the freshwater habitats of the Great Lakes, commercial ships leaving Ijmuiden for US ports may also take on ballast in one or more of the low-salinity ports from the surrounding area. Considering this factor along with the significant proportion of ships leaving from this port makes it a high risk port by proxy.

Table 3.1. Environmental Ranges for North Sea and Baltic Sea Ports with Significant Traffic to the Great Lakes Region.

Port, Country, Sea	***Temperature Range (C)***	***Salinity Range (ppt)***	***Invasion Risk (Low, Med, High)***	***Citation***
Antwerp, Schelde Estuary, Belgium, North Sea	1-25	0.7-10	High	Tackx et al., 2004 Van Damme et al., 2005 http://www.actuelewaterdata.nl/kwaliteit/
Ghent, Schelde Estuary, Belgium, North Sea	5-25	0-1	High	Tackx et al., 2004 Van Damme et al., 2005
Ijmuiden (Velsen), Netherlands, North Sea	0-22	30-35 30.9 28-30	Low	Wetsteyn and Vink, 2001 Leppäkoski and Gollasch, 2006 http://www.mumm.ac.be/EN/Models/Operational/Salinity/maps.php
Rotterdam, Nieuwe Maas, Netherlands, North Sea	5-25	0.3-28.6 0.2-30.9	High	Wetsteyn and Vink, 2001 Leppäkoski and Gollasch, 2006 Paalvast et al., 1998
Bremen and Brake, Weser River, Germany, North Sea	1-24	0-1 Bremen 3-5 Brake	High High	Schuchardt et al., 1993 Schirmer, 2003 Kappenberg and Grabemann, 2001 Leppäkoski and Gollasch, 2006
Uddevalla, Byfjorden Bay, Sweden, Skagerrak Strait Oxelosund, Sweden, Baltic Sea	2-18 0-20	20-22, 18.2 6-8	Low Medium	Bjork et al., 2000 Liungman, 2000 Leppäkoski and Gollasch, 2006 Lehvo et al., 1998
Kotka, Finland, Gulf of Finland, Baltic Sea Oulu, Gulf of Bothnia, Finland	0-25 0-22	0.2-5 0.1-2.5	High High	Inkala and Myrberg, 2002; Bäck and Ruuskanen, 2000; http://www.fimr.fi/en/itamerikanta/bsds/3682.html Lehvo et al., 1998 http://wwwp.ymparisto.fi/scripts/Perameri/Perameri.asp?intLanguage=2
St. Petersburg, Neva Estuary, Russia, Baltic Sea	0-26	0-0.11	High	Alimov 1997 Panov et al., 1999 Lehvo et al., 1998
Stettin, Szczecin Bay, Poland, Baltic Sea	0-23	0-7	High	Jasinska, 1993 Leppäkoski and Gollasch, 2006 Chubarenko et al., 2005
Klaipeda, Curonian Lagoon, Lithuania, Baltic Sea	1-22	0-7	High	Olenin et al., 1999 Zita R. Gasiunaite, 2004-2005
Ventspils, Latvia, Baltic Sea	0-22	1.6 0-7	High	Leppäkoski and Gollasch, 2006 Lehvo et al., 1998

The port of Rotterdam exhibits complex spatial variability in salinity ranging from freshwater zones near the Nieuwe Maas to near full-strength salinity at the opening to the North Sea. Although the number of ships coming directly from Rotterdam to the Great Lakes is less than other North Sea ports, Rotterdam remains as one of the most active ports in the region with planned expansions into 2008. Moreover, the shipping traffic among Rotterdam, Antwerp, and ports of the Baltic Sea serves to dilute faunal differences and promote species homogeneity among all low salinity ports of the North Sea and Baltic Sea.

The Schelde Estuary is bordered by France, Belgium, and The Netherlands. The river mouth is situated near Vlissingen (The Netherlands) where it mixes with the North Sea and exhibits salinities above 30 ppt. The Schelde has been subject to several spatial and temporal studies of its water quality and zooplankton composition (Soetaert and Van Rijswijk, 1993; Tackx et al., 2004; Van Damme et al., 2005). The river is divided into brackish water (0-85km) and freshwater zones (100-160km). Antwerp is one of the busiest ports in the North Sea and is located near kilometer 78.5 of the Scheldt River. Salinity within the port of Antwerp is tidally influenced and exhibits a salinity range that fluctuates between 0-10 ppt (Table 3.1). Further up river (near 160km) is the freshwater port of Ghent (<0.7 ppt, Tackx et al., 2004). Monthly measurements from Tackx et al. (2004) show typical differences for freshwater and brackish water zones. The salinity at Antwerp is unstable and while the winter months exhibit fairly stable low salinity values that rise to 8-9 ppt mid-spring, the summer months are erratic ranging from 0-10 ppt likely due to tidal patterns and river flow.

The Weser River Estuary in Germany empties into the Wadden Sea (North Sea) and contains three major ports, the largest of which is Bremer-Haven. Bremer-haven is situated at the river mouth and has salinity zones strongly influenced by tidal patterns. However, the majority of the ships entering the Great Lakes come from two ports further up river. Brake is located approximately 40 km upstream and exhibits average salinities between 3-5 ppt (Schuchardt et al., 1993). A tidal weir at Bremen (80 km upstream) prevents any tidal influence on this port. Bremen is a freshwater port and due to the presence of a weir, currents are slow and water residence time is longer (Kappenberg and Grabemann, 2001).

At the eastward boundary of the North Sea near the southern coast of Norway and the Swedish west coast is the Skagerrak Strait, where North Sea water mixes with the lower salinity water of the Baltic Sea (Figure 3.3). Due to this mixing, coastal areas within the fjord system of the Swedish west coast have average salinities near 20 ppt (Björk et al., 2000). The port of Uddevalla is sheltered within Byfjorden Bay and is open all year long. The intermediate average salinities found at this port system (20-22 ppt; Liungman, 2000) and proportionately lower shipping traffic classifies it as a low invasion risk for the Great Lakes (Figure 3.2 and Table 3.1).

The Baltic Sea is an intracontinental body of water noted for its low-salinity, brackish water habitats (Paavola et al., 2005). Average salinities within the central Baltic Sea are generally near 7 ppt, but coastal regions are heavily influenced by the outflow of rivers and precipitation trends especially during the spring. The Baltic Sea experiences episodic increases in salinity due to the mixing of higher salinity water through the Kattegat Strait from the North Sea. The driving force behind these saline intrusions is strong westerly winds that occur in the winter months, most often in October and February (Schinke and Matthäus, 1998). It has been suggested that the average lower salinities recorded during recent years within the Baltic Sea were the result of a lower frequency of wind driven events. However, modeling approaches currently favor the hypothesis that this trend is the result of increased precipitation and global warming (Zorita and Laine, 2000). Despite the low proportionate activity to the Great Lakes from ports of the southern Baltic (see Figure 3.2), the high traffic within Baltic Sea and other European ports as well as the overall low salinity habitats makes every Baltic Port a high invasion donor risk to the Great Lakes.

The port of Stettin is located in Szczecin Bay, Poland within the tideless Odra estuary. Average salinities in the outer Pomeranian Gulf are 5-7 ppt. However, the port is located 16 km up river along the Swina Strait where salinities generally range from 1.5-6 ppt (Jasińska 1993). Salinity changes are largely driven by wind directions and seasonal shifts within the Baltic Sea.

The port of Klaipeda, Lithuania is one of the largest ports in the eastern portion of the Baltic Sea and is located at the northern portion of Curonian Lagoon. Salinities are influenced from the outflows of the Nemunas River as well as wind speed and direction. Salinities range from 0-7 ppt and usually increase along with the frequency of salt water intrusions during fall and winter storms or when the water levels are low in summer (Olenin et al., 1999).

The Gulf of Finland contains several large ports such as Turku, Tallinn, Kotka, and St. Petersburg. Ship traffic is greatest to the Great Lakes from Kotka, Finland and St. Petersburg, Russia (Figure 3.1). The salinity gradient within the Gulf of Finland generally increases from its eastern, freshwater portion near the mouth of the Neva River where St. Petersburg is located. Across the westward axis of the Gulf of Finland salinities generally increase from 3 ppt (Kotka, Finland) to 5 ppt near the port of Turku (Östman and Leppäkoski, 1999; Panov et al., 1999; Inkala and Myrberg, 2002). Salinity in the port of Kotka usually ranges from 0.2-5.0 ppt (Bäck and Ruuskanen, 2000; Inkala and Myrberg, 2002). The only other port from Finland with significance for the Great Lakes is that of Oulu that is located in the northern portion of the Gulf of Bothnia (Figure 3.3).

Overall, the Great Lakes and low salinity ports of the North-west Atlantic and West-central Atlantic Ocean share an invasion threat from the North Sea and Baltic Sea. In particular, commercial ships from ports of the Netherlands, Belgium, Germany, Finland, and Russia may represent the greatest threat of invasive species to the freshwater and estuarine ecosystems of the eastern United States. Based on trends of temperature, salinity, and ship traffic the ports of Rotterdam, Antwerp, Ghent, Brake, Bremen, Klaipeda, Kotka, and St. Petersburg have been classified as high invasion risk donor regions. Models predicting global warming and greater precipitation for the coastal regions of the North Sea and Baltic Sea (Zorita and Laine, 2000) would increase the invasion risk status of many ports in the North Sea and all of the ports in the Baltic Sea.

Long-term and Seasonal trends in the Zooplankton Populations of the North Sea and Baltic Sea

Intensive plankton surveys have focused on the North Sea for over 80 years. The Sir Alister Hardy Foundation for Ocean Science (SAHFOS) has continuous monthly samples of near surface phytoplankton and zooplankton for numerous sites within the North Sea since 1958. During this time several long-term shifts in zooplankton composition and abundance have been observed (Hays et al., 2005). Some of the most dramatic trends within the North Sea include the decline of *Calanus finmarchicus* populations, the asynchrony of phytoplankton and meroplankton peaks, and the northward shift of mesoplankton communities. All of these trends are attributed to global climate change and will likely increase the invasion rate of Ponto-Caspian species into the freshwater systems adjacent to the North Sea and Baltic Sea (Bij de Vaate et al., 2002; Mooij et al., 2005).

For the purposes of this study, plankton populations in the coastal regions of Belgium and The Netherlands were investigated over the last ten years (area D1, see Figure 3). Although this region of the North Sea has a salinity of 30-35 ppt, several species of copepods and cladocerans included in this dataset are euryhaline and also occur in brackish water habitats (see Bakker and Pauw, 1975). This dataset also serves as a good baseline for recent seasonal trends in mesoplankton abundance and composition from coastal regions of the North Sea.

Copepods are present throughout the year with peak abundances in the summer months (June). Marine cladocerans are present between April and November and their peak abundance mirrors that of the copepods. Overall, the greatest zooplankton numbers are observed between May through August with the autumn and winter months exhibiting intermediate levels of abundance.

Figure 3.4 breaks down abundance trends across seasons. The dominant species overall are the calanoid copepods *Acartia*, *Parapsuedocalanus*, and *Temora longicornis*. During the summer months the dominant cladocerans are *Podon* spp. and *Evadne* spp. Eriksson (1974) lists the common species from the west coast of Sweden to be *Evadne nordmanni*, *Podon leuckarti*, and *Podon intermedius* that have optimal abundances between 10-18 °C and 8-17 °C respectively. Unlike the previously mentioned species, the copepod *Centropages typicus* and the cladoceran *Penilia avirostris* have their peak abundances between September and December. *P. avirostris* arrived in the southern portion of the North Sea in 1999 through anthroprogenic introduction, a range extension, or both. This species increased abundance since then has been correlated with increased water temperatures in autumn (Johns et al., 2005).

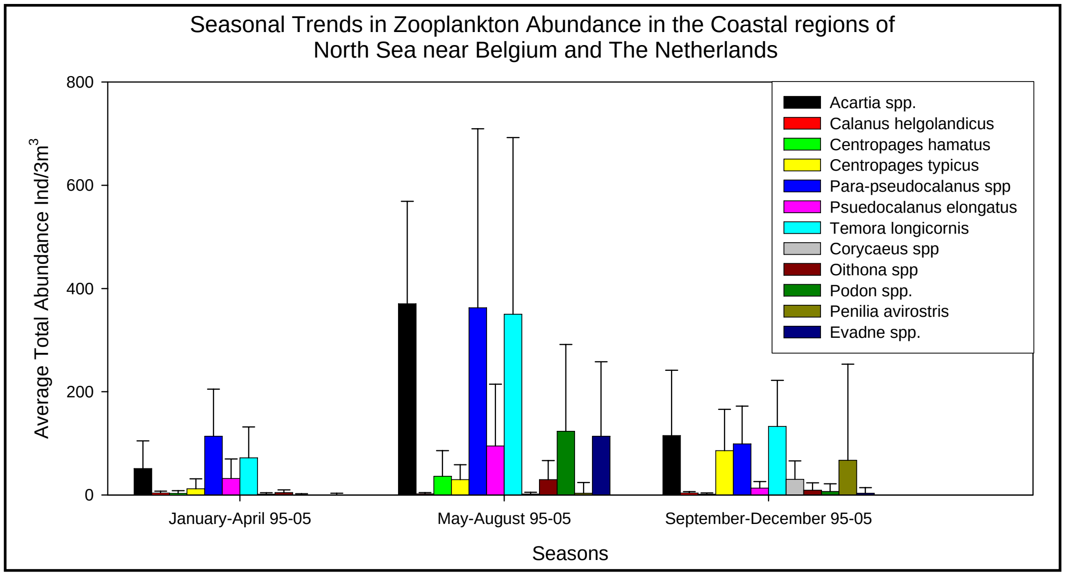


Figure 3.4. Seasonal trends in zooplankton abundance in Coastal regions of the North Sea.

Published accounts of the seasonal changes in the zooplankton fauna and hydrographic conditions from regions adjacent to the low-salinity ports of the North Sea have been described from Antwerp and Ghent (Soetaert and Van Rijswijk, 1993; Tackx et al., 2004; Van Damme et al., 2005). Zooplankton composition and abundance within the freshwater and brackish water zones around ports of both Antwerp and Ghent are dominated by rotifers (see Figure 3.5). Expectedly, the freshwater port of Ghent has significantly greater abundances of rotifers than Antwerp during the spring and summer months. Zooplankton abundance were low between December and February (1995-1996) in Ghent, but exhibited an exponential recovery during March. Rotifer abundances fluctuated significantly between May and September, being completely absent during May and June. Whether this feature is representative of real population fluctuations, species shifts, or just an artifact of sampling cannot be ascertained from the dataset. Cyclopoid copepods abundance correlated with greater water temperatures, but still preceded the arrival of cladocerans in late summer (Figure 3.5). Rotifer abundances did not show distinct trends near Antwerp. Calanoid copepods were evident in spring and polychaete larvae in late summer. The dominant cladoceran and copepod species within Ghent and Antwerp during the peak summer months were *Bosmina longirostris*, *Daphnia pulex*, *Daphnia longispina*, *Acanthocyclops robustus*, and *Cyclops strenuus strenuus* (Figure 3.6).

Published data for zooplankton abundances within other ports of the North Sea are few; however Schuchardt et al. (1993) stated that the abundances of *Eurytemora affinis* and *Bosmina longirostris* may be ten times higher in port of Bremen than surrounding Weser River due to longer water residence times and reduced tidal currents.

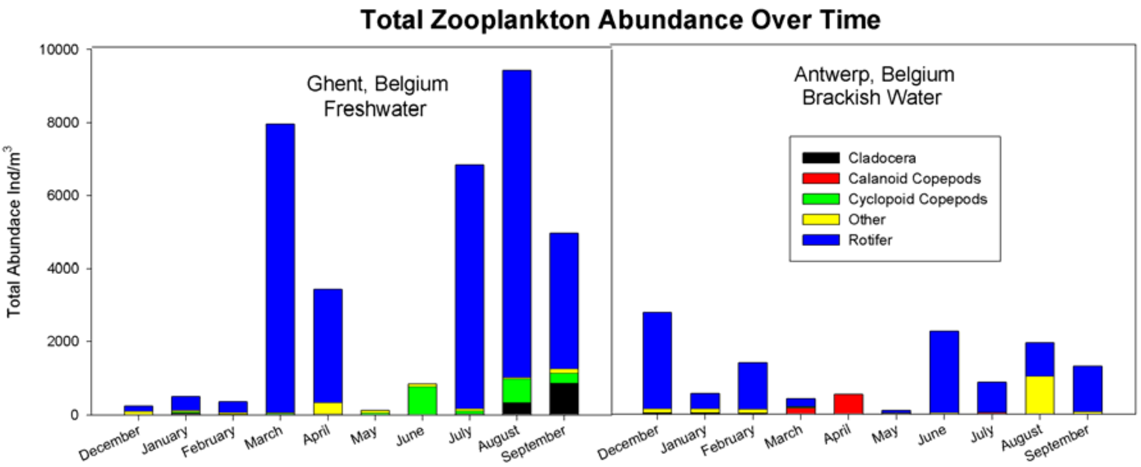


Figure 3.5: Zooplankton Composition and Abundance at Belgium Ports (1995-1996). Data provided by Dr. M. Tackx (see Tackx et al., 2004).

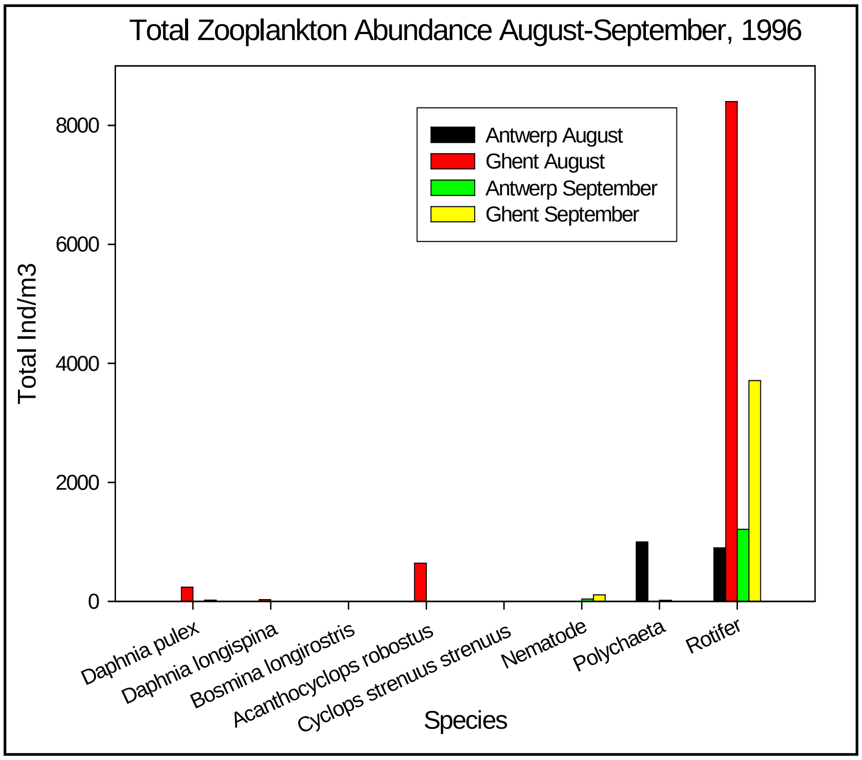


Figure 3.6. Dominant zooplankton species at Ghent and Antwerp during the summer of 1996. Data provided by Dr. M. Tackx (see Tackx et al., 2004). Some fairly abundant species like the mysid, *Mesopodopsis slabberi* did not occur within proximity to the ports.

Seasonal and spatial fluctuations in the zooplankton composition of the Baltic Sea are largely coupled to trends in the phytoplankton populations that are diminished in winter and bloom in mid-summer. The dominant zooplankton species found in less saline coastal zones are the rotifers *Synchaeta* sps., *Keratella* sps., and *Brachionus* sps.; the copepods *Eurytemora affinis* (*hirunoides*) and *Arcartia bifilosa*; and the cladocerans *Pleopsis polyphemoides*, *Eubosmina longispina maritima* (*Eubosmina maritima*), *Bosmina coregoni maritima* (*Eubosmina coregoni*), and *Evadne nordmanni* (Ackefors, 1965; Vuorinen and Ranta, 1987; Viitasalo, 1992). Diversity within cladoceran assemblages are often confused by different taxonomic interpretations and so it can be difficult to compare species accounts created by different authors (especially within the Bosminids although see Taylor et al., 2002 for a revision). The more saline regions of the western and central Baltic are often characterized by species of calanoid copepods such as *Temora longicornis*, *Pseudocalanus elongatus*, *Limnocalanus macurus*, and *Centropages hamatus* (Viitasalo, 1992; Hansen et al., 2006). Similar to what has been observed in the North Sea, climate change is having long-term effects on copepod populations of the Baltic Sea (Alheit et al., 2005). Long-term monitoring in Neva Bay has also demonstrated shifts in the cladoceran species composition as *Bosmina obtusirostris* and *Chydorus sphaericus* are now more dominant than previous years (Primakov and Nikolaenko, 2001).

The Marine Invasions Laboratory at the Smithsonian Environmental Research Center provided a sub-contract to Dr. Zita Rasuole Gasiunaite (Coastal Research and Planning Institute, Klaipeda University, Lithuania) to make monthly measurements of salinity, temperature, and zooplankton abundance at three stations within Curonian Lagoon (Klaipeda, Lithuania, Baltic Sea) from August, 2004 and July, 2005. Approximately 50 different species of zooplankton were recorded during this time, the majority of which were cladocerans, copepods, and rotifers. Based on literature reviews on the composition and relative abundance of the dominant zooplankton species from coastal regions of the Baltic Sea, Curonian Lagoon serves as a useful model port system to represent many of the major port systems of the Baltic Sea. Table 3.2 lists the dominant zooplankton for Klaipeda, and a full list is included in Table 3.3.

Table 3.2. Most abundant zooplankton species for the port of Klaipeda and Curonian Lagoon (1995-1997)

Species	**Maximum Densities (1000 ind/m^3)**	**Species**	**Maximum Densities (1000 ind/m3)**
Daphnia longispina	39.8	*Dreissena polymorpha*	106.1
Chydorus sphaericus	145.9	*Acartia bifilosa*	21.7
Leptodora kindtii	2.3	*Marenzelleria viridis*	520
Mesocyclops leuckarti	48.1		
Keratella sps.	120.1		
Brachionus sps.	254.2		

Similar to the distributions for low-salinity ports of the North Sea, the zooplankton of the Curonian Lagoon is at times dominated by rotifers (Figure 3.7). The winter months exhibit the lowest diversity and abundances of copepods and rotifers. Zooplankton populations begin to bloom in spring with peaks in late spring, although the absolute timing of bloom events often shifts in timing from year to year (Gasiūnaite and Razinkovas, 2004). During 2004-2005, cladoceran species peaked in abundance during August.

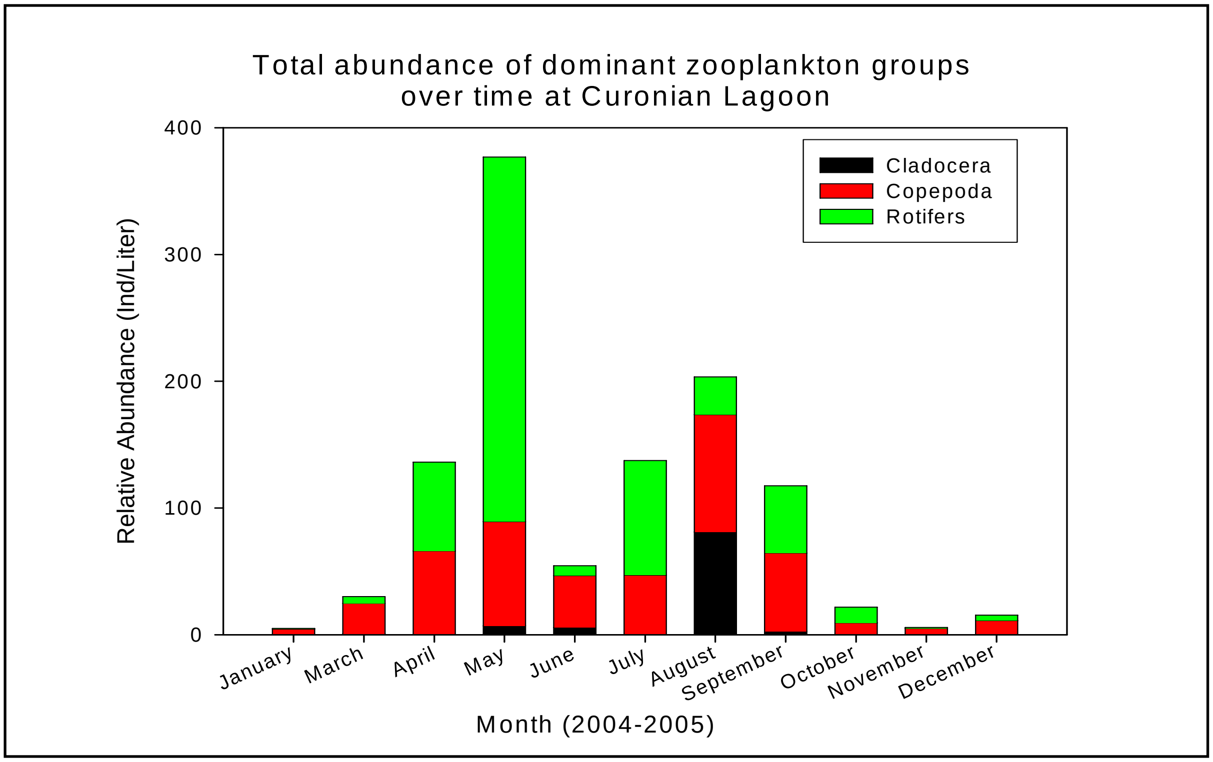


Figure 3.7. Total abundance of the dominant zooplankton groups over time at Curonian Lagoon, Lithuania, Baltic Sea.

When considering only the most dominant zooplankton through seasonal periods, the following trends were observed (Figure 3.8). The greatest overall abundances were observed during the spring when freshwater permeates the lagoon. It was during this time that the zooplankton within the lagoon was comprised mainly of bivalve veligers, rotifer species, and copepod copepodites. Also present at this time were *Cyclops vicinus*, *Mesocyclops leukarti*, *Daphnia longispina*, and *Brachionus angularis* (Figure 3.9). As the water warmed and increased in salinity during the summer, the community shifted to more salinity tolerant species such as *Chydorus sphaericus* and *Marenzelleria viridis* larvae. *Acartia bifilosa* and *Evadne nordmanni* also occur between June and September. As the water cooled in winter and again increased in salinity due to pulses from the North Sea, the zooplankton population greatly decreased. Temperature plays a major role in structuring the seasonal patterns, but the shifts in total abundance and species composition within the lagoon are likely driven by changes in salinity. Both the species diversity and abundance of cladocerans decreases with increasing salinity (Gasiūnaite, 2000).

The peak abundances and species diversity of zooplankton among the ports of the Baltic Sea and the North Sea occur during late spring through late summer, although the exact timing is difficult to predict due to seasonal and annual fluctuations in temperature and salinity. Thus, inoculate in ships' ballast and sediment to the Great Lakes from these regions is expected to mimic this seasonality.

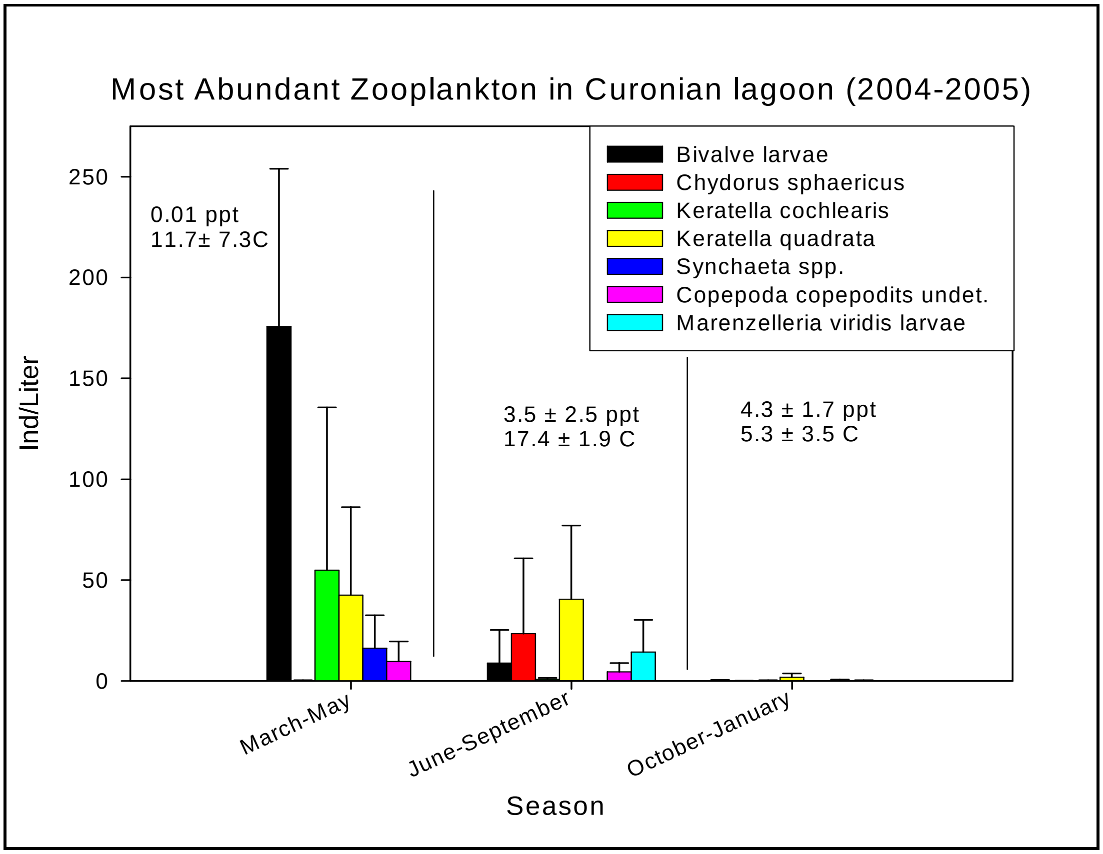


Figure 3.8. Seasonal Patterns in the dominant zooplankton forms within Curonian Lagoon (2004-2005).

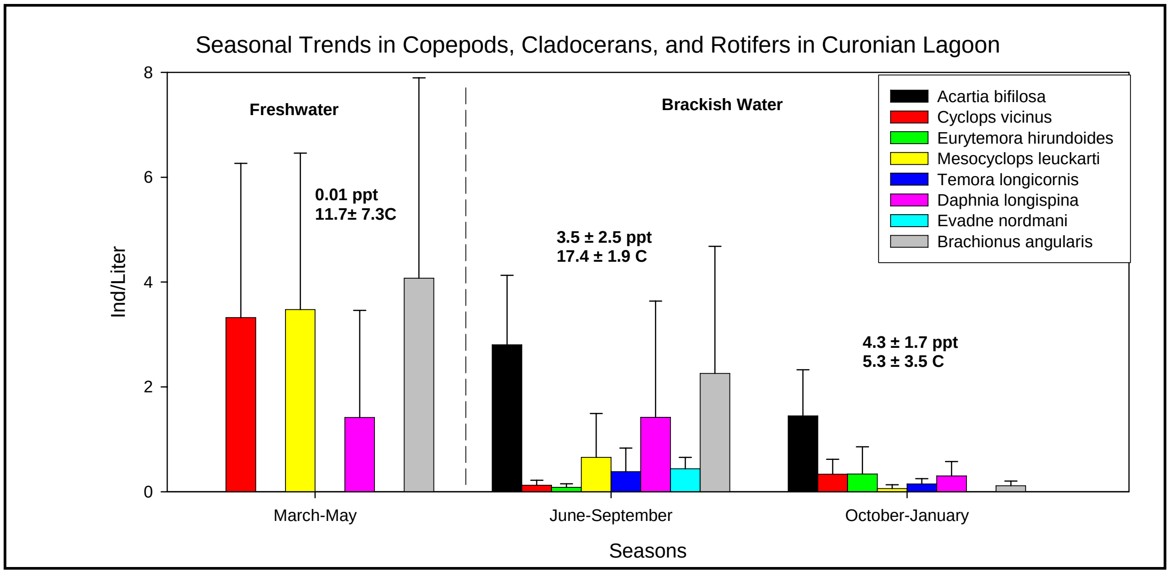


Figure 3.9: Seasonal patterns in less abundant taxa within Curonian Lagoon (2004-2005).

Objective 3.2: To synthesize literature-based information on species composition, life-history characteristics, and reported environmental tolerances of zooplankton from European source ports.

Overview

One of our long-term goals is to track the source populations for species most likely to invade the Great Lakes and other low-salinity ports of the United States. For these reasons, we compiled a list of benthic and planktonic organisms from major ports in the North Sea and Baltic Sea that may best tolerate the environmental conditions of the Great Lakes (Appendix 5). Of the 269 species listed in Table 1 of Appendix 4, the Great Lakes and port systems of the North Sea and Baltic Sea have at least 37% (n=100) species in common and only 18% (n=19) of shared species are considered exotic to the Great Lakes region. At least 5 species (27% of introduced species from these regions) listed are considered to have negative impacts upon the indigenous fauna (invasive). This estimate is proportionally higher than that for all exotic species and source regions as a whole for the Great Lakes region (15/162, 9.3%). It may be that the rate of introduction, establishment, and impact is greater for the North Sea, Baltic Sea, and Black-Azov Sea regions to the Great Lakes than other donor regions. Alternatively, this may reflect a bias in our analysis and understanding of the species communities among these regions. Taxonomic groups such as oligochaetes and rotifers have species in common but no clear introductions have occurred from the North Sea and Baltic Sea into the Great Lakes. This may reflect the difficulty in identifying species from these taxonomic groups. However, the lack of decapods from estuarine habitats introduced into the Great Lakes likely reflects the inability most of these brackish water species to survive and reproduce within freshwater. Finally, sampling effort and techniques may be biased against some invertebrate groups such as flatworms, isopods, and cumaceans.

Based on species diversity and environmental tolerances the most likely taxonomic groups to invade the Great Lakes are the amphipods, isopods, harpacticoid copepods, cladocerans, mysids, and mollusks. We then gathered further life-history information for species of amphipods, cladocerans, and mysids pertinent to their abundance and reproduction within the Ponto-Caspian, North Sea, and Baltic Sea regions. These data are summarized in Table 1 of Appendix 4. Although some of the species identified are reported from the Gulf of St. Lawrence, none of them have been recorded within the Great Lakes region but are considered potential invaders. The majority of species identified are capable of survival in freshwater and many species have salinity tolerances equaling or exceeding full-strength seawater. Our intention was to compare the life history characteristics of past and potential invaders to investigate whether there are convergent physiological, reproductive, or behavioral properties among successful invaders to the Great Lakes region and low-salinity ports of the United States. Presently, this analysis is still ongoing within our laboratory and requires further descriptive and experimental data. Preliminary review of these characteristics suggests that a combination of features such as relative abundance near multiple source regions, wide environmental tolerances, and reproductive capacity all play a role in the invasion success of particular species. Through this review, it also became clear that information regarding the salinity tolerance, reproductive capacity, and relative abundance for particular species fluctuated within and among populations. Estimates of these characters are often expressed as the collective range exhibited by a few investigated populations and often do not reflect within-population fluctuations as correlated with environmental shifts. Similar conclusions were reached by Paavola et al. (2005) during their investigation of successful invasive species found within European brackish-water habitats. Furthermore, in terms of ballast water management strategies, invasion success of particular species may be more influenced by physiological parameters such as salinity tolerance. Considering that reported salinity tolerances may not reflect a species' ability to withstand dramatic short-term changes, we designed an experimental approach to test this factor among numerous freshwater and estuarine taxa from several high-risk populations (see following sections).

Objective 3.3: To conduct laboratory-based toxicity experiments on species that occur at low-salinity ports of the United States and Europe, to test the efficacy of saltwater exposure on survivorship, simulating the time course experienced for both BWE and NOBOB BMP's.

Overview

Ballast Water Management Policies

Numerous studies have attempted to find the most economical and effective techniques for ridding ship's ballast water of both microscopic and macroscopic organisms (Tamburri et al., 2002; Waite et al., 2003; Hunt et al., 2005). Many of these ballast water management practices are effective in reducing the density of viable propagules in ballast tanks, but universal agreement among the international community on the best technology type has not been forthcoming. Currently, the most widely employed method of reducing the density of organisms in ballast tanks is open ocean ballast water exchange (BWE). However, the effectiveness of the open-ocean BWE policy has come into question in the North American Great Lakes (Holeck et al., 2004). This argument is based on the increased rate of nonidigenous species found in the Great Lakes after 1989 when ballast water regulations were initiated. Although Drake et al. (2005) stated that the conclusions of Holeck et al. (2004) were biased by time lags between nonindigenous species discovery and establishment rates and estimated the 'acceleration date' in the discovery rate of nonidigenous species in the Great Lakes to be 1982, before the start of BWE programs.

Direct observations and experimental studies aboard ships provide estimates of the efficacy of BWE and indicate that (a) BWE routinely removes 88-99% of the original coastal water and zooplankton and (b) the empty-refill method of BWE is more effective than the flow-through method (Ruiz et al., 2004 and 2005; Choi et al., 2005). Despite the efficacy of BWE, it is also evident that residual organisms remain in ballast tanks, including both organisms in the water column as well as bottom sediments (Minton et al., 2005). The response of these residual organisms to oceanic water conditions (following BWE), and their subsequent risk of invasion success is poorly understood.

For the Great Lakes and other low-salinity estuaries, the highest risk of ballast-mediated invasion is from vessels with low salinity and freshwater organisms. BWE operates both to reduce the concentration of such low-salinity organisms and also exposes them to high salinity conditions. The latter is expected to result in high mortality due to osmotic shock, thereby enhancing the efficacy of BWE (i.e., resulting from the additive effects of removal and mortality). Although Bailey et al. (2004 and 2005) explored the effect of high salinity conditions experienced during BWE on resting stages (in sediments), there was very little prior information regarding the effects of such high salinity exposure on low-salinity, waterborne assemblages. Our attempts to conduct these types of experiments in our previous NOBOB Study were only marginally successful as it was difficult to find appropriate ships that matched our experimental design and originated in a freshwater port (see Johengen et al., 2005). Experimental results described above under Objective 2.3 and 2.4, and in the following Objectives 3.3 and 3.4 provide some of the most definitive proof about the effects of such high salinity exposure on low-salinity, waterborne assemblages.

Environmental Tolerances of Nonindigenous Species: The Importance of Experimental Design

The Marine Invasions Laboratory at SERC designed salinity tolerance experiments with a diversity of planktonic and benthic animals that match the conditions of most tank types undergoing BWE by both flow-through and empty-refill methods. Details for the experimental methods are listed in Appendix 6. To briefly summarize here, salinity tolerance experiments were conducted using three treatments, each of which contained 40 individuals and survivorship is monitored at times of 1 hour, 2 hours, 3 hours, 24 hours, and 48 hours. In some of our experiments when all individuals within a given treatment appeared dead or 48 hours was reached, these specimens were transferred back to their ambient water as a final check of survivorship. Treatments included:

- *Control* - animals were kept at ambient salinity and temperature at the time of collection.
- *Flow-Through* - animals experienced a stepwise increase in salinity from ambient conditions to 14, 24, and 34 ppt seawater over a period of three hours
- *Empty-Refill* - animals experienced an instantaneous shift to full-strength seawater.

It was our intention to identify the minimum time point and salinity required to eliminate various taxa based on BWE conditions. We chose to investigate ‘high risk taxa’ such as copepods, mysid shrimps, amphipods, rotifers, bivalve veligers, decapod zoea, and cladocerans based on their reported high abundances within the ballast tanks of commercial ships from low salinity ports (or the port areas themselves) of the North Sea and Baltic Sea (Smith et al., 1999; Olenin et al., 2000; Levings et al., 2004; Tackx et al., 2005; Van Damme et al., 2005). We further selected species that were present in low salinity waters of several port systems with high shipping traffic. The experiments were carried out in several different regions known for high invasion rates and commercial ship traffic. Initial experiments were conducted in the Chesapeake Bay (Maryland) and San Francisco Bay (California), due to their close proximity and ready access, allowing us to develop and refine methods as well as broaden the scope of our analysis to multiple regions. The primary focus of our analysis was on European ports, as a major source of ship-mediated invasions to the Great Lakes (note, however, that the Chesapeake Bay and the eastern U. S. are also sources of shipping traffic to the Great Lakes). For Europe, experimental analyses were conducted on organisms from three locations: Curonian Lagoon, Klaipeda, Lithuania, Baltic Sea; The Vistula River, Poland, Baltic Sea; The port of Rotterdam, The Netherlands, North Sea.

Our experiments measured the survival of all individuals through time in a stepwise series of increasing salinity conditions. Overall, meaningful comparisons across taxa are best expressed as the proportion surviving at the time point when all or the majority of individuals undergo mortality under flow-through and empty-refill conditions. This critical time point places species into salinity functional groups that can also vary across populations and with fluctuating environmental conditions within populations.

Below, we present experimental results by source region and taxonomic group. The data are displayed in a series of figures, arranging species from left to right according to their tolerance level (survivorship) to increasing concentrations of seawater and exposure time. Photos of some of the species in our experiments are contained in appendices 2, 3, and 4.

Results and Discussion

A. Chesapeake Bay, U. S. A.

Initial experiments in the Chesapeake Bay were aimed at gathering critical salinity levels for a variety of brackish water animals. Mixed species of rotifers and cladocerans were completely eliminated by both flow-through and empty-refill treatments, although at different final salinities (see Figure 3.10). Two of the four copepod species from the Chesapeake Bay underwent 100 percent mortality in both the flow-through and empty-refill treatments. However, *Leptinogaster major* only experienced significant mortality in the empty-refill treatment, and the survivorship of a harpactoid copepod was not negatively affected by either treatment.

Mixed flatworm species were unaffected by the flow-through treatment but showed complete mortality under empty-refill conditions (Figure 3.10, Platyhelminthes). These flatworms represent a collection of species that normally prefer lower salinities (6-7ppt) but are capable of survival at higher salinities when the change is more gradual.

Although polychaete species exhibited proportionately more mortality in the empty-refill treatments than flow-through trials, these differences are not statistically significant. However, spionid polychaetes exhibited significant differences among treatments. Significant proportions of larval stages from crabs, shrimps, barnacles, and bivalves survived in both salinity treatments. *Palaemonetes pugio* zoea develop best at salinities between 10 and 35 ppt, but a small proportion of larvae can withstand salinities as low as 3 ppt (Broad and Hubschman, 1962; McKenney and Neff, 1979). Our own experiments agree with these findings in that significant mortality occurs below 3 ppt. However, a related North American species *P. paludosus* is found predominantly in freshwater and can tolerate salinities as high as 30 ppt (Beck and Cowell, 1976). Palaemonid shrimps are also invasive within the brackish water and freshwater habitats of the Baltic Sea (Grabowski, 2006). The zoea of *Rhithropanopeus harrisii* also have a developmental salinity range between 2 and 30 ppt and similar to the results for palaemonid shrimps, larval development among distant populations showed some favorability toward local environmental conditions (McKenney and Neff, 1979; Laughlin and French, 1989).

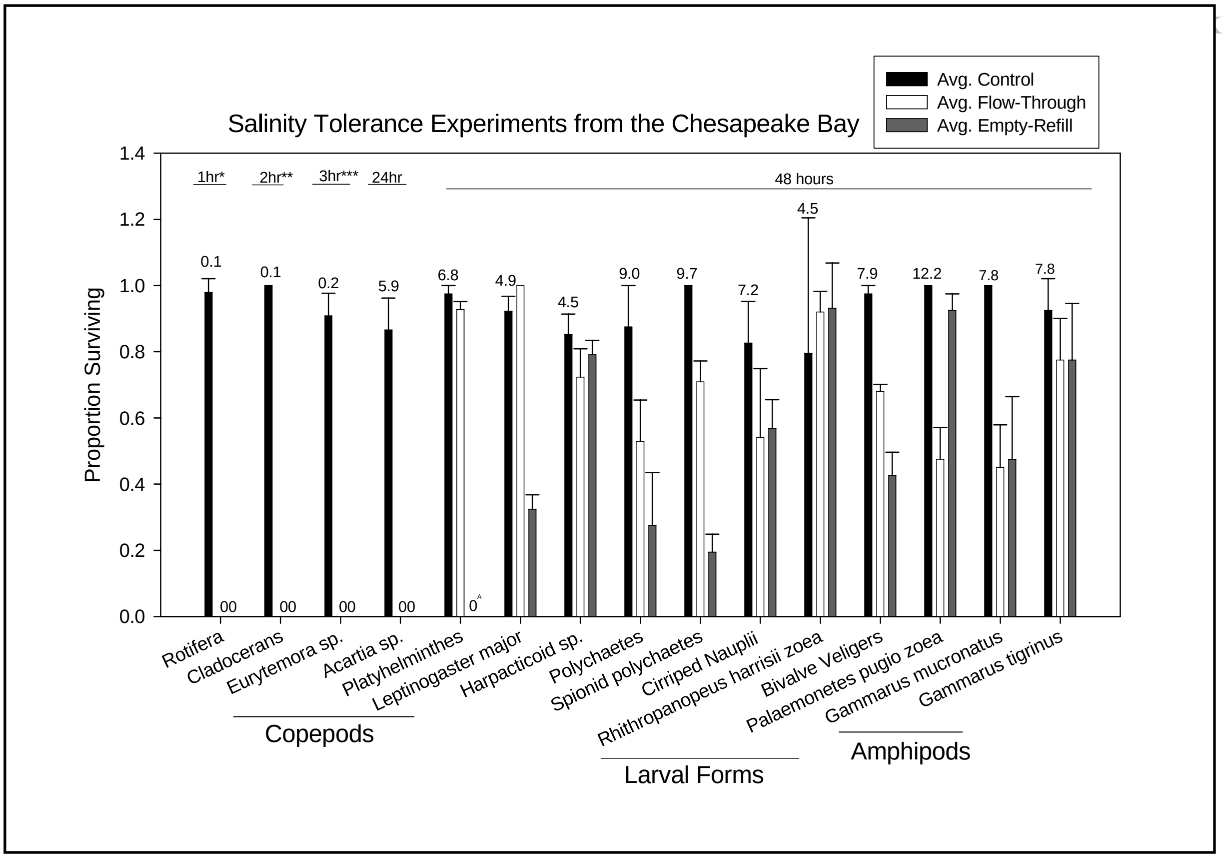


Figure 3.10: Salinity tolerance experiments from the Chesapeake Bay. Species are grouped by their relative survivorship according to the time and salinity required for maximum mortality. Unless specified minimum salinity concentration reached was 34 ppt. Ambient salinities are listed above each control. Error bars equal one standard deviation. * - minimum salinity exposure required was only 14 ppt; ** - minimum salinity exposure required was only 24 ppt, all animals died in E-R treatment at 1 hour. *** - All animals died in E-R treatment at 1 hour. ^{A} – All flatworms in the E-R treatment died at 1 hour.

B. San Francisco Bay, U. S. A.

The results for species from San Francisco Bay exhibit similar, although more complex trends in salinity tolerance than that of the Chesapeake Bay. The least salinity tolerant organisms were two introduced species of copepods from Asia, *Sinocalanus doerri* and *Tortanus dextrilobatus*, both were eliminated when exposed to 34 ppt seawater (24 hours for F-T treatment and 1 hour for E-R treatment; Figure 3.11). First stage zoea of *Rhithropanopeus harrisii* did not survive past the 24 hour exposure to full strength seawater (both F-T and E-R treatments). *Balanus* larvae and the copepods *Limnoithona tetraspina*, *Acartia hudsonica*, and *Eurytemora affinis* all experienced complete mortality over different periods of time with a direct exposure to full-strength seawater, but did survive at significant proportions when the salinity increase was gradual under flow-through conditions. This may reflect physiological attributes shared by wide-spread species that are known to favor lower salinities (Uye et al., 2000; Bouley and Kimmerer, 2006). The most salinity tolerant organisms were the native isopod, *Gnorimosphaeroma insulare,* and the introduced cumacean, *Nippoleucon hinumensis*. Both species did not undergo any significant mortality in either of the experimental treatments. Overall, five of the six introduced species to San Francisco Bay shown in Figure 3.11 (*Sinocalanus doerri*, *Tortanus dextrilobatus*, *Rhithropanopeus harrisii*, *Balanus improvisus*, and *Limnoithona tetraspina*) were eliminated by empty-refill treatments. Two experiments with a native amphipod species from the San Francisco Bay, *Eogammarus confervicolus*, exhibited varying survivorship. Experiments with this species run during April, 2004 with animals gathered at a salinity of 5 ppt survived in both the flow-through and empty-refill treatments at proportions greater than 50 percent. However, animals that were hand-reared at a salinity of 1 ppt during June, 2004 were completely eliminated by both experimental treatments after two days. *Eogammarus confervicolus* is an abundant amphipod within estuarine habitats of North American Pacific Coast (Bousfield, 1979), and it has been recorded from habitats with a salinity range of approximately 0-17 ppt (Furota and Emmett, 1993; Simenstad et al., 2001). Another native amphipod species, *Americorophium spinicorne*, was moderately tolerant of full-strength seawater but all individuals were dead by 24 hours in both treatments.

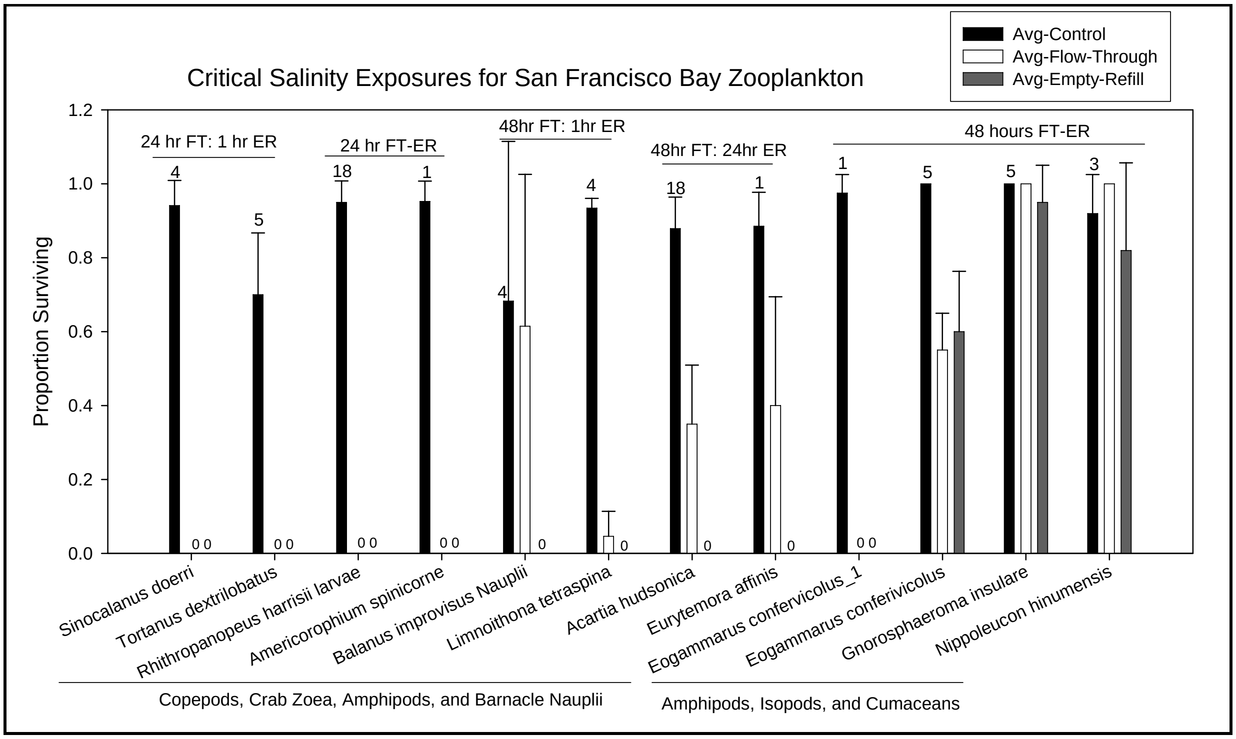


Figure 3.11: Critical salinity exposures for San Francisco Bay invertebrates. All experiments reached a minimum salinity of 34 ppt before maximum mortality was observed. Several species exhibited significant survivorship within the flow-through treatment (FT), but were eliminated by the empty-refill treatment (ER) over different periods of time. Ambient salinities are listed above each control. Species are grouped by their relative survivorship according to the time and salinity required for maximum mortality. Error bars equal one standard deviation.

C. Curonian Lagoon, Lithuania

Salinity tolerance experiments were performed with taxa from Curonian Lagoon by Dr. Zita Rasuole Gasiunaite (Coastal Research and Planning Institute, Klaipeda University, Lithuania), following our protocols. Nine of the 14 species shown in Figure 3.12 are not tolerant of full-strength salinity. The cladocerans *Chydorus sphaericus* and *Daphnia longispina* as well as the copepod *Thermocyclops dybowskii* were all eliminated with a one hour exposure to 14 ppt seawater. The cladocerans *Bosmina coregoni maritima* (*Eubosmina martima*, see Taylor et al., 2002), *Diaphanosoma brachyurum*, *Leptodora kindtii* and the copepods *Eudiaptomus graciloides* and *Mesocyclops leuckarti* and the mysid shrimp *Paramysis lacustris* were slightly more tolerant surviving up to 24 ppt. The copepods *Acartia bifilosa* and *Eurytemora hirunoides* were able to survive for longer periods in 34 ppt seawater when acclimated under flow-through conditions but died immediately if exposed directly to 34 ppt seawater. In contrast, two other species of mysid shrimps (*Limnomysis* and *Praunus*) and the nauplii of *Balanus improvisus* survived both experimental treatments at proportions greater than 50 percent. There were no significant differences between the flow-through and empty refill treatments for *Limnomysis benedeni* and *Praunus* sp. Nauplii of the barnacle *Balanus improvisus* were unaffected by the flow-through treatment but experienced significant mortality (>40%) in the empty-refill treatment. All species were collected in Curonian Lagoon where salinities can fluctuate between 0 and 7.5 ppt. Those species that are capable of surviving below 1 ppt (see Gasiūnaite, 2000) are usually eliminated by either the 14 or 24 ppt treatment. However, other species such as copepods and mysids that often occur above 1 ppt and much greater salinities required significantly longer exposure times or did not exhibit significant mortality in full-strength seawater.

D. Rotterdam, The Netherlands

Experiments were performed at the port of Rotterdam by Scott Santagata and Gemma Quilez-Badia during July, 2006 with the assistance and facilities provided by the National Museum of Natural History of the Netherlands (Naturalis) and the Rotterdam Zoo/Aquarium. *Daphnia galeata galeata*, a recently introduced subspecies of cladoceran to the Great Lakes that hybridizes with the native *Daphnia galeata mendotae* (Taylor and Hebert, 1993), did not survive in the switch to 14 ppt seawater (Figure 3.13). Consistent with some of the experiments on *Rhithropanopeus* zoea from other Atlantic and Pacific Ocean populations, zoea collected from Rotterdam Harbor exhibited intermediate levels of survival when exposed to full-strength seawater for two days. The most abundant mysid shrimp in Rotterdam Harbor during July, *Neomysis integer*, was also moderately salinity tolerant. Other species collected in the harbor, but not abundant enough to use in experiments, were the cladocerans *Leptodora kindtii* and *Bythotrephes longimanus*, and the isopod *Cyathura carinata*.

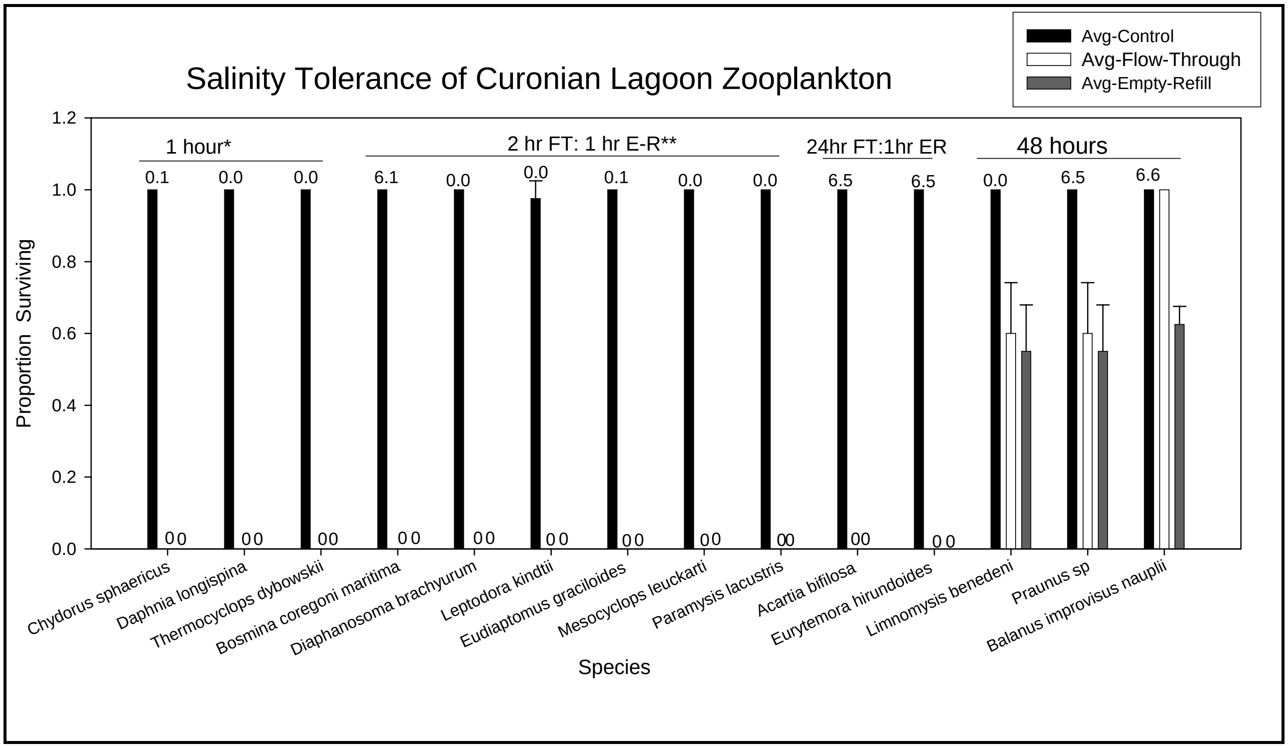


Figure 3.12: Salinity tolerance of Curonian Lagoon zooplankton. Both flow-through (FT) and empty-refill (ER) treatments reached a minimum salinity of 34 ppt unless specified. Ambient salinities are listed above each control. Species are grouped by their relative survivorship according to the time and salinity required for maximum mortality. Error bars equal one standard deviation. * - minimum salinity required to cause complete mortality was 14 ppt; ** - minimum salinity required to cause complete mortality was 24 ppt.

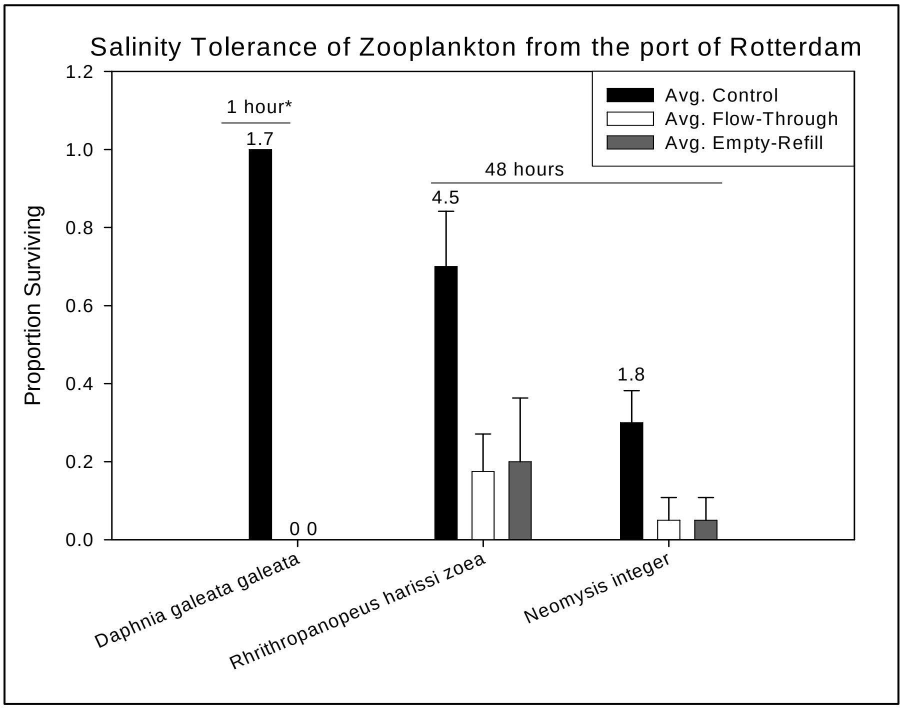


Figure 3.13: Salinity tolerance of zooplankton from the port of Rotterdam. Both flow-through (FT) and empty-refill (ER) treatments reached a minimum salinity of 34 ppt unless specified. Ambient salinities are listed above each control. Species are grouped by their relative survivorship according to the time and salinity required for maximum mortality. Error bars equal one standard deviation. * - minimum salinity required to cause complete mortality was 14 ppt.

Amphipods Across Sites

Here we focus particular attention on our experimental results for amphipods across all sites, examining the previous results from San Francisco Bay and Chesapeake Bay, as well as additional experiments from the Baltic Sea (Curonian Lagoon, Lithuania and The Vistula River, Poland). This taxonomic group includes several species that have invaded the Great Lakes, and introduced amphipods have extended their ranges rapidly within the Baltic and North Sea (Wawrzyniak-Wydrowska and Gruszka, 2005; Daunys and Zettler, 2006). Of the 10 species shown in Figure 3.14 the least tolerant species were *Chelicorophium curvispinum* and *Chaetogammarus warpachowskyi* from the Baltic Sea. Neither species survived in full-strength seawater. A species from the San Francisco Bay, *Americorophium spinicorne*, and two species from the Baltic, *Pontogammarus crassus* and *Dikerogammarus villosus* were more tolerant of full-strength seawater but were dead by 24 hours.

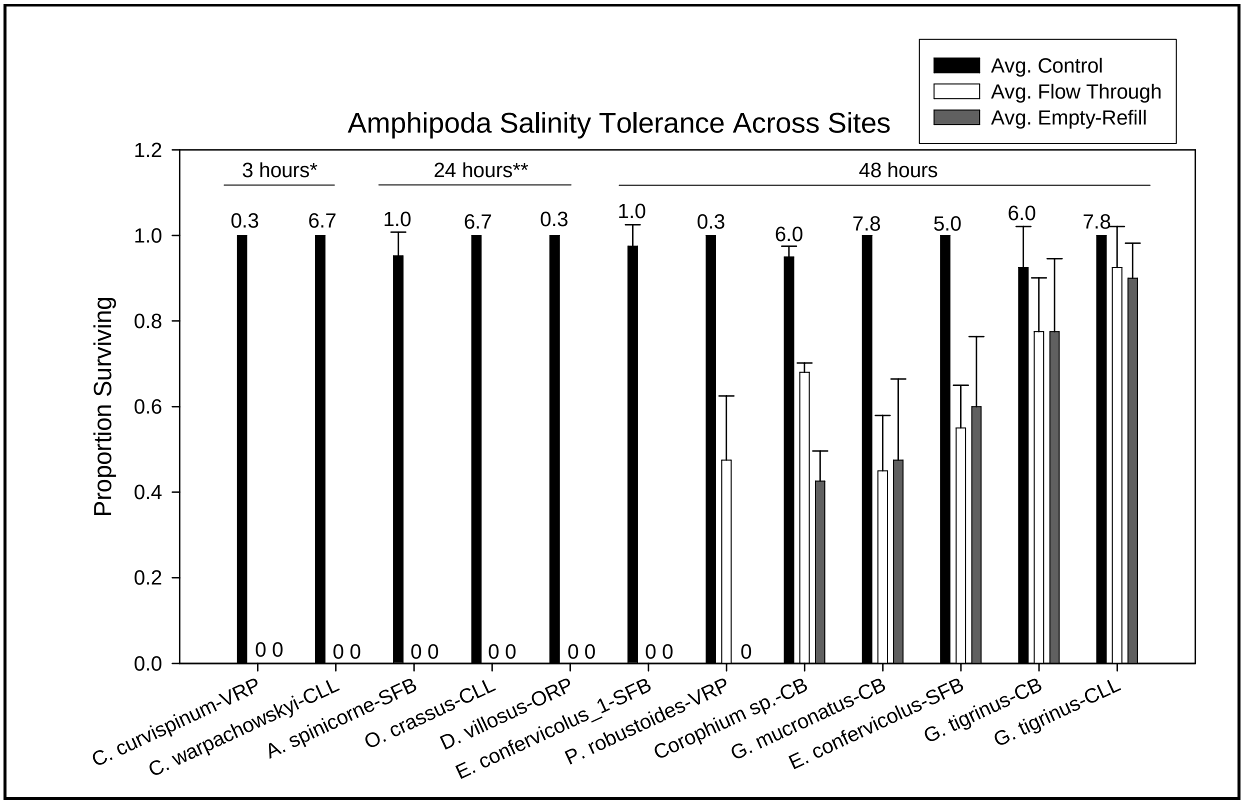


Figure 3.14: Salinity tolerance of amphipod species across sites. Both flow-through (FT) and empty-refill (ER) treatments reached a minimum salinity of 34 ppt in all experiments. Ambient salinities are listed above each control. Species are grouped by their relative survivorship according to the time and salinity required for maximum mortality. Error bars equal one standard deviation. * - *Chaetogammarus warpachowskyi* and *Chelicorophium curvispinum* both died at 1 hour in the E-R treatment. ** - *O. crassus* died at 3 hours in the E-R treatment. CB – Chesapeake Bay, U. S. A.; SFB – San Francisco Bay, U. S. A.; CLL – Curonian Lagoon, Lithuania; VRP - Vistula River, Poland.

Two experiments with another amphipod species from the San Francisco Bay, *Eogammarus confervicolus*, exhibited varying survivorship. Experiments with this species run during April, 2004 with animals gathered at a salinity of 5 ppt survived in both the flow-through and empty-refill treatments at proportions greater than 50 percent. However, animals that were hand-reared at a salinity of 1 ppt during June, 2004 were completely eliminated by both experimental treatments. Differences were also observed between closely related species of *Pontogammarus* and *Obessogammarus* that have overlapping ranges in the Baltic Sea as only *P. robustoides* was able to survive in full-strength seawater within the flow-through treatment but *O. crassus* (formerly *Pontogammarus crassus*) did not. Two species of *Gammarus* from the Chesapeake Bay were widely salinity tolerant and also survived in the final switch to ambient water after two days at 34 ppt. Introduced populations of *Gammarus tigrinus* are particularly wide spread in the Baltic and North Sea (Kukert, 1984; Daunys and Zettler, 2006). This species has also been introduced to the Great Lakes presumably from native populations along the east coast of the United States (Grigorovich et al., 2005; Kelly et al., 2006). Populations of this species from the Chesapeake Bay (Rhode River) and the Baltic Sea (Curonian Lagoon) are equally tolerant of exposure to full-strength seawater for two days then being transferred directly back into ambient seawater.

Rhithropanopeus Larvae Across Sites

The zoea of *Rhithropanopeus harrisii* have a developmental salinity range between 2 and 30 ppt, and their larval development among distant populations shows favorability toward local environmental conditions (McKenney and Neff, 1979; Laughlin and French, 1989). Considering these factors, we compared its survivorship in our experiments across three temperatures (16°C, 20°C, and 24°C) at salinities commonly observed within the Rhode River, Chesapeake Bay during summer. These data along with the experiments discussed previously are summarized in Figure 3.15. Most larvae at 16°C were eliminated by 24 hours regardless of treatment, and underwent complete mortality by 48 hours. Larvae at 20°C survived at low levels in both treatments by 48 hours, including an additional switch back into ambient seawater. Experiments at 24°C eliminated all larvae but at different times, 24 and 48 hours respectively. Overall, the differences in survivorship among experiments due to temperature are overwhelmed by exposure to salinities above 30 ppt. Interestingly, zoea reared from adult broods within the San Francisco Bay at 18 ppt showed equal (or less) survivorship when exposed to full-strength seawater than zoea reared at lower salinities. Zoea collected from the plankton of the Chesapeake Bay and the port of Rotterdam were more tolerant of full-strength seawater than zoea reared from adult broods. These differences in survivorship may be due to cohort quality or genetic differences.

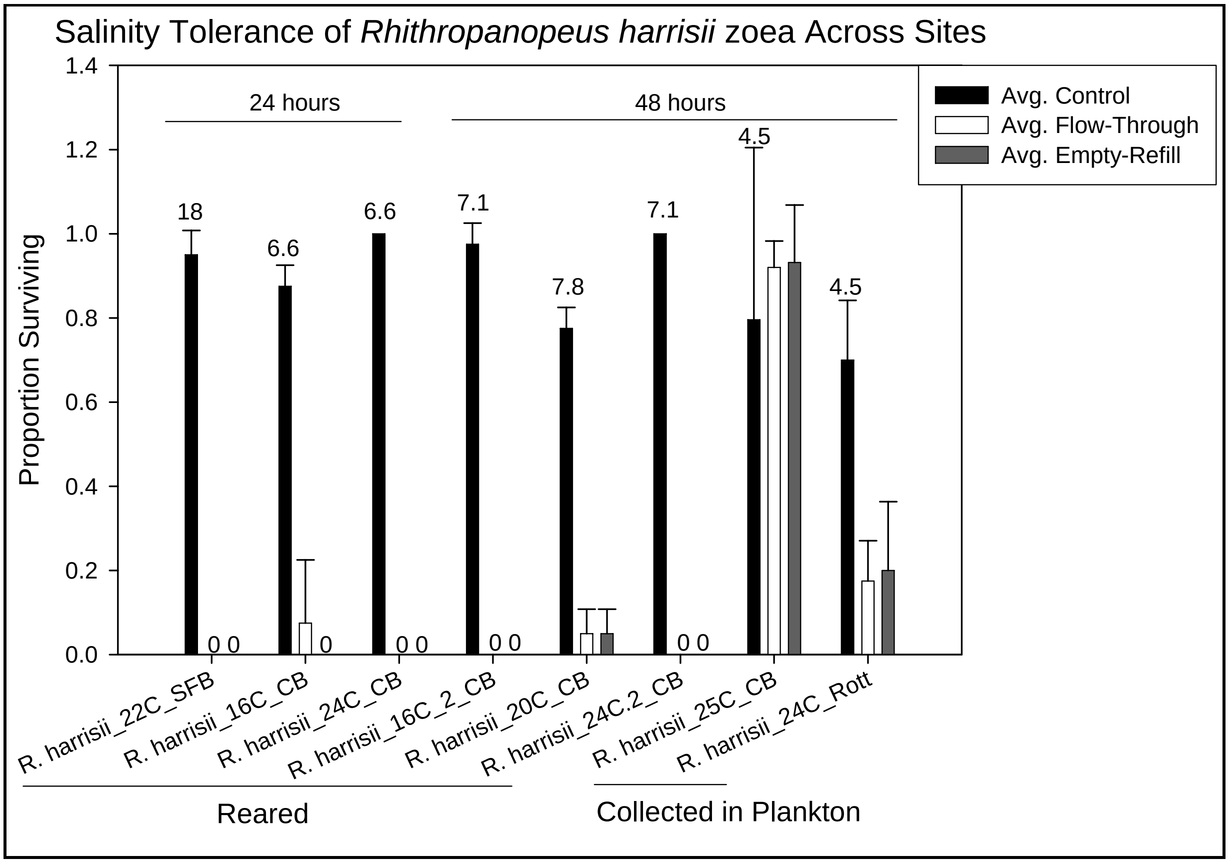


Figure 3.15: Salinity tolerance of *Rhithropanopeus harrisii* zoea (stage one) across sites. Both flow-through (FT) and empty-refill (ER) treatments reached a minimum salinity of 34 ppt in all experiments. Ambient salinities are listed above each control and ambient temperatures are listed along the bottom axis. Species are grouped by their relative survivorship according to the time and salinity required for maximum mortality. Error bars equal one standard deviation. CB – Chesapeake Bay, U. S. A.; SFB – San Francisco Bay, U. S. A.; Rott – the port of Rotterdam, The Netherlands.

Recovery of Copepods

Experiments with the widely distributed cyclopoid copepod, *Acanthocyclops robustus*, were difficult to score. In both of the experiments shown in Figure 3.16, specimens appeared dead in the 24 ppt seawater. However, when switched back to ambient water 5-25% of these copepods recovered in the flow-through treatments. The empty-refill treatments were also inconsistent, in one trial this treatment eliminated all individuals within one hour and in another trial a small proportion recovered when placed back into ambient water (*Acanthocyclops robustus*_2). Similar inconsistent results were yielded for *Acartia tonsa* from the Chesapeake Bay. This species appeared to have a critical salinity tolerance at 34 ppt since specimens in both treatments became unresponsive at this salinity concentration. However, when these animals were returned to ambient water (7.1 ppt), over twenty percent on average recovered.

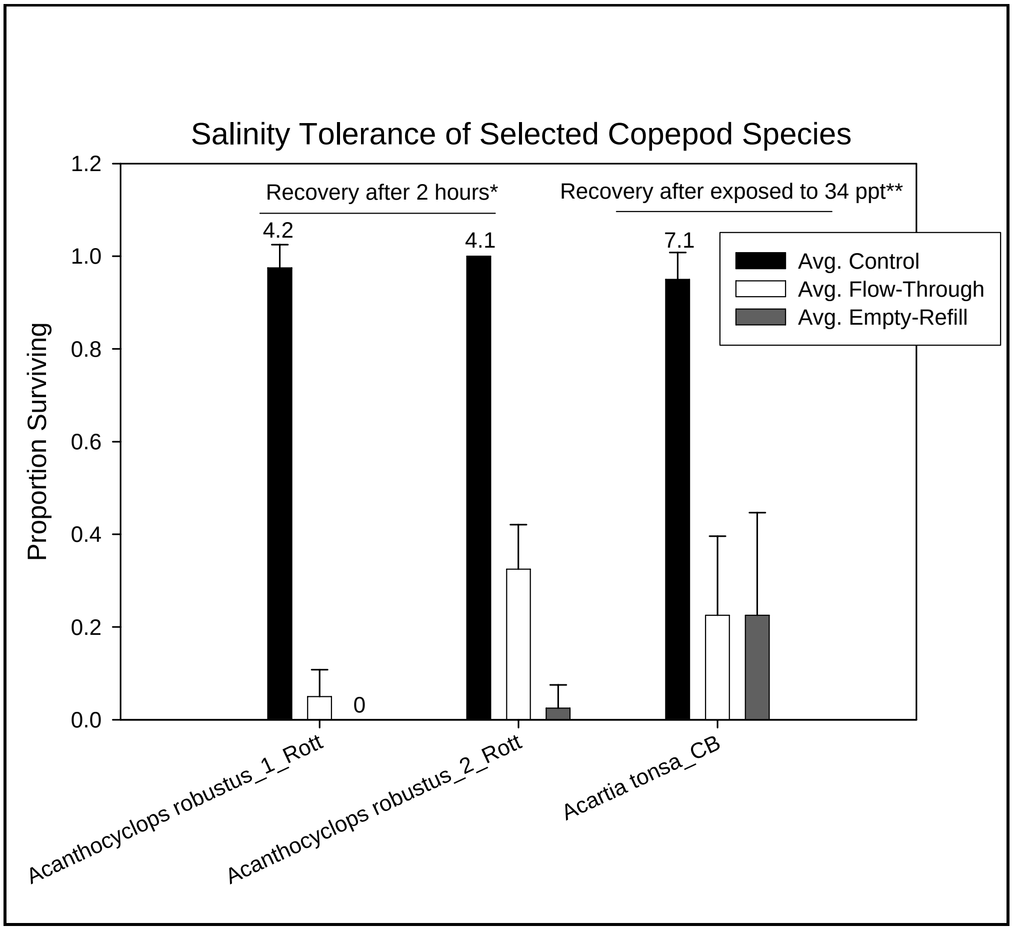


Figure 3.16: Salinity tolerance of selected copepod species. Ambient salinities are listed above each control and ambient temperatures are listed along the bottom axis. Species are grouped by their relative survivorship according to the time and salinity required for maximum mortality. Error bars equal one standard deviation. * - Flow-through treatments reached a minimum salinity of 24 ppt, Empty-refill treatments were 34 ppt for 2 hours. ** - Flow-through treatments reached a salinity of 34 ppt at 3 hours, exposure for empty-refill treatments were 34 ppt for one hour. CB – Chesapeake Bay, U. S. A.; Rott – the port of Rotterdam, The Netherlands.

Objective 3.4: To conduct laboratory-based salinity-tolerance experiments on species indigenous to the Great Lakes and species that have already invaded the Great Lakes, providing a retrospective analysis of the efficacy of BWE and saltwater flushing to prevent past invaders associated with shipping.

3.4.1 SERC Salinity Tolerance Experiments

Overview

Salinity tolerance experiments were performed by both SERC and the University of Windsor as part of this objective. SERC, with the participation of CILER and GLERL, conducted experiments on 10 species collected from the Great Lakes. In addition, the University of Windsor conducted experiments on 4 species from the Great Lakes, two of which were in-common with those for SERC.

Two sets of experiments were conducted in Lake Erie and Lake Michigan during the summer of 2006 to test the effect of ballast water exchange on native and non-native species of the Great Lakes. Some of the most common native cladoceran species in the Lakes, *Bosmina longirostris*, *Leptodora kindtii*, and *Daphnia retrocurva* were all eliminated in the initial exposure to 14 ppt seawater. This was also true for the highly abundant rotifer, *Asplachna priodonta*. Two of the most problematic invasive species in the Great Lakes, the predatory cladocerans *Cercopagis* and *Bythotrephes*, were slightly more tolerant of higher salinities and survived until the 24 ppt treatment (Figure 3.17). This was also true for the widely distributed cladoceran species of *Polphemus*, *Alona*, and *Eurycercus*. Furthermore, late stage juveniles brooded within adults of *Bosmina longirostris* and *Eurycercus lamellatus* survived in some of these short-term salinity treatments when returned to ambient water (0.1 ppt or 270 µS). The only full-strength salinity tolerant species encountered were the abundant quagga and zebra mussel veligers. During the course of our experiments, veligers responded behaviorally by the closing their valves and sinking to the bottom of the bowl. Survivorship in these situations was judged by looking for signs of ciliary beat through the valves. However, as a final check of viability we transferred these animals to freshwater at the end of the experiment and left them overnight. No individuals survived this final treatment.

Figure 3.18 summarizes our experimental data broken down into salinity range categories. Both flow-through and empty-refill treatments were effective in nearly all of the experiments against animals from oligohaline habitats (87 and 94% respectively, 0-2 ppt). The effectiveness of both treatment methods against animals from lower-salinity habitats (2-5 ppt) decreased significantly as compared to oligohaline taxa. Although not significant, empty-refill treatments were slightly more effective than flow-through treatments against animals collected from low salinity and mesohaline habitats (Figure 3.18). There are significant differences in the frequency of effectiveness within each treatment type across the three salinity ranges (Chi square for F-T=12.1 and E-R=13.5, both have a $p<0.01$). However, this is due to the results from the oligohaline habitats (0-2 ppt). When this category is removed there are no significant differences between the remaining salinity categories. Although flow-through and empty-refill treatments were equally effective against species within each salinity range, empty-refill treatments required significantly less exposure time.

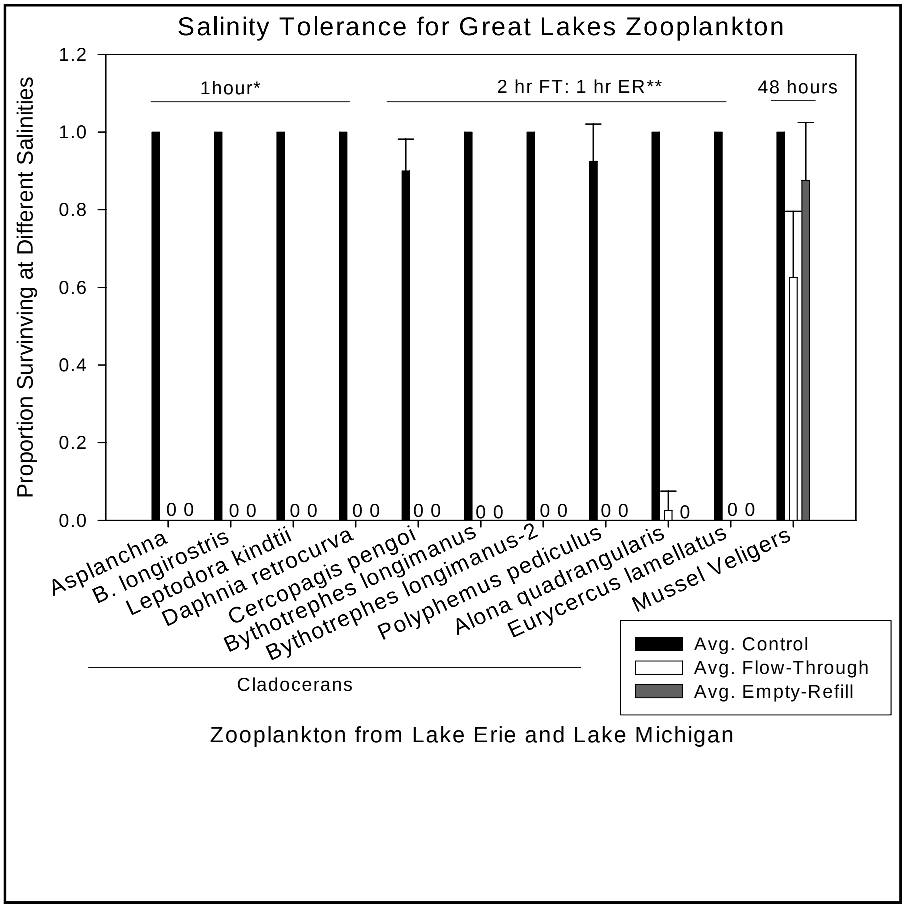


Figure 3.17: Salinity tolerance for Great Lakes zooplankton. The majority of these species were eliminated before exposure to full-strength seawater. Species are grouped by their relative survivorship according to the time and salinity required for maximum mortality. Ambient water in all experiments was 0.1 ppt or 270-290 µS. Error bars equal one standard deviation. * - minimum salinity required to cause complete mortality was 14 ppt; ** - minimum salinity required to cause complete mortality was 24 ppt.

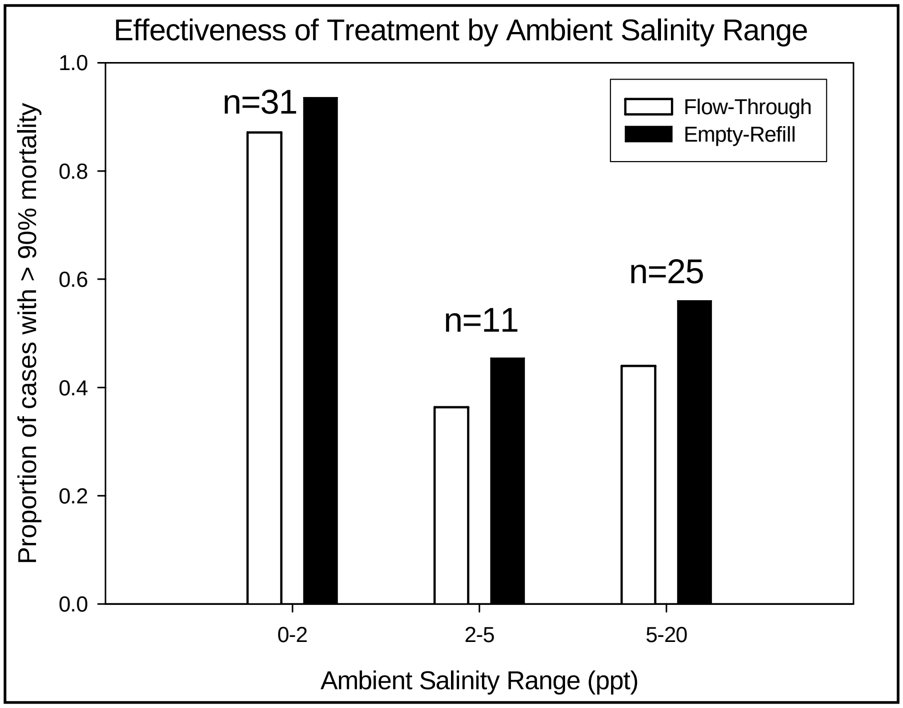


Figure 3.18: Effectiveness of treatment by ambient salinity range. The sample size is listed above each category. Bars represent the proportion of experiments within a given salinity range that yielded >90% mortality by treatment. Experiments with recovery were not included.

Table 3.3 summarizes our experimental results by different taxa. All of the cladocerans in our experiments were eliminated by either 14 or 24 ppt seawater. There are marine cladocerans that can survive in salinities greater than 24 ppt such as species of *Podon*, *Pseudoevadne*, *Evadne*, *Penilia*, and *Pleopsis*. However, these species are rarely found within freshwater habitats or cannot survive in constant freshwater systems (Frey, 1993). The majority of copepods in our experiments were not tolerant of full-strength seawater, but considering the ability of some species to recover from short-term exposures to dramatic salinity shifts, exposure duration should be at least a day for all copepod species. The larvae of crabs, shrimps, barnacles, and bivalves as well as adult amphipods, isopods, cumaceans, and mysids were generally tolerant of full-strength seawater (or higher salinities). For these taxa, it is a better discriminator of invasion risk for the Great Lakes region to determine species that are capable of establishing populations within a constant freshwater habitat.

Table 3.3: Summary of Salinity Limits for Numbers of Species from Different Taxonomic Groups.

Taxon	**<14 ppt**	**14-24 ppt**	**24-34 ppt**	**>34 ppt**
Cladocerans	5	8	0	0
Copepods	1	2	9	2
Amphipods, Isopods, Cumaceans, or Mysids	0	0	6	10
Larvae of crabs, shrimps, barnacles, or bivalves	0	0	2	6

Discussion

In agreement with numerous other salinity tolerance studies, our data show that the tolerance range for a species is influenced by both temperature and prior salinity conditions (Laughlin and French, 1989; Fockedey et al., 2005). While acclimation effects are clearly important in determining the widest physiological range for a particular species, the increased salinity range often exhibited by animals from low-salinity environments acclimated to higher salinities are either too narrow to accommodate survival at full-strength seawater; or expands the salinity range of a euryhaline species that normally occupies habitats with full-strength salinity at some point in its life history. Furthermore, many euryhaline organisms often possess lower salinity limits near 1 to 2 ppt and are incapable of surviving in fresh water for extended periods of time. For these reasons, differences in survivorship for estuarine taxa during ballast water exchange due to acclimation conditions or full-strength-salinity adapted species are of less importance for introductions to freshwater habitats such as the Great Lakes. With regard for ballast water exchange methods, the greater risk for the Great Lakes lies with species or particular life stages that can tolerate full-strength seawater for at least two days and establish viable populations within a constant freshwater system. Considering our and other published observations, zebra and quagga mussel veligers may have these characteristics under certain environmental conditions (Padilla, 2005). However, this is not the case for the adult forms of the invasive cladocerans *Cercopagis pengoi*. This species establishment in the Great Lakes may be due to several reasons such as ballast water exchange practices have not been followed rigorously, it was introduced from low-salinity residual water of NOBOBs after BWE regulations were implemented, or perhaps their resting stages have more physiological resistance than the adults. Experiments designed to test the efficacy of ballast water exchange on the hatching success of resting stages of other species of cladocerans (not *Cercopagis*) from the Great Lakes have yielded mixed results, but overall the viability of diapausing eggs of several freshwater cladoceran species was most reduced by exposure to 8 ppt seawater at 20 °C (Bailey et al., 2005 and 2006).

Although wide reaction norms (phenotypic plasticity) has been implicated many times as one of the reasons behind invasion success (Lee et al., 2003; Sexton et a;, 2002; Richards et al., 2006), several empirical studies have concluded that fluctuating environmental factors are acting as an evolutionary force on natural populations and ballast-water communities, selecting for low frequency genotypes that survive and propagate in the new environmental conditions (Lee, 2002; Dybdahl and Kane, 2005). The low frequency of genotypes permissive under stressed conditions is one reason why propagule pressure is so important (Ruiz et al., 2000). In particular, salinity tolerance within a species is also variable within a population, and these phenotypic differences can be heritable (Lee et al., 2003). Within cladocerans, fluctuating salinities often result in genotypes differentially adapted to local conditions (Ortells et al., 2005). Overall, a more significant factor for the invasion success of estuarine species into freshwater habitats may be low frequency genotypes with wide environmental tolerances from a few or several key source populations, rather than the total set of genetic and phenotypic characters for a species across its entire range. Evidence based on the molecular phylogeography of a few recent ballast water invaders to the Great Lakes would support this hypothesis as their invasive populations are the result of several invasion events from multiple source populations (Cristescu et al. 2001; Colautti et al., 2005; Stepien et al., 2005; Kelly et al., 2006).

Considering these factors, predicting the potential invasion success of individual species in a new habitat based on published accounts of their environmental tolerances in their native range or experimental estimates from a single population can be misleading (Paavola et al., 2005; see also Grigorvich et al., 2003 where the invasion risk probability of *Gammarus tigrinus* was classified as low). Often, the limits of important environmental variables such as temperature and salinity documented for a species reflects the total range for all sampled populations and habitats. In order for more realistic predictions to be made about the potential 'invasiveness' of a particular species, environmental limits must be documented during seasonal fluctuations within a population as well across populations. This information will allow us to test what role local adaptations to fluctuating estuarine environments contributes to the environmental tolerance and potentially to the invasion success of organisms commonly dispersed via the ballast water operations of commercial ships.

Although not a complete barrier against all exotic species, these experiments clearly show that many taxa that originate from low-salinity ports can be eradicated from ballast tanks relatively quickly through exposure to full-strength seawater (34 ppt). This is especially true for several species of rotifers, cladocerans, and copepods that are more likely to occur in freshwater or oligohaline habitats (0-2 ppt, see Figure 3.18 and Table 3.3). It is not surprising that our experiments with animals from habitats with higher average salinities (2-5 and 5-10 ppt) exhibit greater resistance to treatments of full-strength seawater. These findings support similar conclusions drawn from previous ballast water exchange experiments conducted in the Chesapeake Bay and San Francisco Bay (Smith et al, 1999; Choi et al., 2005). Invertebrates from our experiments identified as salinity-tolerant species (34 ppt) include mysid shrimps, amphipods, isopods, harpacticoid copepods, bivalve veligers, and decapod zoea. Members of these taxonomic groups often experience dramatic fluctuations in salinity and temperature as part of their normal life histories and these factors have contributed to their ability to invade estuarine habitats (Lockwood, 1976; Hamer et al., 1998; Wittmann and Ariani, 2000; Bruijs et al., 2001; Torres et al., 2006). Of these estuarine animals, only a subset of salinity-tolerant species are capable of surviving and reproducing in a constant freshwater habitat such as the Great Lakes. Identifying species and populations with these characteristics from the port systems of the North-West Atlantic, North Sea, and Baltic Sea is paramount for preventing problematic species from invading the Great Lakes region via the operations of commercial ships.

Acknowledgements

This study represents the work of the Marine Invasions Laboratory and some of our international collaborators. We greatly appreciate the efforts of current and past members of the Smithsonian Environmental Research Center as well as our outside collaborators. In particular, we thank Karolina Bacela, Chris Brown, Jillian Cohen, Jeff Cordell, Scott Cowen, Piet de Bruin, Karen Eason, Zita Gasiunaite, Amanda Gilborn, Adriaan Gittenberger, Francis Kerckhof, Margaret Kramer, Jan Kros, Michael Laterveer, Robert Leewis, Kelly Lion, Whitman Miller, Mark Minton, Irina Ovcarenko, Peter Paalvast, Dirk Petersen, Kelly Ryan, George Smith, Carin Stringer, Micky Tackx, Gemma Quilez-Badia, Heidi van der Meij, Gerard van der Velde, and Emma Verling. A portion of this research was funded by Prince William Sound Regional Citizens' Advisory Council.

3.4.2 University of Windsor Salinity Tolerance Experiments

Overview

Laboratory simulations of both empty-refill and flow-through BWE were conducted on four species of nonindigenous crustaceans to the Great Lakes; three cladoceran species, *Bosmina coregoni*, *Bythotrephes longimanus* and *Cercopagis pengoi* and one amphipod species, *Echinogammarus ischnus*. Of these species, *B. coregoni* and *B. longimanus* were introduced before BWE regulations were put into place, most likely via ballast water (Wells 1970, Bur et al 1986). *E. ischnus* and *C. pengoi* were both discovered in the Great Lakes basin after BWE was mandated, potentially due to survival in ballast tanks despite regulatory measures. Retrospective analysis of the salinity tolerance of *B. coregoni* and *B. longimanus* will demonstrate if these species would have been likely candidates to enter the Great Lakes if BWE had been in place prior to their introductions. Analysis of the survival of *E. ischnus* and *C. pengoi* under BWE conditions may indicate that these species are able to survive BWE and that novel ballast water treatment methods are required to prevent NIS introductions.

Methods

Animals were collected from the field for use in laboratory experiments. Details regarding collection dates and methods are found in Table 3.4. Collection methods varied based on different morphologies and life histories of the target animal.

Table 3.4. Collection date, location, method and water conditions at time of collection for all species studied.

Taxa	Collection Date and location	Collection Methods	Ambient Salinity (ppt)	Ambient Temperature (deg C)
Amphipoda				
Echinogammarus ischnus	March 21 2006 Detroit River	300 µm mesh kick net	0.1	7.0
Cladocera				
Bosmina coregoni	August 4 2006 Lake Erie	253 µm plankton net vertical tow	0.1	23.1
Bythotrephes longimanus	August 28 2006 Peninsula Lake	950 µm plankton net horizontal tow	0.0	23.4
Cercopagis pengoi	August 16 2006 Lake Ontario	500 µm plankton net horizontal tow	0.2	19.2

Samples were brought back to the lab and slowly acclimated to 20ºC, sorted under a dissecting microscope and healthy adults were placed, 10 per jar, into 100mL of filtered site water. Animals were left for a maximum of 24 hours in an environmental chamber at 20ºC and 16:8 light:dark regime before experimental treatments began and were not fed during this time interval.

To simulate flow-through BWE, animals were introduced to gradually increasing salinity, beginning at 4ppt and ending at 30ppt. To simulate empty-refill BWE, animals were directly introduced to 30ppt. A control treatment was employed in which animals were exposed to ambient filtered site water. Each treatment consisted of four replicates containing 10 individuals per 100mL of water for the species *B. coregoni, B. longimanus,* and *E. ischnus*. Due to negative intraspecific interactions, experiments with *C. pengoi* included 8 replicates containing 5 individuals in 100mL of water. All replicates were covered loosely with cellophane to limit evaporation, which may alter salinity.

Experiments were started by placing healthy adult individuals into filtered water of the appropriate salinity. In the flow-through treatment individuals were transferred to new water of increasing salinity. To ensure all replicates endured the same amount of handling, replicates from both the empty-refill and control treatments were also transferred to new water at 30ppt and ambient salinity, respectively. All treatments took place at 20ºC and in the dark except during observation periods. Survivorship was recorded at each time of transfer and one hour after the final transfer. In experiments with *B. coregoni, B. longimanus* and *E. ischnus* animals were monitored for survival at 24 and 48 hours, after which time all treatments were transferred to ambient water to monitor for recovery. After 1 hour in freshwater, survivorship was observed, then any animals alive were preserved in 95% ethanol. Due to high mortality in experiments with *C. pengoi,* after 24 hours all replicates were transferred to freshwater for 1 hour, after which time survivorship was assessed and experiments were terminated. Range finding trials were used to narrow the number of salinity gradients used in the final LC_{50} experiments. Each treatment included 3 replicates consisting of 10 individuals in 100mL of water prepared to various salinities by mixing filtered site water with Instant Ocean. *E. ischnus*, which has a high salinity tolerance, was exposed to salinities of 0, 16, 18, 20, 22 and 24ppt. *B. longimanus* was exposed to lower salinity water of 0, 4, 6, 7, 8 and 12ppt. All replicates were put in an environmental chamber set to 20ºC and 16:8 light:dark regime and covered with cellophane. After 1 hour, 24 and 48 hours replicates were monitored for survival, and any dead individuals were removed. After 48 hours any live individuals were preserved in 95% ethanol and the experiment was terminated.

Results

Four NIS were used in laboratory trials to ascertain the ability of crustaceous plankton to survive flow-through or empty-refill ballast water exchange. Trials for species were conducted over a 49-hour period with the exception of *Cercopagis pengoi* (terminated after 25 hours due to high mortality in control treatments). Individuals were considered to be dead when unresponsive to probing and no beating of gills was observed.

Table 3.5. Percent of individuals to end of trial surviving in all treatments. p value and Chi Square for survival analysis comparing multiple samples. § 49-hour trial * 25 hour trial.

Species	Flow-through	Empty-refill	Control	p value	Chi Square
Bosmina coregoni§	0.00	0.00	71.01	0.00	104.37
Bythotrephes longimanus§	0.00	0.00	30.00	0.00	93.71
*Cercopagis pengoi**	0.00	0.00	16.78	0.00	95.72
Echinogammarus ischnus§	3.33	0.00	77.50	0.00	69.89

Significant differences ($p<0.00$) were found between all treatments for each species using a multiple group survival analysis (Table 3.5). Significant differences ($p<0.05$) were also found between all combinations of treatments in all species with the exception of the flow-through and empty-refill treatment comparison for *Echinogammarus ischnus* ($p>0.5$). All cladocerans (*Bosmina coregoni, Cercopagis pengoi, Bythotrephes longimanus*) were killed after one hour in the empty-refill treatment (30ppt). In the flow-through experiments for these species all individuals were dead upon observation after one hour at 14ppt. While statistical analyses indicate significantly different survival curves the resulting mortality for both flow-through and empty-refill treatments is 100% for these species. For the amphipod *E. ischnus*, one individual survived to the end of the empty-refill simulation while two survived to the end in the flow-through treatment. 95% of individuals had died when monitored after 48 hours in the flow-through treatment, while at the same time in the empty-refill treatment 97.5% of individuals had died.

Discussion

Shipboard experiments to test BWE can be very difficult to organize and demand significant resources. Furthermore, shipboard experiments may be limited by the number and type of taxa found within ballast tanks. In order to explore the ability of a wide variety of species to survive BWE it is practical to conduct laboratory simulations. Laboratory experiments also provide better control over experimental conditions, which can vary between ballast tanks and ships, making replication of shipboard trials difficult.

Laboratory based experiments on 4 crustacean species showed varying degrees of salinity tolerance in relation to BWE. Three cladoceran species, *B. coregoni*, *B. longimanus*, and *C. pengoi* all experienced 100% mortality in both flow-through and empty-refill BWE treatments. However, *B. longimanus* and *C. pengoi* also experienced high mortality in control treatments. High mortality in controls is probably partly due to sensitivity of these animals to handling in the laboratory and our protocols of not feeding the animals during acclimation. As well, *C. pengoi* and *B. longimanus* are both predaceous species which function as cannibals. In the experiment, at least one individual was eaten in each replicate in control treatments, and in some cases more than one was eaten. Therefore, the mortality in the flow-through and empty-refill treatments is probably due to a combination of sensitivity to handling and salinity toxicity and it is difficult to obtain accurate estimates of survival during BWE.

It is also important to point out that the results for Cercopagis and Bythotrephes obtained by the University of Windsor differ somewhat from those of SERC. Experimental results from SERC indicate that to reliably eliminate Cercopagis and Bythotrephes required a second inoculation step from 14 to 24 ppt (2 hour total experiment time). Although 14 ppt is beyond these species "salinity comfort zone", animals in the SERC experiments were clearly still alive at 14 ppt. Considering the SERC results during recovery experiments and the added factor of tolerance differences between the adult cladocerans and their brooded juveniles, it would be more appropriate and conservative to not consider 14 ppt as a minimum exposure level for Cercopagis and Bythotrephes.

B. coregoni and *E. ischnus* experienced less mortality in controls than *B. longimanus* and *C. pengoi*, consequently survivorship in BWE treatments is most likely due to sensitivity to salinity. Flow-through and empty-refill treatments were equally effective in causing mortality for *B. coregoni* but not for *E. ischnus.* Test results indicate that *B. coregoni* would not survive complete BWE; providing salinity reached at least 14ppt. *E. ischnus* is a euryhaline species with a European distribution from the open Black Sea through inland waters to the Black and Caspian seas (Jazdzewski, 1980). Consequently, this species has the ability to acclimate to fluctuating salinities such as those that occur during BWE. However, the present study demonstrates that *E. ischnus* would not survive complete BWE. Only 3.33% of individuals survived in the flow-through BWE experiment, half of these individuals were unresponsive to probing and potentially would not have survived if the experiment had continued. In ship board experiments using *E. ischnus* in ballast tanks all individuals were killed by empty-refill BWE (see above Obj. 2.3). The agreement of the results of shipboard and laboratory experiments suggests that laboratory trials can be used to predict the outcome of BWE for other species.

Conclusions

It does not appear that *E. ischnus* or *C. pengoi* would have entered the Great Lakes via ballast water discharge after mandatory BWE if salinity requirements were met. It is likely that *E. ischnus*, with a benthic life history, entered through ships declaring no ballast on board (NOBOB) which were not required to conduct BWE (Kohn 1990, Duggan et al 2005). *C. pengoi* was not observed in Lake Ontario until 1998, it is possible that it was present before this time and simply not abundant enough to be discovered (MacIsaac et al 1999). Many researchers believe this is highly unlikely due to its unique caudal appendage which causes it to foul fishing lines and become clearly visible (Ricciardi, 2006, MacIsaac et al 1999). Other possible entry mechanisms for *C. pengoi* include through ships declaring NOBOB status and as resting eggs in NOBOB or BOB ships (Duggan et al 2003, Bailey et al 2003). *B. coregoni* and *B. longimanus*, which entered the Great Lakes before BWE regulations would have been unlikely to enter from the discharge of ballasted ship had BWE been required at the time. However, NOBOB ships provide an additional vector that was not regulated and also could have been responsible for their introduction. This study demonstrates that BWE is an effective method for reducing NIS introduction. Species with salinity tolerance <14ppt appear to be equally affected by flow-through and empty-refill BWE; while the species with a broad salinity tolerance was more affected by empty-refill BWE. It is likely that coastal species, with their ability to survive fluctuations in salinity, would be more apt to survive flow-through exchange, and empty-refill exchange may prove more effective in preventing entry of such species. More studies involving coastal species will assist in discerning the ability of these species to acclimate to salinity during BWE and are necessary to determine if other ballast treatments are necessary. We also wish to point out that the effects of saltwater exposure may serve as an effective treatment for the organisms' resident in the residual water and sediment of empty NOBOB tanks and encourage the adoption of this practice as an enhancement to the current list of precautionary BMPs. Furthermore, we emphasize that NOBOB ships pose an additional risk not addressed with the BWE regulations.

References

Ackefors, H. 1965. On the zooplankton fauna at Asko (The Baltic - Sweden). *Ophelia* 2: 269-280.

Ackefors, H. 1971. *Podon polyphemoides* Leuckart and *Bosmina coregoni maritima* (P. E. Muller) in relation to temperature and salinity in field studies and laboratory experiments. *Journal of Experimental Marine Biology and Ecology* 7: 51-70.

Aladin, N. V. 1982a. Salinity adaptations and osmoregulation abilities of the Cladocera. 1. Forms from open Seas and oceans. *Zoologicheskii Zhurnal* 61: 341-351.

Aladin, N. V. 1982b. Salinity adaptation and osmoregulation abilities of the Cladocera. 2. Forms from Caspian and Aral seas. *Zoologicheskii Zhurnal* 61: 507-514.

Aladin, N. V. 1983. Displacement of the Critical Salinity Barrier in the Caspian and Aral Seas (the Branchiopoda and Ostracoda Taken as Examples). *Zoologichesky Zhurnal* 62: 689-694.

Aladin, N. V. & Potts, W. T. W. 1995. The Osmoregulatory Capacity of the Cladocera. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* 164: 671-683.

Alheit, J., Mollmann, C., Dutz, J., Kornilovs, G., Loewe, P., Mohrholz, V. & Wasmund, N. 2005. Synchronous Ecological Regime Shifts in the Central Baltic and the North Sea in the Late 1980s. *Ices Journal of Marine Science* 62: 1205-1215.

Alimov, A. F. 1997. Monitoring the Neva bay and the eastern Gulf of Finland. Technical Report, 127 pp. Zoological Institute of the Russian Academy of Sciences, St. Petersburg.

Anger, K. 1991. Effects of Temperature and Salinity on the Larval Development of the Chinese Mitten Crab *Eriocheir sinensis* (Decapoda, Grapsidae). *Marine Ecology-Progress Series* 72: 103-110.

Arner, M. & Koivisto, S. 1993. Effects of salinity on metabolism and life history characteristics of *Daphnia magna*. *Hydrobiolgia* 259: 69-77.

Audzijonyte, A., Daneliya, M. E. & Väinolä, R. 2005. Comparative phylogeography of Ponto-Caspian mysid crustaceans: isolation and exchange among dynamic inland sea basins. *Molecular Ecology* 15: 2969–2984.

Back, S. & Ruuskanen, A. 2000. Distribution and Maximum Growth Depth of Fucus Vesiculosus along the Gulf of Finland. *Marine Biology* 136: 303-307.

Bailey, S. A., Duggan, I. C., Jenkins, P. T. & MacIsaac, H. J. 2005a. Invertebrate Resting Stages in Residual Ballast Sediment of Transoceanic Ships. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 1090-1103.

Bailey, S.A., Duggan, I.C., van Overdijk, C.D.A. 2003. Viability of invertebrate diapausing eggs collected from residual ballast sediment. *Limnology and Oceanography* 48: 1701-1710.

Bailey, S. A., Duggan, I. C., van Overdijk, C. D. A., Johengen, T. H., Reid, D. F. & MacIsaac, H. J. 2004. Salinity Tolerance of Diapausing Eggs of Freshwater Zooplankton. *Freshwater Biology* 49: 286-295.

Bailey, S. A., Nandakumar, K., Duggan, I. C., Van Overdijk, C. D. A., Johengen, T. H., Reid, D. F. & MacIsaac, H. J. 2005b. In Situ Hatching of Invertebrate Diapausing Eggs from Ships' Ballast Sediment. *Diversity and Distributions* 11: 453-460.

Bailey, S. A., Nandakumar, K. & MacIsaac, H. J. 2006. Does Saltwater Flushing Reduce Viability of Diapausing Eggs in Ship Ballast Sediment? *Diversity and Distributions* 12: 328-335.

Bainbridge, V. 1958. Some observations on *Evadne nordmanni* Loven. *Journal of the Marine Biological Association of the United Kingdom* 37: 349-370.

Bakker C. & Pauw N. de 1975. Comparison of plankton assemblages of identical salinity ranges in estuarine tidal, and stagnant environments II. Zooplankton. *Netherlands Journal of Sea Research* 9: 145-165.

Barnes, R. S. K. 1994. The brackish-water fauna of northwestern Europe. Cambridge (UK): Cambridge University Press.

Beck, J. T. & Cowell, B. C. 1976. Life history and ecology of the freshwater caridean shrimp, *Palaemonetes paludosus* (Gibbes). *American Midland Naturalist* 96: 52-65.

Benider, A., Tifnouti, A. & Pourriot, R. 1998. Reproduction parthénogénétique de *Moina macrocopa* (Strauss 1820) (Crustacea: Cladocera). Influence des conditions trophiques, de la densité de la population, du groupement, et de la température. *Annales de Limnologie* 34: 387-399.

Berezina, N. A. 2005. Seasonal dynamics of structure and fecundity of the Baikalian amphipod (*Gmelinoides fasciatus,* Amphipoda, Crustacea) population in reedbeds of the Neva Bay. *Zoologicheskii Zhurnal* 84: 411-419.

Berizina, N. A. & Panov, V. E. 2004. Distribution, population structure and salinity tolerance of the invasive amphipod *Gmelinoides fasciatus* (Stebbing) in the Neva Estuary (Gulf of Finland, Baltic Sea). *Hydrobiologia* 514: 199-206.

Bij de Vaate, A., Jazdzewski, K., Ketelaars, H. A. M., Gollasch, S. & van der Velde, G. 2002. Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. *Canadian Journal of Fisheries and Aquatic Science* 59: 1159-1174.

Bjork, G., Liungman, O. & Rydberg, L. 2000. Net Circulation and Salinity Variations in an Open-Ended Swedish Fjord System. *Estuaries* 23: 367-380.

Boersma, M. & Vijverberg, J. 1994. Resource depression in *Daphnia galeatea, Daphnia cucullata*, and their interspecific hybrid: life history consequences. *Journal of Plankton Research* 16: 1741-1758.

Bollens, S. M., Cordell, J. R., Avent, S. & Hooff, R. 2002. Zooplankton Invasions: a Brief Review, Plus Two Case Studies from the Northeast Pacific Ocean. *Hydrobiologia* 480: 87-110.

Borodich, N. D. & Havlena, F. K. 1972. The biology of mysids acclimatized in the reservoirs of the Volga River. *Hydrobiologia* 42: 527-539.

Boronat, L., Miracle, M. R. & Armengol, X. 2001. Cladoceran Assemblages in a Mineralization Gradient. *Hydrobiologia* 442: 75-88.

Bouley, P. & Kimmerer, W. J. 2006. Ecology of a highly abundant, introduced cyclopoid copepod in a temperate estuary. *Marine Ecology Progress Series* 324: 219-228.

Bousfield, E. L. 1958. Freshwater amphipods of glaciated North America. *Canadian Field-Naturalist* 72: 55-113.

Bousfield, E. L. 1973. Shallow-water gammaridean Amphipoda of New England. Ithaca, NY: Comstock Publishing Associates.

Bousfield, E. L. 1979. The amphipod superfamily Gammaroidea in the Northeastern Pacific region: systematics and distributional ecology. *Bulletin of the Biological Society of Washington* 3: 297-357.

Briggs, J. C. 1974. Marine Zoogeography. New York: McGraw-Hill.

Broad, A. C. & Hubschuman, J. H. 1962. A comparison of larvae and larval development of species of Eastern U.S. *Palaemonetes intermedius* Holthuis. *American Zoologist* 2: 394-395.

Brooks, J. L. 1957. The systematics of North American *Daphnia*. *Memoirs of the Connecticut Academy of Arts and Sciences* 13: 1-180.

Bruijs, M. C. M., Kelleher, B., Van Der Velde, G. & De Vaate, A. B. 2001. Oxygen Consumption, Temperature and Salinity Tolerance of the Invasive Amphipod

Dikerogammarus villosus: Indicators of Further Dispersal via Ballast Water Transport. *Archiv Fur Hydrobiologie* 152: 633-646.

Bryan B. B. & Grant G. C. 1979. Parthenogenesis and the distribution of the Cladocera. *Bulletin of the Biological Society of Washington* 3: 54–59.

Buckley, P., Dussart, G. & Trigwell, J. 2004. Invasion and expansion of Corophiidae (Amphipoda) in the Stour estuary (Kernt, UK). *Crustaceana* 77: 425-433.

Bur, M.T., Klarer, D.M., and Krieger, K.A. 1986. First record of a European cladoceran, Bythotrephes cederstroemi, in Lakes Erie and Huron. *Journal of Great Lakes Research* 12: 144-146.

Carlton, J. T. & Geller, J. B. 1993. Ecological Roulette - the Global Transport of Nonindigenous Marine Organisms. *Science* 261: 78-82.

Caudill, C. C. & Bucklin, A. 2004. Molecular Phylogeography and Evolutionary History of the Estuarine Copepod, Acartia Tonsa, on the Northwest Atlantic Coast. *Hydrobiologia* 511: 91-102.

Chinnery, F. E. & Williams, J. A. 2004. The Influence of Temperature and Salinity on Acartia (Copepoda: Calanoida) Nauplii Survival. *Marine Biology* 145: 733-738.

Choi, K. H., Kimmerer, W., Smith, G., Ruiz, G. M. & Lion, K. 2005. Post-Exchange Zooplankton in Ballast Water of Ships Entering the San Francisco Estuary. *Journal of Plankton Research* 27: 707-714.

Chojnacki, J. C. 1999. Description of Ecosystem of the Lower Odra and the Odra Estuary. *Acta Hydrochimica et Hydrobiologica* 27: 257-267.

Chubarenko, B., Chubarenko, I. & Baudler, H. 2005. Comparison of Dars-Zingst Bodden Chain and Vistula Lagoon (Baltic Sea) in a view of hydrodynamic numerical modeling. *Baltica* 18: 56-67.

Colautti, R.I., Bailey, S.A., van Overdijk, C.D.A., Amundsen, K., and MacIsaac, H.J. 2006. Characterised and projected costs of nonindigenous species in Canada. Biological Invasions 8: 45-59.

Colautti, R. I., Manca, M., Viljanen, M., Ketelaars, H. A. M., Burgi, H., MacIsaac, H. J. & Heath, D. D. 2005. Invasion Genetics of the Eurasian Spiny Waterflea: Evidence for Bottlenecks and Gene Flow Using Microsatellites. *Molecular Ecology* 14: 1869-1879.

Colautti, R. I., Niimi, A. J., van Overdijk, C. D. A., Mills, E. L., Holeck, K. & MacIsaac, H. J. 2003. Spatial and temporal analysis of transoceanic shipping vectors to the Great Lakes. In: Invasive Species: Vectors and Management Strategies. (pp. 227-246). Island Press.

Costello, C. J. & Solow, A. R. 2003. On the Pattern of Discovery of Introduced Species. *Proceedings of the National Academy of Sciences of the United States of America* 100: 3321-3323.

Cristescu, M. E. A., Herbert, P. D. N. & Witt, J. 2001. An invasion history for *Cercopagis pengoi* based on mitochondrial gene sequences. *Limnology and Oceanography* 46: 224-229.

Czeczuga, B. & Kozµowska, M. 2002. Fertility of *Eudiaptomus, Bosmina* and *Daphnia* (Crustacea) Representatives in lakes of varied trophic states in the Suwaµki District. *Polish Journal of Environmental Studies* 11: 23-32.

Daunys D. & Zettler M.L. 2006. Invasion of the North American amphipod (*Gammarus tigrinus* Sexton, 1939) into the Curonian Lagoon, South Eastern Baltic Sea. *Acta Zoologica Lituanica* 16: 20-26.

De Gelas, K. & De Meester, L. 2005. Phylogeography of *Daphnia magna* in Europe. *Molecular Ecology* 14: 753-764.

De Melo, R. & Hebert, P. D. N. 1994. Taxonomic reevaluation of North American Bosminidae. *Canadian Journal of Zoology* 72: 1808-1825.

Dediu, I. I. 1980. Amphipods of fresh and brackish waters of the southwestern USSR. Dediu, I. I. Amphipods of fresh and brackish waters of the southwestern USSR. 1980. Shtiintsa, Kishinev, Mold. SSR.

Deevey, E. S. & Deevey, G. B. 1971. The American species of *Eubosmina* Seligo. (Crustacea, Cladocera). *Limnology and Oceanography* 16: 201-28.

Demelo, R. & Hebert, P. D. N. 1994. A Taxonomic Reevaluation of North-American Bosminidae. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 72: 1808-1825.

DeMott, W. R. 1987. An analysis of the precision of birth and death rate estimates for egg-bearing zooplankters. *American Society of Limnology and Oceanography Special Symposium* 3: 337-345.

Den Hartog, C. 1964. The amphipods of the deltaic region of the rivers Rhine, Meuse and Scheldt, in relation to the hydrography of the sea. Part III. The Gammaridae. *Netherlands Journal of Sea Research* 2-3: 407-457.

Den Hartog, C., van den Brink, F. W. B. & van der Velde, G. 1993. Why was the invasion of the river Rhine by *Corophium curvispinum* and *Corbicula* species so successful? *Journal of Natural History* 26: 1121-1129.

Dooh, R. T., Adamowicz, J., & Hebert, P. D. N. 2006. Comparative phylogeography of two North American 'glacial relict' crustaceans. *Molecular Ecology* 15: 4459-4475.

Dorgelo, J. 1974. Comparative ecophysiology of gammarids (Crustacea: Amphipoda) from marine, brackish, and fresh-water habitats, exposed to the influence of salinity-temperature combinations. 1. Effects on survival. *Netherlands Journal of Aquatic Ecology* 8: 90-109.

Drake, J. M., Costello, C. & Lodge, D. M. 2005a. When Did the Discovery Rate for Invasive Species in the North American Great Lakes Accelerate? *Bioscience* 55: 4.

Drake, L. A., Jenkins, P. T. & Dobbs, F. C. 2005b. Domestic and International Arrivals of NOBOB (No Ballast on Board) Vessels to Lower Chesapeake Bay. *Marine Pollution Bulletin* 50: 560-565.

Duggan, I.C., Bailey, S.A., Colautti, R.I., Gray, D.K., Makarewicz, C., and MacIsaac, H.J. 2003. Biological invasions in Lake Ontario: past, present and future. In State of Lake Ontario: Past, Present and Future. Edited by M. Munawar. pp. 132-141.

Duggan, I. C., Van Overdijk, C. D. A., Bailey, S. A., Jenkins, P. T., Limen, H. & MacIsaac, H. J. 2005. Invertebrates Associated With Residual Ballast Water and Sediments of Cargo-Carrying Ships Entering the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 2463-2474.

Dybdahl, M. F. & Kane, S. L. 2005. Adaptation Vs. Phenotypic Plasticity in the Success of a Clonal Invader. *Ecology* 86: 1592-1601.

Eggers, T. O. & Anlauf, A. 2003. *Obesogammarus crassus* (G.O. Sars 1894, Crustacea, Amphipoda) erreichte die Elbe. *Lauterbornia* 55: 125-128.

Eriksson, S. 1974. The occurrence of marine Cladocera on the west coast of Sweden. *Marine Biology* 26: 319-327.

Filippov, A. A. 2006. Adaptability of the amphipod *Pontoporeia affinis* (Crustacea: Amphipoda) to salinity changes. *Russian Journal of Marine Biology* 32: 198–200.

Fockedey, N., Mees, J., Vangheluwe, M., Verslycke, T., Janssen, C. R. & Vincx, M. 2005. Temperature and Salinity Effects on Post-Marsupial Growth of Neomysis Integer (Crustacea : Mysidacea). *Journal of Experimental Marine Biology and Ecology* 326: 27-47.

Fofonoff, P. W. Marine Cladocerans in Narragansett Bay. 1994. Kingston. University of Rhode Island. 1994.

Frey, D. G. 1993. The Penetration of Cladocerans into Saline Waters. *Hydrobiologia* 267: 233-248.

Furota, T. & Emmett, R. L. 1993. Seasonal Changes in the Intertidal and Subtidal Macrobenthic Invertebrate Community Structure in Baker Bay, Lower Columbia River Estuary. Seasonal Changes in the Intertidal and Subtidal Macrobenthic Invertebrate Community Structure in Baker Bay, Lower Columbia River Estuary. U.S. Department of Commerce, NOAA Tech. Memo. NMFS-NWFSC-5, 68 p.

Garton, D. W., Berg, D. J., Stoeckman, A. M. & Haag, W. R. 1987. Biology of recent invertebrates invading species in the Great Lakes: the spiny water flea, *Bythotrephes cederstroem*i, and the zebra mussel, *Dreissena polymorpha.* McKnight, Bill N. Biological Pollution: The Control and Impact of Invasive Exotic Species. 63-85. Indianapolis, Indiana Academy of Science.

Gasiunaite, Z. R. 2000. Coupling of the Limnetic and Brackishwater Plankton Crustaceans in the Curonian Lagoon (Baltic Sea). *International Review of Hydrobiology* 85: 653-661.

Gasiunaite, Z. R. & Razinkovas, A. 2004. Temporal and Spatial Patterns of Crustacean Zooplankton Dynamics in a Transitional Lagoon Ecosystem. *Hydrobiologia* 514: 139-149.

Giere, O. W. 1978. Tolerance and Preference Reactions of Oligochaeta in Relation to Their Distribution. Giere, O. W. Tolerance and Preference Reactions of Oligochaeta in Relation to Their Distribution. New York and London, Plenum Press.

Gollasch, S., Lenz, J., Dammer, M. & Andres, H. G. 2000. Survival of Tropical Ballast Water Organisms during a Cruise from the Indian Ocean to the North Sea. *Journal of Plankton Research* 22: 923-937.

Grabowski, M. 2006. Rapid colonization of the Polish Baltic coast by an Atlantic palaemonid shrimp *Palaemon elegans* Rathke, 1837. *Aquatic Invasions* 1: 116-123.

Grigorovich, I. A., Colautti, R. I., Mills, E. L., Holeck, K., Ballert, A. G. & MacIsaac, H. J. 2003. Ballast-Mediated Animal Introductions in the Laurentian Great Lakes: Retrospective and Prospective Analyses. *Canadian Journal of Fisheries and Aquatic Sciences* 60: 740-756.

Grigorovich, I. A., Kang, M. & Ciborowski, J. J. H. 2005. Colonization of the Laurentian Great Lakes by the Amphipod Gammarus Tigrinus, a Native of the North American Atlantic Coast. *Journal of Great Lakes Research* 31: 333-342.

Grigorovich, I. A. 1998. *Bythotrephes longimanus* in the Commonwealth of Independent States: variability, distribution and ecology. *Hydrobiologia* 739: 183-198.

Gruszka P. 1999. The River Odra estuary as gateway for alien species immigration to the Baltic Sea basin. *Acta Hydrochimica Hydrobiologica* 27: 347-382.

Hackstein, E., Schirmer, M. & Liebsch, H. 1986. Untersuchungen zur Populationsdynamik von *Gammarus tigrinus* Sexton (Crustacea: Amphipoda) in der Weser bei Bremen. *Archiv fuer Hydrobiologie.* 105: 443-458.

Hamer, J. P., McCollin, T. A. & Lucas, I. A. N. 1998. Viability of Decapod Larvae in Ships' Ballast Water. *Marine Pollution Bulletin* 36: 646-647.

Hansen, F. C., Mollmann, C., Schutz, U. & Neumann, T. 2006. Spatio-Temporal Distribution and Production of Calanoid Copepods in the Central Baltic Sea. *Journal of Plankton Research* 28: 39-54.

Hanski, I. & Ranta, E. 1983. Coexistence in a patchy environment: Three species of *Daphnia* in rock pools. *Journal of Animal Ecology* 52: 263-279.

Holeck, K. T., Mills, E. L., MacIsaac, H. J., Dochoda, M. R., Colautti, R. I. & Ricciardi, A. 2004. Bridging Troubled Waters: Biological Invasions, Transoceanic Shipping, and the Laurentian Great Lakes. *Bioscience* 54: 919-929.

Holmes, S. P. & Miller, N. 2006. Aspects of the Ecology and Population Genetics of the Bivalve Corbula Gibba. *Marine Ecology-Progress Series* 315: 129-140.

Horvath, T. G., Whitman, R. L. & Last, L. L. 2001. Establishment of Two Invasive Crustaceans (Copepoda : Harpacticoida) in the Nearshore Sands of Lake Michigan. *Canadian Journal of Fisheries and Aquatic Sciences* 58: 1261-1264.

Hunt, C. D., Tanis, D. C., Stevens, T. G., Frederick, R. M. & Everett, R. A. 2005. Verifying Ballast-Water Treatment Performance. *Environmental Science & Technology* 39: 321A-328A.

Inkala, A. & Myrberg, K. 2002. Comparison of Hydrodynamical Models of the Gulf of Finland in 1995: a Case Study. *Environmental Modelling & Software* 17: 237-250.

Ioffe, Ts. I., Salazkin, A. A. & Petrov, V. V. 1968. Biological foundations for enrichment of fish food resources in the Gorkyi, Kuibyshev and Volgograd reservoirs. *Izv. Gos. Nauchno-Issled. Inst. Ozern. Rechn. Rybn. Khoz.* 67: 30–80.

Jasinska, E. 1993. Motion of salt water and associated fronts in tideless estuaries. *Estuaries* 16: 53-67.

Jazdzewski, K. 1970. Biology of Crustacea Malacostraca in the Bay of Puck, Polish Baltic Sea. *Zoologica Poloniae* 20: 423-479.

Jazdzewski, K. 1973. Ecology of gammarids in the Bay of Puck. *Oikos* 15.

Jazdzewski, K. 1980. Range extensions of some gammaridean species in European inland waters caused by human activity. *Crustaceana* Suppl. 6: 84-107.

Jazdzewski, K., Konopacka, A. & Grabowski, M. 2005. Native and alien malacostracan Crustacea along the Polish Baltic Sea coast in the twentieth century. *Oceanological and Hydrobiological Studies* 34: 175-193.

Jeczmien, W. & Szaniawska, A. 2000. Changes in species composition of the genus *Gammarus Fabricius* in Puck Bay. *Oceanologia* 42: 71-87.

Johannsson, O. E., Mills, E. L. & O'Gorman, R. 1991. Changes in the nearshore and offshore zooplankton communities in Lake Ontario: 1981-1988. *Canadian Journal of Fisheries and Aquatic Sciences* 48: 1546-1557.

Johns, D. G., Edwards, M., Greve, W. & Sjohn, A. W. G. 2005. Increasing Prevalence of the Marine Cladoceran Penilia Avirostris (Dana, 1852) in the North Sea. *Helgoland Marine Research* 59: 214-218.

Jorgensen, O. M. 1933. On the marine Cladocera from the Northumbrian plankton. *Journal of the Marine Biological Association of the United Kingdom* 19: 179-226.

Kankaala, P. 1983. Resting eggs, seasonal dynamics, and production of B*osmina longispina maritima* (P. E. Müller) (Cladocera) in the northern Baltic proper. *Journal of Plankton Science* 5: 53-69.

Kappenberg, J. & Grabemann, I. 2001. Variability of the Mixing Zones and Estuarine Turbidity Maxima in the Elbe and Weser Estuaries. *Estuaries* 24: 699-706.

Karpevich, A. F. 1975. Theory and practice of acclimatization of aquatic organisms. Karpevich, A. F. Theory and practice of acclimatization of aquatic organisms. Moscow, Pishchevaya Promyshlennost.

Kelleher, B., Van der Velde, G., Wittman, K. J., Faase, M. A. & bij de Vaate, A. 1999. Current status of the freshwater Mysidae in the Netherlands, with the records of *Limnomysis benedeni* Czerniavsky, 1882, a Pontocaspian species in Dutch Rhine branches. *Bulletin Zoölogisch Museum, Universiteit van Amsterdam* 16: 89-94.

Kelly, D. W., Muirhead, J. R., Heath, D. D. & MacIsaac, H. J. 2006. Contrasting Patterns in Genetic Diversity Following Multiple Invasions of Fresh and Brackish Waters. *Molecular Ecology* 15: 3641-3653.

Ketelaars, H. A. M., Lambregts-van de Clundert, F. E., Carpentier, C. J., Wagenvoort, A. J. & Hoogenboezem, W. 1999. Ecological effects of the mass occurrence of the Ponto-Caspian invader, *Hemimysis anomala* G.O. Sars, 1907 (Crustacea: Mysidacea), in a freshwater storage reservoir in the Netherlands, with notes on its autoecology and new records. *Hydrobiologia* 394: 233-248.

Khemeleva, N. N. & Baicharov, V. M. 1987. Patterns of reproduction of Pontocaspian relict *Paramysis lacustris* with distribution area. *International Revue de Gesamten Hydrobiologie*.

Kley, A. & Maier, G. 2003. Life history characteristics of the invasive freshwater gammarids *Dikerogammarus villosus* and *Echinogammarus ischnus* in the river Main and the Main-Donau canal. *Archiv für Hydrobiolgie* 156: 457–469.

Klink, A. G. & Bij de Vaate, A. 1996. *Hypania invalida* (Grube, 1860) (Polychaeta: Ampharetidae) a freshwater polychaeta in the Lower Rhine, new to the Dutch fauna. *Lauterbornia* 25: 57-60.

Kohn, J. and Waterstraat, A. 1990. The amphipod fauna of Lake Kummerow (Mecklenburg, German Democratic Republic) with reference to Echinogammarus ischnus Stebbing, 1899. *Crustaceana* 1: 74-82.

Kolding, S. 1973. Habitat selection and life cycle characteristics of five species of the amphipod genus *Gammarus* in the Baltic. *Oikos* 173-178.

Komarova, T. I. 1984. Mysids of the delta of the Dneipr and the Dneipr-Bug Lagoon. *Vestnik Zoologii* 35-38.

Komarova, T. I. 1991. Mysidaceans (Mysidacea). Vol. 26. Malacostracans. Kiev, Ukraine. Naukova Dumka. Fauna of Ukraine.

Koski, M., Viitasalo, M. & Kuosa, H. 1999. Seasonal development of mesozooplankton biomass and production on the SW coast of Finland. *Ophelia* 50: 69-91.

Kotta, J. & Kotta, I. 1997. Do the towns of Helsinki and Tallinn oppress the zoobenthos in the adjacent sea? *EMI Report Series* 8: 55-71.

Kukert K 1984. Die Crustaceen der Brackwassertumpel im Aubendeichsland zwischen Spieka-Neufeld und Arensch-Berensch/Cuxhaven und ihre Verteilung in Beziehung zum Salzgehalt (Crustacea: Cladocera, Copepoda, Amphipoda, Decapoda). *Abhandlungen Naturwissenschaftlichen Verein zu Bremen* 40: 115-136.

Lance, J. 1963. The salinity tolerance of some estuarine planktonic copepods. *Limnology and Oceanography* 8: 440-449.

Lance, J. 1964. The Salinity Tolerances of Some Estuarine Planktonic Crustaceans. *Biological Bulletin* 127: 108-118.

Laughlin, R. B. & French, W. 1989. Differences in Responses to Factorial Combinations of Temperature and Salinity by Zoeae from 2 Geographically Isolated Populations of the Mud Crab Rhithropanopeus-Harrisii. *Marine Biology* 102: 387-395.

Lavoie, D. M., Smith, L. D. & Ruiz, G. M. 1999. The Potential for Intracoastal Transfer of Non-Indigenous Species in the Ballast Water of Ships. *Estuarine Coastal and Shelf Science* 48: 551-564.

Lee, C. E. 1999. Rapid and Repeated Invasions of Fresh Water by the Copepod Eurytemora Affinis. *Evolution* 53: 1423-1434.

Lee, C. E. 2002. Evolutionary Genetics of Invasive Species. *Trends in Ecology & Evolution* 17: 386-391.

Lee, C. E. & Petersen, C. H. 2002. Genotype-by-Environment Interaction for Salinity Tolerance in the Freshwater-Invading Copepod Eurytemora Affinis. *Physiological and Biochemical Zoology* 75: 335-344.

Lee, C. E., Remfert, J. L. & Gelembiuk, G. W. 2003. Evolution of Physiological Tolerance and Performance during Freshwater Invasions. *Integrative and Comparative Biology* 43: 439-449.

Lehvo, A., Ekebom, J. & Back, S. 1998. Introduction to the marine and coastal environment of Finland. Baltic Sea Environmental Proceedings No. 75: Red List of Marine and Coastal Biotopes and Biotope Complexes of the Baltic Sea, Belt Sea, and Kattegat. Helsinki, Helsinki Commission. Baltic Marine Environment Protection Commission.

Leppakoski, E., Gollasch, S., Gruszka, P., Ojaveer, H., Olenin, S. & Panov, V. 2002. The Baltic - a Sea of Invaders. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1175-1188.

Leppäkoski, E. & Gollasch, S. 2006. Risk Assessment of Ballast Water Mediated Species Introductions: a Baltic Sea Approach. Risk Assessment of Ballast Water Mediated Species Introductions into the Baltic Sea.

Levings, C. D., Cordell, J. R., Ong, S. & Piercey, G. 2004. The Origin and Identity of Invertebrate Organisms Being Transported to Canada's Pacific Coast by Ballast Water. *Canadian Journal of Fisheries and Aquatic Sciences* 61: 1-11.

Liungman, O. 2000. Tidally Forced Internal Wave Mixing in a K-Epsilon Model Framework Applied to Fjord Basins. *Journal of Physical Oceanography* 30: 352-368.

Locke, A., Reid, D.M., van Leeuwen, H.C., Sprules, W.G., and Carlton, J.T. 1993. Ballast water exchange as a means of controlling dispersal of freshwater organisms by ships. *Canadian Journal of Fisheries and Aquatic Sciences* 50: 2086-2093.

Lockwood, A. P. M. 1976. Physiological adaptations to life in estuaries. (pp. 315-392). London: Butterworths.

Lopez, T., Toja, J. & Gabellone, N. A. 1991. Limnological Comparison of 2 Peridunar Ponds in the Donana-National-Park (Spain). *Archiv Fur Hydrobiologie* 120: 357-378.

Lougheed, V. L. & Chow-Fraser, P. 2002. Development and use of a zooplankton index to monitor wetland quality in Canadian marshes of the Great Lakes basin. *Ecological Applications* 12: 474-486.

MacIsaac, H.J., Grigorovich, I.A., Hoyle, J.A., Yan, N.D., and Panov, V.E. 1998. Invasion of Lake Ontario by the Ponto-Caspian predatory crustacean Cercopagis pengoi. *Canadian Journal of Fisheries and Aquatic Sciences* 56: 1-5.

MacIsaac, H. J., Robbins, T. C. & Lewis, M. A. 2002. Biological Invasions of Aquatic Habitats in Europe and the Great Lakes. Modeling Ships' Ballast Water as Invasion Threats to the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1245-1256.

Maier, G. 1992. Development, reproduction, and growth pattern of two coexisting pond dwelling cladocerans. *Internationale Revue der Gesamten Hydrobiologie* 77: 621-632.

Mann, R. & Harding, J. M. 2003. Salinity Tolerance of Larval *Rapana venosa*: Implications for Dispersal and Establishment of an Invading Predatory Gastropod on the North American Atlantic Coast. *Biological Bulletin* 204: 96-103.

Marques, S. C., Azeiteiro, U. M., Marques, J. C., Neto, J. M. & Pardal, M. A. 2006. Zooplankton and Ichthyoplankton Communities in a Temperate Estuary: Spatial and Temporal Patterns. *Journal of Plankton Research* 28: 297-312.

Mauchline, J. 1965. Breeding and fecundity of Praunus inermis (Crustacea, Mysidacea). *Journal of the Marine Biological Association of the United Kingdom* 45: 663-671.

Mauchline, J. 1971. The biology of *Praunus flexuosus* and *P. neglectus* (Crustacea, Mysidacea). *Journal of the Marine Biological Association of the United Kingdom* 51: 641-652.

McKenney, C. L. Jr. & Neff, J. M. 1979. Individual effects and interactions of salinity, temperature, and zinc on larval development of the grass shrimp *Palaemonetes pugio*. I. Survival and development duration through metamorphosis. *Marine Biology* 52: 177-188.

McLusky, D. S. & Heard, V. E. J. 1971. Some effects of salinity on the mysid *Praunus flexuosus*. *Journal of the Marine Biological Association of the United Kingdom* 51: 709-715.

Mees, J., Abdulerim, Z. & Hamerlynck, O. 1993. Distribution and abundance of the shallow-water mysids (Crustacea, Mysidacea) and euphausiids (Crustacea, Euphausiacea) in the Voordelta and the Westerschelde, Southwest Netherlands. *Marine Ecology Progress Series* 109: 43-57.

Meijering, M. P. D. 1983. On the occurrence of 'Arctic' Cladocera with special reference to those along the Strait of Belle Isle (Quebec, Labrador, Newfoundland). *Internationale Revue der Gesamten Hydrobiologie.* 68: 85-89.

Mills, E.L., Leach, J.H., Carlton, J.T., and Secor, C.L. 1993. Exotic Species in the Great Lakes: A history of biotic crises and anthropogenic introductions. *Journal of Great Lakes Research* 19: 1-54.

Mills, E. L., Rosenberg, G., Spidle, A. P., Ludyanskiy, M., Pligin, Y. & May, B. 1996. A Review of the Biology and Ecology of the Quagga Mussel (Dreissena bugensis), a Second Species of Freshwater Dreissenid Introduced to North America. *American Zoologist* 36: 271-286.

Minton, M. S., Verling, E., Miller, A. W. & Ruiz, G. M. 2005. Reducing Propagule Supply and Coastal Invasions via Ships: Effects of Emerging Strategies. *Frontiers in Ecology and the Environment* 3: 304-308.

Montschenko, V. 1967. Beitrag zur Kenntnis der Gattung Schizopera (Crustacea, Harpacticoida) im Schwarzen. *Meeresforschung Zoologische Anzeiger* 178: 367–374.

Mooij, W. M., Hulsmann, S., Domis, L. N. D., Nolet, B. A., Bodelier, P. L. E., Boers, P. C. M., Pires, L. M. D., Gons, H. J., Ibelings, B. W., Noordhuis, R., Portielje, R., Wolfstein, K. & Lammens Ehrr 2005. The Impact of Climate Change on Lakes in the Netherlands: a Review. *Aquatic Ecology* 39: 381-400.

Mordukhai-Boltovskoi 1968. Caspian fauna beyond the Caspian Sea. *Internationale Revue der Gesamten Hydrobiologie* 49: 139-170.

Mordukhai-Boltovskoi, Ph. D. & Rivier, I. K. 1987. Predatory Cladocera of the world's fauna. Leningrad, Nauka.

Moroz, T. G. 1994. Aquatic Oligochaeta of the Dnieper-Bug Estuary System. *Hydrobiologia* 278: 133-138.

Moroz, T. R. 1977. Oligochaetes of the estuarine zone of the North-West tributaries of the Black Sea. *Hydrobiological Journal*.

Nehring, S. 2006. Four Arguments Why So Many Alien Species Settle Into Estuaries, With Special Reference to the German River Elbe. *Helgoland Marine Research* 60: 127-134.

Niimi, A.J. 2004. Role of container vessels in the introduction of exotic species. *Marine Pollution Bulletin* 49: 778-782.

Niimi, A. J. & Reid, D. M. 2003. Low Salinity Residual Ballast Discharge and Exotic Species Introductions to the North American Great Lakes. *Marine Pollution Bulletin* 46: 1334-1340.

Normant, M., Kubicka, M., Lapucki, T., Czarnowski, W. & Michalowska, M. 2005. Osmotic and Ionic Haemolymph Concentrations in the Baltic Sea Amphipod Gammarus Oceanicus in

Relation to Water Salinity. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* 141: 94-99.

O'Connor, W. A. & Lawler, N. F. 2004. Salinity and Temperature Tolerance of Embryos and Juveniles of the Pearl Oyster, *Pinctada imbricata* Roding. *Aquaculture* 229: 493-506.

Ojaveer, H., Leppakoski, E., Olenin, S. & Ricciardi, A. 2002. Ecological impact of Ponto-Caspian invaders in the Baltic Sea, European inland waters and the Great Lakes: an inter-ecosystem comparison. Leppakoski, E, Gollasch, S., and Olenin, S. Invasive Aquatic Species of Europe. 412-425. Dordrecht, Boston, London. Kluwer Academic Publishers.

Olenin, S., Gollasch, S., Jonusas, S. & Rimkute, I. 2000. En-Route Investigations of Plankton in Ballast Water on a Ship's Voyage from the Baltic Sea to the Open Atlantic Coast of Europe. *International Review of Hydrobiology* 85: 577-596.

Olenin, S., Olenina, I., Daunys, D. & Gasiunaite, Z. 1999. The harbour profile of Klaipeda, Lithuania. The risk assessments of alien species in Nordic waters.

Ortells, R., Reusch, T. B. H. & Lampert, W. 2005. Salinity Tolerance in Daphnia Magna: Characteristics of Genotypes Hatching From Mixed Sediments. *Oecologia* 143: 509-516.

Ostman, M. & Leppakoski, E. 1999. The ports of southwest Finland - Turku, Naantali, and Paragas. Risk assessment of alien species in Nordic coastal waters.

Ovčarenko, I., Audzijonyte, A. & Rasuole Gasiunaite, Z. 2006. Tolerance of *Paramysis lacustris* and *Limnomysis benedeni* (Crustacea, Mysida) to sudden salinity changes: implications for ballast water treatment. *Oceanologia* 48: 231-242.

Paalvast, P., Ledema, W., Ohm, M. & Posthoorn, R. 1998. MER Beheer Haringvlietsluizen, Deelrapport ecologies en landschap. RIZA rapport 98.051.

Paavola, M., Olenin, S. & Leppakoski, E. 2005. Are Invasive Species Most Successful in Habitats of Low Native Species Richness Across European Brackish Water Seas? *Estuarine Coastal and Shelf Science* 64: 738-750.

Padilla, D. K. 2005. The Potential of Zebra Mussels as a Model for Invasion Ecology. *American Malacological Bulletin* 20: 123-131.

Panov, V. E., Alimov, A. F., Balushkina, E. V., Golubkov, S. M., Nikulina, V. N., Telesh, V. I. & and Finogenova, N. P. 1997. Monitoring biodiversity in bottom and planktonic communities of the Neva Estuary. Monitoring of biodiversity (pp. 228–294). Moscow: Pensoft.

Panov, V. E., Krylov, P. I. & Telesh, I. V. The St. Petersburg Harbour profile. Risk assessment of alien species in Nordic coastal waters. 1999.

Panov, V. Ye. 1986. Growth and production of *Gammarus lacustris* in the Neva Inlet. *Hydrobiological Journal* 22: 37-42.

Panov, V. *Cercopagis pengoi* (Ostroumov, 1891). 2003. Nov. 15, 2006.

Panov, V. E. & Berizina, N. A. 2002. Invasion history, biology, and impacts of the Baikalian amphipod *Gmelinoides fasciatus*. In Leppakoski, E., Gollasch, S. & Olenin, S. (Eds) Invasive Aquatic Species of Europe (pp. 96-103). Dordrecht, Boston, London. Kluwer Academic Publishers.

Panov, V. E., Ketelaars, H. E. & MacIsaac, H. *Bythotrephes longimanus* Leydig 1860. 2003. November 15, 2006.

Pienimäki, M., Haleavuori, M. & Leppäkoski, E. 2004. First findings of the North American amphipod *Gammarus tigrinus* along the Finnish Coast. *Memoranda Societatis pro Fauna et Flora Fennica.* 80: 17-19.

Pimentel, D. 2005. Aquatic Nuisance Species in the New York State Canal and Hudson River Systems and the Great Lakes Basin: an Economic and Environmental Assessment. *Environmental Management* 35: 692-701.

Pimentel, D., Lach, L., Zuniga, R., and Morrison, D. 2000. Environmental and economic costs of nonindigenous species in the United States. *Bioscience* 50: 53-65.

Pinkster, S. & Platvoet, D. 1983. Further observations on the distribution and biology of two alien amphipods, *Gammarus tigrinus,* Sexton 1939 and *Crangonyx pseudogracilis*, Bousfield 1958, in the Netherlands (Crustacea, Amphipoda). *Bulletin Zoologisch Museum Universiteit van Amsterdam* 9: 154-164.

Pinkster, S., Scheepmaker, M., Platvoet, D. & Broodbakker, N. 1992. Drastic changes in the amphipod fauna (Crustacea) of Dutch inland waters during the last 25 years. *Bijdragen tot de Dierkunde* 61: 193-204.

Pliüraitë, V. 2003. Species diversity of zooplankton in the Curonian Lagoon in 2001. *Acta Zoologica Lithuanica* 13: 106-113.

Poggensee, P. a. L. J. 1981. On the population dynamics of two brackish-water cladocera *Podon leuckarti* and *Evadne nordmanni* in Kiel Fjord. *Kieler Meeresforschung. Sonderheft* 5: 268-273.

Ponomareva, S. A. 1975. Effect of salinity on the fresh-water shrimp *Dikerogammarus haemobaphes* (Eichwald) from the mouth of the Dnepr. *Hydrobiological Journal* 11: 67-69.

Potts, W. T. W. & Durning 1980. Physiological evolution in the branchiopods. *Comparative Biochemistry and Physiology B. - Comparative Biochemistry.* 67: 475-484.

Preece, G. S. 1971. The ecophysiological complex of Bathyporeia pilosa and B. pelagica (Crustacea: Amphipoda). II. Effects of exposure. *Marine Biology* 11: 28-34.

Primakov, I. M. & Nikolaenko, P. 2001. Plankton Communities in the Neva Bay during the 20th Century. *Ambio* 30: 292-296.

Reid, D. & Orlova, M. 2002. Geological and Evolutionary Underpinnings for the Success of Ponto-Caspian Species Invasions in the Baltic Sea and North American Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1144-1158.

Remane, A. & Schlieper, C. 1971. Die Binnengewasser. *E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart* 25: 211-350.

Reznichenko, O. G. 1959. On the ecology and morphology of the mysidaceans of the genus *Hemimysis* (Crustacea, Malacostraca). *Trudy Vsesoyuznye Gidrobiolichesckovo Obshchestva* 9: 320-343.

Rhodes, A. 2003. Methods for high density batch culture of *Nitokra lacustris*, a marine harpacticoid copepod. Howard I. Browman and Anne Berit Skiftesvik. The Big Fish Bang.

Proceedings of the 26th Annual Larval Fish Conference. Bergen, Norway, Institute of Marine Research.

Ricciardi, A. 2001. Facilitative Interactions Among Aquatic Invaders: Is an "Invasional Meltdown" Occurring in the Great Lakes? *Canadian Journal of Fisheries and Aquatic Sciences* 58: 2513-2525.

Ricciardi, A. 2006. Patterns of invasions in the Laurentian Great Lakes in relation to changes in vector activity. *Diversity and Distributions* **12**: 425-433.

Ricciardi, A. and MacIsaac, H.J. 2000. Recent mass invasion of the North American Great Lakes by Ponto-Caspian species. *Trends in Ecology and Evolution* 15: 62-65.

Ricciardi, A. and Rasmussen, J.B. 1998. Predicting the identity and impact of future biological invaders: a priority for aquatic resource management. *Canadian Journal of Fisheries and Aquatic Sciences* 55: 1759-1765.

Richter, B.D., Braun, D.P., Mendelson, M.A., and Master, L.L. 1997. Threats to imperiled freshwater fauna. *Conservation Biology* 11: 1081-1093.

Rivier, I. K. 1998. The predatory cladocera (Onychopoda: Podonudae, Polyphemidae, Cercopagidae) and Leptodorida of the world. Leiden, Backhuys. Guides to the identification of the microinvertebrates of the continental waters of the world.

Rodionova, N. V., Krylov, P. I. & Panov, V. E. 2005. Invasion of the Ponto-Caspian predatory cladoceran *Cornigerius maeoticus maeoticus* into the Baltic Sea. *Oceanology* 45: 73-75.

Rodionova, N. V. & Panov, V. E. 2006. Establishment of the Ponto-Caspian predatory cladoceran *Evadne anonyx* in the eastern Gulf of Finland, Baltic Sea. Aquatic Invasions 1, 7-12.

Ruiz, G. M., Fofonoff, P. W., Carlton, J. T., Wonham, M. J. & Hines, A. H. 2000. Invasion of Coastal Marine Communities in North America: Apparent Patterns, Processes, and Biases. *Annual Review of Ecology and Systematics* 31: 481-531.

Ruiz G. M., Murphy, K. R., Verling, E., Smith, G., Chaves, S. & Hines A. H. 2004. Ballast water exchange: Efficacy of treating ships' ballast water to reduce marine species transfers and invasion success? Ballast water exchange: Efficacy of treating ships' ballast water to reduce marine species transfers and invasion success? Report submitted to Prince William Sound Regional Citizens' Advisory Council and the US Fish & Wildlife Service, 14p.

Ruiz, G. M. & Smith, G. 2005. Biological study of container vessels at the Port of Oakland: (a) biota associated with ballast water of container ships arriving to the Port of Oakland, (b) ballast water exchange efficacy on eight container ships, and (c) analysis of biofouling organisms associated with the hulls of container ships arriving to the Port of Oakland. Biological study of container vessels at the Port of Oakland: (a) biota associated with ballast water of container ships arriving to the Port of Oakland, (b) ballast water exchange efficacy on eight container ships, and (c) analysis of biofouling organisms associated with the hulls of container ships arriving to the Port of Oakland. Final Report submitted to the Port of Oakland. 151p.

Sala, O.E., Chapin III, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Osterheld, M., LeRoy Poff, N., Sykes, M.T., Walker, B.H., Walker, M., and Wall, D.H. 2000. Global Biodiversity Scenarios for the Year 2100. *Science* 287: 1770-1774.

Salemaa, H. & Hietalahti, V. 1994. *Hemimysis anomala* G.O. Sars (Crustacea: Mysidacea) - Immigration of a Pontocaspian mysid into the Baltic Sea. *Annales Zoologici Fennici* 30: 271-276.

Sarviro, V. S. 1980. Temperature aspects of the ecology of the amphipod *Gammarus lacustris*. *Soviet Journal of Ecology* 1: 57-64.

Schinke, H. & Matthaus, W. 1998. On the Causes of Major Baltic Inflows - an Analysis of Long Time Series. *Continental Shelf Research* 18: 67-97.

Schirmer, M. 2003. Limnologische funktionskontrolle der ausgleichs- und Ersatzmabnahme auf der Kleinensieler Plate. Bremen, Germany, University of Bremen.

Schuchardt, B., Haesloop, U. & Schirmer, M. 1993. The tidal freshwater reach of the Weser Estuary: riverine or estuarine? *The Netherlands journal of aquatic ecology* 2: 215-226.

Schwenk, K., Posada, D. & Hebert, P.D.N. 2000. Molecular systematics of European *Hyalodaphnia*: the role of contemporary hybridization in ancient species. *Proceedings of the Royal Society of London B.* 267: 1822-1842.

Segersträle, S. G. 1950. The amphipods on the coasts of Finland- Some facts and problems. *Societas Scientarum Fennica Commentationes Biologicae* 10: 2-27.

Seys, J., Vincx, M. & Meire, P. 1999. Spatial Distribution of Oligochaetes (Clitellata) in the Tidal Freshwater and Brackish Parts of the Schelde Estuary (Belgium). *Hydrobiologia* 406: 119-132.

Shoemaker, C. R. 1947. Further notes on the amphipod genus *Corophium*, from the east coast of North America. *Journal of the Washington Academy of Sciences* 37: 47-63.

Simenstad, C. A., Wick, A. J., Cordell, J. R., Thom, R. M. & Williams, G. D. 2001. Decadal Development of a Created Slough in the Chehalis River Estuary: Year 2000 Results. Decadal Development of a Created Slough in the Chehalis River Estuary: Year 2000 Results. Report to U.S. Army Corps of Engineers, Seattle District.

Simm, M. & Ojaveer, H. 2006. Taxonomic status and reproduction dynamics of the non-indigenous *Cercopagis* in the Gulf of Riga (Baltic Sea). *Hydrobiologia* 554: 147–154.

Smith, L. D., Wonham, M. J., McCann, L. D., Ruiz, G. M., Hines, A. H. & Carlton, J. T. 1999. Invasion pressure to a ballast-flooded estuary and an assessment of inoculant survival. *Biological Invasions* 1: 67-87.

Soetaert, K. & Vanrijswijk, P. 1993. Spatial and Temporal Patterns of the Zooplankton in the Westerschelde Estuary. *Marine Ecology-Progress Series* 97: 47-59.

Soldatova, I. N. 1986. Eco-physiological properties of *Pontogammarus maeoticus* (Amphipoda) in a salinity gradient. *Marine Biology* 92: 115-123.

Spittler, P. & Schiller, H. 1984. The Effect of Salinity on the Distribution and Population Growth of Chydorus Sphaericus (Cladocera). *Limnologica* 15: 507-512.

StatSoft, Inc. 2003. STATISTICA (data analysis software system), version 6. www.statsoft.com.

Stepien, C. A., Taylor, C. D. & Dabrowska, K. A. 2002. Genetic Variability and Phylogeographical Patterns of a Nonindigenous Species Invasion: a Comparison of Exotic vs. Native Zebra and Quagga Mussel Populations. *Journal of Evolutionary Biology* 15: 314-328.

Straale, D. & Halbich, A. 2000. Life history and multiple antipredator defenses of an invertebrate pelagic predator, Bythotrephes longimanus. 81: 150-163.

Sutcliffe, D. W. 1967. Sodium regulation in the fresh-water amphipod, *Gammarus pulex* (L.). *Journal of Experimental Biology* 46: 499-S18.

Sutcliffe, D. W. 1968. Sodium Regulation and Adaptation to Fresh Water in Gammarid Crustaceans. *Journal of Experimental Biology* 48: 359-380.

Sutcliffe, D. W. & Shaw, J. 1967. The sodium balance mechanism in the fresh-water amphipod, *Gammarus lacustris* Sars. *Journal of Experimental Biology* 46: 519-528.

Tackx, M. L. M., De Pauw, N., Van Mieghem, R., Azemar, F., Hannouti, A., Van Damme, S., Fiers, F., Daro, N. & Meire, P. 2004. Zooplankton in the Schelde Estuary, Belgium and the Netherlands. Spatial and Temporal Patterns. *Journal of Plankton Research* 26: 133-141.

Tamburri, M. N., Wasson, K. & Matsuda, M. 2002. Ballast Water Deoxygenation Can Prevent Aquatic Introductions While Reducing Ship Corrosion. *Biological Conservation* 103: 331-341.

Tattersall, W. M. & Tattersall, O. M. 1951. The British Mysidacea. London: Ray Society.

Taylor, D. J., Ishikane, C. R. & Haney, R. A. 2002. The Systematics of Holarctic Bosminids and a Revision That Reconciles Molecular and Morphological Evolution. *Limnology and Oceanography* 47: 1486-1495.

Taylor, D. J. & Hebert, P. D. N. 1993. Cryptic intercontinental hybridization in *Daphnia* (Crustacea): the ghost of introductions past. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 254: 163-168.

Telesh, I. V. 1996. Species Composition of Planktonic Rotifera, Cladocera and Copepoda in the Littoral Zone of Lake Ladoga. *Hydrobiologia* 322: 181-185.

Teschner, M. 1995. Effects of Salinity on the Life History and Fitness of Daphnia-Magna - Variability Within and Between Populations. *Hydrobiologia* 307: 33-41.

Therriault, T. W., Grigorovich, I. A., Cristescu, M. E., Ketelaars, H. A. M., Viljanen, M., Heath, D. D. & MacIsaac, H. J. 2002. Taxonomic resolution of the genus *Bythotrephes* Leydig using molecular markers .and re-evaluation of its global distribution. *Diversity and Distributions* 8: 87-84.

Torres, G., Anger, K. & Gimenez, L. 2006. Effects of Reduced Salinities on Metamorphosis of a Freshwater-Tolerant Sesarmid Crab, Armases Roberti: Is Upstream Migration in the Megalopa Stage Constrained by Increasing Osmotic Stress? *Journal of Experimental Marine Biology and Ecology* 338: 134-139.

US Coast Guard 1993. Ballast water management for vessels entering the Great Lakes. 33-CFR Part 151.1510. 1993. Code of Federal Regulations.

US Environmental Protection Agency. 1994. Probit Analysis. http://www.epa.gov/nerleerd/stat2.htm

Uye, S., Shimazu, T., Yamamuro, M., Ishitobi, Y. & Kamiya, H. 2000. Geographical and Seasonal Variations in Mesozooplankton Abundance and Biomass in Relation to Environmental Parameters in Lake Shinji-Ohashi River-Lake Nakaumi Brackish-Water System, Japan. *Journal of Marine Systems* 26: 193-207.

Vader, W., Christopherson, C., Kempe, J. & Skadsheim, A. 1984. *Gammarus inaequicauda*, in Norway (Crustacea, Amphipoda). *Fauna Norvegica Ser. A.* 5: 9-13.

Van Damme, S., Struyf, E., Maris, T., Ysebaert, T., Dehairs, F., Tackx, M., Heip, C. & Meire, P. 2005. Spatial and Temporal Patterns of Water Quality along the Estuarine Salinity Gradient of the Scheldt Estuary (Belgium and the Netherlands): Results of an Integrated Monitoring Approach. *Hydrobiologia* 540: 29-45.

Van der Brink, F. W. B., Van der Velde, G. & Bij de Vaate, A. 1993. Ecological aspects, explosive range extension and impact of a mass invader, *Corophium curvispinum* Sars, 1895 (Crustacea: Amphipoda), in the Lower Rhine (The Netherlands). *Oecologia* 93: 224-232.

Van Maren, M. J. Some notes on the intertidal gammarids Crustacea: Amphipoda from the Atlantic Coast of the Iberian Peninsula Spain, Portugal. *Beaufortia* 23[305], 153-168. 1975.

Vanden Bossche, J. P., Cherot, F., Delooz, E., Grisez, F. & Josens, G. 2001. First Record of the Pontocaspian Invader Hypania Invalida (Grube, 1860) (Polychaeta: Ampharetidae) in the River Meuse (Belgium). *Belgian Journal of Zoology* 131: 183-185.

Verling, E., Ruiz, G. M., Smith, L. D., Galil, B., Miller, A. W. & Murphy, K. R. 2005. Supply-Side Invasion Ecology: Characterizing Propagule Pressure in Coastal Ecosystems. *Proceedings of the Royal Society B-Biological Sciences* 272: 1249-1256.

Verslycke, T., Janssen, C., Lock, K. & Mees, J. 2005. First occurrence of the Ponto-Caspian invader *Hemimysis anomala* (Sars, 1907) in Belgium (Crustacea: Mysidacea). *Belgian Journal of Zoology* 130: 157-158.

Viitasalo, M. 1992. Mesoplankton of the Gulf of Finland and Northern Baltic proper - a review of monitoring data. *Ophelia* 35: 147-168.

Vuorinen, I. & Ranta, E. 1987. Dynamics of Marine Meso-Zooplankton at Seili, Northern Baltic Sea, in 1967-1975. *Ophelia* 28: 31-48.

Waite, T. D., Kazumi, J., Lane, P. V. Z., Farmer, L. L., Smith, S. G., Smith, S. L., Hitchcock, G. & Cap, T. R. 2003. Removal of Natural Populations of Marine Plankton by a Large-Scale Ballast Water Treatment System. *Marine Ecology-Progress Series* 258: 51-63.

Wawrzyniak-Wydrowska, B. & Gruszka, P. 2005. Population Dynamics of Alien Gammarid Species in the River Odra Estuary. *Hydrobiologia* 539: 13-25.

Weider, L. J. 1993. A Test of the General-Purpose Genotype Hypothesis - Differential Tolerance to Thermal and Salinity Stress among Daphnia Clones. *Evolution* 47: 965-969.

Wells, L. 1970. Effects of alewife predation on zooplankton populations of Lake Michigan. *Limnology and Oceanography* 556-565.

Wetsteyn, L. P. M. J. & Vink, M. 2001. An investigation into the presence of plankton organisms in the ballast water of ships arriving in Dutch Ports, and the survival of these organisms in Dutch surface and port waters. Ballast Water. Directorate-General of Public Works and Water Management.

Wijnhoven, S., Van Riel, M. C. & van der Velde, G. 2003. Exotic and indigenous freshwater gammarid species: physiological tolerance to water temperature in relation to ionic content of the water. *Aquatic Ecology* 37: 151-158.

Williams-Howze, J. 1996. Biology and Morphology of the Marine Harpacticoid Copepod Heteropsyllus Nunni Coull, During Encystment Diapause. *Hydrobiologia* 320: 179-189.

Witt, J. D. S., Hebert, P. D. N. & Morton, W. B. 1997. *Echinogammarus ischnus:* another crustacean invader in the Laurentian Great Lakes system. *Canadian Journal of Fisheries and Aquatic Sciences* 54: 264-268.

Wittmann, K. J. & Ariani, A. P. 2000. *Limnomysis benedeni* Czerniavsky: a Pontocaspian Mysid New for the Freshwaters of France (Crustacea, Mysidacea). *Vie et Milieu-Life and Environment* 50: 117-122.

Wonham, M. J., Bailey, S. A., MacIsaac, H. J. & Lewis, M. A. 2005. Modelling the Invasion Risk of Diapausing Organisms Transported in Ballast Sediments. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 2386-2398.

Wonham, M. J., Lewis, M. A. & MacIsaac, H. J. 2005. Minimizing Invasion Risk by Reducing Propagule Pressure: a Model for Ballast-Water Exchange. *Frontiers in Ecology and the Environment* 3: 473-478.

Wouters, K. 2002. On the distribution of alien non-marine and estuarine macro-crustaceans in Belgium. *Bulletin Van Het Koninklin Instituut Voor Naturwetenschappen, Biologie.* 72: 119-129.

Wright, D. A., Setzlerhamilton, E. M., Magee, J. A., Kennedy, V. S. & McIninch, S. P. 1996. Effect of Salinity and Temperature on Survival and Development of Young Zebra (Dreissena Polymorpha) and Quagga (Dreissena Bugensis) Mussels. *Estuaries* 19: 619-628.

Xi, Y.-L., Hagiwara, Atsushi & Sakakura, Y. 2005. Combined effects of food level and temperature on life table demography of *Moina macrocopa* Straus (Cladocera). *Internationale Review of Hydrobiology* 90: 546-554.

Ysebaert, T., De Neve, L. & Meire, P. 2000. The Subtidal Macrobenthos in the Mesohaline Part of the Schelde Estuary (Belgium): Influenced by Man? *Journal of the Marine Biological Association of the United Kingdom* 80: 587-597.

Zhao, Y. and Newman, M.C. 2004. Shortcomings of the laboratory-derived median lethal concentration for predicting mortality in field populations: exposure duration and latent mortality. Environmental Toxicology and Chemistry 23: 2147-2153

Zinovyeh, A. N. 1931. Salt and brackish water bodies of the Troitskiy district, Ural region. *Isv. Permskago bio., nauchno-issled. in-ta* 9: 271-295.

Zorita, E. & Laine, A. 2000. Dependence of Salinity and Oxygen Concentrations in the Baltic Sea on Large-Scale Atmospheric Circulation. *Climate Research* 14: 25-41.

Summary and Recommendations

Although not a complete barrier against all exotic species, our salinity tolerance and on-board ballast water exchange experiments clearly show that many taxa that originate from low-salinity ports can be eradicated from ballast tanks relatively quickly through exposure to full-strength seawater (34 ppt). This conclusion is especially true for species of rotifers, cladocerans, and copepods that are likely to occur in freshwater habitats (0-2 ppt). Animals from habitats with higher average salinities (2-5 and 5-10 ppt) exhibited greater resistance to treatments of full-strength seawater. Similar conclusions were drawn from our previous ballast water exchange experiments conducted in the Chesapeake Bay and San Francisco Bay.

Invertebrate species we identified as salinity-tolerant include mysid shrimps, amphipods, isopods, harpacticoid copepods, bivalve veligers, and decapod zoea. These taxonomic groups often experience dramatic fluctuations in salinity and temperature as part of their normal life histories, which has contributed to their ability to invade estuarine habitats. However, of these salinity-tolerant estuarine species, only a subset is also capable of surviving and reproducing in a constant freshwater habitat such as the Great Lakes. Identifying species and populations with these characteristics from the port systems of the east coast of the U.S. and Canada, North Sea, and Baltic Sea is paramount for preventing problematic species from invading the Great Lakes region via commercial ships. It should be emphasized, however, that these results only address live organisms and that salinity toxicity on resting stages is much more variable. Some experimental evidence suggests that saltwater exposure is unlikely to significantly reduce the risk from resting stages as a potential source of propagules.

The results of our previous NOBOB study and this study strongly supports the implementation of new Canadian Ballast Management Regulations adopted in 2006 and the Policy Statement issued by The United States Coast Guard in 2005 requiring/recommending mid-ocean tank flushing. In order to reduce ANS risk, vessels operating outside the Great Lakes should conduct saltwater flushing of their empty (NOBOB) ballast tanks prior to each entry and as soon as possible after any subsequent ballast operations within the Lakes. This recommendation would apply to both foreign vessels and U.S. coastal trade vessels that may operate from other fresh or brackish water ports within US waters. Flushing is accomplished by allowing a limited amount of seawater to slosh around in an individual ballast tank as a result of the ship's rolling and pitching motion during passage, and is then discharged in the open ocean. This procedure loosens and resuspends trapped sediments and subjects biota to seawater salinity exposure.

Our analysis of BMPs as a management tool to reduce the ANS risk on commercial vessels suggests that this approach is unlikely to provide a reliable, consistent protection against nonindigenous species introductions, but could result in some decrease in overall risk. Many of the recommendations put forth in Item 6 of the Code of Best Management Practices require information on local water quality conditions that is not generally available to the shipping industry, or are often not practical to conduct due to cargo loading and unloading requirements. However, we recognize that consistent application of ballast management practices can help to reduce sediment accumulations and associated populations of organisms and resting eggs.

Therefore, while BMPs, if consistently and repeatedly applied, can reduce the risk of introductions from NOBOB vessels by minimizing the amount of sediment and associated organisms that are transported within ballast tanks, the practical realities and limitations associated with vessel operations makes the existing BMPs inadequate as the lone strategy for reducing the risk of nonindigenous species introductions from NOBOB vessels. The designation and routine use of saltwater flushing as an official BMP would greatly improve the protection framework for the Great Lakes, <u>if aggressively implemented</u> by the shipping industry.

Lastly, we acknowledge the cooperation of the industry to support this research, although constraints on our experimental design due to operational logistics did not allow us to conduct a rigorous scientific evaluation as to the effectiveness of BMPs.

APPENDIX 1

The Shipping Federation of Canada
Code of Best Practices for Ballast Water Management
September 28, 2000

Contributing PI:
Dr. David Reid, NOAA Great Lakes Environmental Research Lab

The Shipping Federation of Canada
Code of Best Practices for Ballast Water Management
September 28, 2000

RECOGNIZING that discharge of ballast water from ships is viewed as a principle vector for the introduction and spread of harmful aquatic organisms and pathogens,

RECOGNIZING the role ship-owners and vessel operators can play in minimizing the introduction and spread of non-indigenous organisms and protecting the Great Lakes waters,

CONSIDERING the current status of technology for the treatment of ballast water and the need to develop standards against which to measure efficiency of management procedures;

VESSELS entering into the Great Lakes commit to the following Code of Best Practices For Ballast Water Management.

> 1. to conduct ballast water management whenever practical and at every opportunity even if the vessel is not bound for a port where such a procedure may be required. This process will ensure that residual ballast on board will, to the greatest extent possible, be subjected to these practices. This process will also aid to minimize sediment accumulations in ballast tanks, and there mid-ocean exchange is practiced, subject fresh-water organisms to an extended exposure to salt water.
>
> Where mid-ocean ballast water exchange is the, or one of the management practices used as required by IMO, USCG, Canadian or other regulations, the safety of the ship shall be a top priority and management shall be practiced according to recognized safe practices.
>
> 2.to regular inspection of ballast tanks and removal of sediment, if necessary, to at least the level comparable to that required by the vessel’s Classification Society in order to conduct a "close-up" Enhanced Survey, Ballast Tank Structural and Coating Inspection.
>
> 3. to ballast water exchange procedures as provided for in US legislation and approved and enforced through United States Coast Guard Regulations.
>
> 4. to record keeping and reporting according to United States Coast Guard Regulations (ballast water report forms) – the master to record all uptake and discharge of ballast water in an appropriate log book; Ballast Water Report Forms to be completed and submitted as per Regulations; inspection and cleaning of ballast tanks to be recorded and records to be made available to inspectors upon request.
>
> 5. to provide information and logs to authorized inspectors and regulators for the purposes of verifying the vessel’s compliance with this Code of Best Practices.
>
> 6. to apply a precautionary approach in the uptake of ballast water by minimizing ballasting operations under the following conditions:
>
> > a. In areas identified in connection with toxic algal blooms, outbreaks of known populations of harmful aquatic organisms and pathogens, sewage outfalls and dredging activity.
> >
> > b. In darkness, when bottom dwelling organisms may rise in the water column.
> >
> > c. In very shallow water.
> >
> > d. Where a ship’s propellers may stir up sediment.
> >
> > e. In areas with naturally high levels of suspended sediments, e.g. river mouths, and delta areas, or in locations that have been affected significantly by soil erosion from inland drainage.

    f. In areas where harmful aquatic organisms or pathogens are known to occur.

7. to the disposal of accumulated sediments as provided for in the existing IMO Ballast Water Protocols during ocean passages outside International Ballast Water Management Areas or as otherwise approved by Port State Authorities.

8. to foster and support scientific research sampling programs and analysis – Facilitate access to on board sampling and testing of ballast water and sediment including opening of ballast tank covers and safe access to ballast tanks following safety procedures for entering enclosed spaces. Sampling, testing and inspection to be planned and coordinated to fit within vessels' operational program and minimize any delays.

9. to cooperate and participate in standards development and treatment systems testing and approval processes, including, but not limited to mechanical management and treatment systems, and pesticide management systems as well as improved techniques for ballast water exchange and their scientific assessment.

10. to strive toward global, integrated ballast water management strategies in conformity with internationally agreed principles that respect national and regional aquatic ecosystems.

This Code of Best Practices is endorsed by the undersigned and represents our common goal to attain the highest standards of safe ballast water management to minimize the introduction and spread of aquatic nuisance species in the Great Lakes.

Shipping Federation of Canada, September 28, 2000

Identifying, Verifying, and Establishing Options for Best Management Practices for NOBOB Vessels

APPENDIX 2

Summary of Ballast History Activities for the MV Irma and MV Lady Hamilton for the 2005 -2006 field years of study

Contributing PIs:
Dr. Thomas Johengen – University of Michigan
Philip Jenkins – Jenkins and Associates, Ltd.

IRMA Ballast History Summary (Tank 5S)

Sonde Data				Ship Ballast History		
Date	Time	Depth	Conductivity	Date	Port	Ballast Activity
7/29/04	Instruments Started			29-Jul	Cleveland, US	
8/2/04	10:31	33.4	1			
8/2/04	18:01	51.0	289	2-Aug	Burns Harbor	Ballast FW
8/3/04	9:01	51.0	294			
8/3/04	10:01	82.7	405		Burns Harbor	Ballast FW
8/5/04	15:31	82.4	340			
8/5/04	17:01	33.7	332 / 1	6-Aug	Thunder Bay	Deballast
8/26/04	7:31	34.2	0			
8/26/04	9:01	74.9	56040	26-Aug	Marseille, France	Ballast SW
8/26/04	10:01	47.8	56050		Adjusting trim at port	
8/31/04	5:31	52.0	56220			
8/31/04	8:01	33.2	56190 / 30	31-Aug	Manfredonia, Italy	Deballast
9/8/04	7:01	34.4	11			
9/8/04	10:31	80.4	57050	8-Sep	Manfredonia, Italy	Ballast SW
9/10/04	22:01	84.5	57050			
9/10/04	23:01	40.2	57050	12-Sep	Mid Ocean	SW Exchange
9/11/04	0:31	86.6	58110			
9/13/04	20:01	87.0	57720			
9/13/04	22:31	34.2	217	14-Sep	Ashdod, Israel	Deballast
10/6/04	2:01	34.1	63			
10/6/04	2:31	33.8	53930	6-Oct	Mid-ocean	SW Flushing
10/6/04	5:31	34.2	182			
10/30/04	16:31	34.2	62			
10/30/04	18:31	83.6	54830	30-Oct	Aratu, Brazil	Ballast SW
11/6/04	21:01	82.3	55290			
11/7/04	0:01	33.2	55280 / 87	6-Nov	Maceo, Brazil	Deballast
11/30.04	End Deployment			30-Nov	Cleveland, US	

Sonde Data				Ship Ballast History		
Date	Time	Depth	SpCond	Date	Port	Ballast Activity
4/26/05	Instruments Started			26-Apr	Cleveland, US	
5/1/05	11:01	33.8	0			
5/1/05	14:01	43.3	206	1-May	Montreal, Canada	Ballast
5/3/05	3:31	43.5	216			

5/3/05	4:31	61.8	39620	no record bu	Gulf of St. Lawrence	Ballast BrW (top-up)
5/14/05	6:01	64.0	8587			
5/14/05	12:01	34.9	8555	14-May	Casablanca, Morocco	Deballast
5/20/05	20:31	34.1	8542			
5/20/05	22:01	36.4	54020	21-May	Casablanca, Morocco	Ballast SW
5/25/05	6:01	36.6	49380			
5/25/05	7:31	83.4	53950	25-May		Ballast SW (top-up)
6/6/05	18:01	83.4	53100			
6/6/05	21:31	33.5	52580 / 41	6-Jun	Vitoria, Brazil	Deballast
7/9/05	8:01	34.1	10			
7/9/05	19:31	53.1	48600	9-Jul	Acajutla, El Salvador	Ballast SW
7/10/05	13:01	52.7	48420			
7/10/05	14:01	82.8	49030	11-Jul		Ballast SW (top-up)
7/18/05	18:31	82.5	49360			
7/18/05	20:31	34.1	144	18-Jul	Port Esquivel, Jamaca	Deballast
8/4/05	6:01	33.9	61			
8/4/05	7:31	36.1	39910	4-Aug	Ardanstangel, Norway	Ballast Vary SW
8/4/05	17:01	80.3	37170		Ardanstangel, Norway	Ballast Vary SW
8/18/05	20:01:00	86.359	51410			
8/18/05	22:31:00	33.33	39270 / 60	19-Aug	Ijmuiden, Netherlands	Deballast
9/6/05		End Deployment		6-Sep	Cleveland, US	

Sonde Data				**Ship Ballast History**		
Date	**Time**	**Depth**	**SpCond**	**Date**	**Port**	**Ballast Activity**
9/6/05		Instruments Started		6-Sep	Cleveland, US	
9/10/05	11:31:00	33.668	0			
9/10/05	15:31:00	84.017	298	9-Oct	Burns Harbor	Ballast FW
9/13/05	2:16:00	83.791	408			
9/13/05	4:46:00	33.215	389	13-Sep	Duluth	Deballast
10/4/05	10:16:00	34.327	0			
10/4/05	12:31:00	36.604	55360	4-Oct	Barcelona, Spain	Ballast SW
10/5/05	8:01:00	37.28	55660			
10/6/05	10:31:00	37.638	55260			
10/6/05	11:01:00	73.599	55580	6-7 Oct	Barcelona, Spain	Ballast SW (top-up)
10/6/05	12:01:00	49.245	55340			Adjusting Stresses
10/7/05	4:31:00	82.154	55110	6-7 Oct	Barcelona, Spain	Ballast SW (top-up)
10/10/05	9:16:00	81.569	55020			
10/10/05	10:31:00	33.671	55470 / 12	10-Oct	Casablanca, Morroco	Deballast
10/19/05	9:31:00	34.308	74			
10/19/05	10:46:00	60.921	1077		Police, Poland	Ballast FW

10/21/05	12:31:00	56.272	2200			
10/21/05	13:31:00	83.836	1192	21-Oct	Police, Poland	Ballast FW
10/28/05	16:01:00	84.967	1117	Not recorded		
10/28/05	16:46:00	54.52	1118			
10/29/05	3:01:00	35.417	1111 / 3	29-Oct	Riga, Latvia	Deballast
11/5/05	11:16:00	34.047	0			
11/5/05	12:31:00	59.137	27380	5-Nov	Hull, UK	Ballast, BrW
11/6/05	4:16:00	84.206	27030		Hull, UK	Ballast, BrW
11/10/05	1:01:00	84.68	27470			
11/10/05	1:46:00	52.937	27360			
11/11/05	4:46:00	33.926	27550 / 117	12-Nov	Ijmuiden, Netherlands	Deballast
12/2/05		End Deployment		2-Dec	Cleveland, US	

Sonde Data				Ship Ballast History		
Date	**Time**	**Depth**	**SpCond**	**Date**	**Port**	**Ballast Activity**
12/2/05		Instruments Started				
12/8/05	10:31:00	0.129	0			
12/8/05	18:01:00	5.691	344	12/8/2005	Milwaukee	Ballast, FW
12/12/05	14:31:00	5.236	412	13/12/05	Burns Harbor	Deballasting
12/12/05	20:16:00	-0.009	410 / 2			
1/6/06	4:31:00	0.243	218			
1/6/06	12:31:00	15.351	2403	1/5/2006	Ghent, Belgium	Ballast, FW
1/12/06	2:46:00	15.391	50304			
1/12/06	5:31:00	6.276	49807			Deballast
1/14/06	2:16:00	6.16	49960			paused
1/14/06	3:16:00	0.445	34137 / 143	1/14/2006	Riga, Latvia	continued
1/27/06	1:46:03	0.276	318			
1/27/06	8:01:05	13.604	15977	1/25/2006	Amsterdam, Netherlands	Ballast BrW
2/1/06	6:46:00	16.02	52129			
2/1/06	7:46:00	10.629	50691			Deballast
2/2/06	13:31:00	10.556	41880			paused
2/2/06	17:31:00	0.192	39341 / 241	2/2/2006	Aheim, Norway	continued
2/7/06	2:16:00	0.194	279			
2/7/06	5:16:00	15.327	44520	2/7/2006	Ijmuiden, Netherlands	Ballast BR/SW
2/14/06	4:01:00	13.494	49413			
2/14/06	4:46:00	6.291	49275			Deballast
2/15/06	9:01:00	6.327	49495			pause
2/15/06	21:01:00	0.022	25571 / 76	2/14/2006	Gdansk, Poland	continued
3/25/06	20:30:00		27			

3/25/06	21:15:00		74024	3/20/2006	Barranquilla, Colombia	Ballast SW
3/28/06	21:00:00		60677			
3/28/06	23:15:00		239	3/28/2006	Puerto Predeco,Colombia	Deballast
4/22/06	7:45:00		246			
4/22/06	8:00:00		46030	4/22/2006	Brayton Point, USA	Ballast SW
4/26/06	20:45:00		48209			
4/27/06	0:00:00		220	4/26/2006	Albany, USA	Deballast
		End Deployment				

Sonde Data				**Ship Ballast History**		
Date	**Time**	**Depth**	**SpCond**	**Date**	**Port**	**Ballast Activity**
		Instruments Started				
4/29/2006	22:46:00	10.553	654	29-Apr	Hudson River	Ballasting
4/30/2006	0:16:00	20.019	266 / 834	30-Apr		
5/13/2006	4:01:00	19.678	407			
5/13/2006	5:31:00	10.453	406 / 1	14-May	Sfax	Deballasting
5/21/2006	10:16:00	10.362	0			
5/21/2006	17:31:00	23.198	60173	21-22 May	La Goulette	Ballasting
6/7/2006	18:31:00	26.256	60389			
6/7/2006	22:46:00	10.378	60392 / 84	22-Jun	Ashdod	Deballasting
6/22/2006	14:01:00	10.378	135			
6/22/2006	21:31:00	25.712	13233	22-Jun	Amsterdam	Ballasting
6/28/2006	2:31:00	25.794	13221			
6/28/2006	7:31:00	10.41	12015 / 29	27-Jun	Ijmuiden	Deballasting
7/3/2006	13:31:00	10.467	57156			
7/4/2006	11:16:00	10.459	388	3-4 Jul	Mid Ocean Flushing	SW Flushing
		End Deployment				

Lady Hamilton Ballast History (Tank 5S)

Sonde Data				Ship Ballast Logs			
Date	Time	Depth	SpCond	Date	Local Time	Port/ Country	Ballast Activity
6/3/05	10:00	Instrument Started		3-Jun		Hamilton, Canada	
6/14/05	16:46	33.7	212				
6/14/05	18:16	73.4	4190	Not recorded		Burns Harbor, US	Ballast FW
6/17/05	7:46	74.8	4436				
6/17/05	9:31	33.5	854	17-Jun	1030 - 1145	Goderich, Canada	Deballast (900T)
6/20/05	8:16	33.42	808				No ballasting
6/20/05	8:31	33.43	3	20-Jun	0900 - 1830	Thunder Bay, Canada	Changing Trim
7/12/05	14:46	34.6	4				
7/12/05	15:46	77.2	29914	12-Jul	1100 - 1130	Tillbury, UK	Ballast BrW (530T)
				NB: very polluted water, lead to atmosphere problems in July entry			
				NB: Depth declines slowly over next two days to 40, suspect leaking pipes			
7/18/05	11:46	39.0	31463				
7/18/05	12:46	76.3	30336	18-Jul	1700 - 1730	Antwerp,Netherlands	Ballast BrW (450 T)
7/19/05	4:46	74.6	31129				
7/19/05	5:01	50.0	30670	19-Jul	0800 - 0900	Antwerp,Netherlands	Deballast
7/19/05	7:01	49.4	30904				
7/19/05	23:46	70.7	30914	Not recorded		Antwerp,Netherlands	Adjusting trim for stress
7/20/05	3:31	33.9	23917				
8/17/05		Terminated		17-Aug		Cleveland, US	

Sonde Data				Ship Ballast Logs			
Date	Time	Depth	SpCond	Date	Local Time	Port/ Country	Ballast Activity
8/17/05	11:00	Instruments Redeployed		17-Aug		Cleveland, US	
NB: Conductivity sensor calibrated incorrectly for this deployment - note relavtive changes only							
8/17/05	21:16	0.0	0				
8/17/05	23:16	22.4	4	Not recorded		Cleveland, US	Ballast FW
8/18/05	14:16	3.8	6				
8/18/05	14:31	39.3	4	19-Aug	1300 - 1430	underway to Detroit	Ballast FW (850T)
				NB: Time mismatch with sensor data			
8/18/05	19:46	30.5	9				
8/19/05	0:46	8.1	8	Not recorded		Detroit, US	Adjusting trim
8/20/05	12:46	0.1	6				
8/20/05	15:01	45.5	2	20-Aug	1300 - 1400	Detroit, US	Ballast FW
8/23/05	17:01	44.4	2				
8/23/05	21:01	0.5	3	23-Aug	2000 - 2130	Thunder Bay, Canada	Deballast
9/27/05	3:16	1.0	450				
9/27/05	4:31	47.6	452	27-Sep	1600 - 1640	Tarragona, Spain	Ballast SW (830T)
9/27/05	23:31	47.2	452				
9/28/05	7:16	48.5	452	28-Sep	0830 - 0910	Tarragona, Spain	Ballast SW (550T)
10/8/05	3:46	50.1	514				
10/8/05	5:31	3.3	514	8-Oct	0825 - 1030	Mid-ocean	Exchange SW
10/8/05	6:46	50.2	483				
10/14/05	9:16	46.8	442				
10/14/05	10:01	5.2	463	14-Oct		Vitoria, Brazil	Deballast topside (365T)
10/14/06	15:16	5.3 - 45.2		Not recorded		Vitoria, Brazil	Adjusting ballast-heavy weather
10/16/05	1:31	46.1 - 0.9		Not recorded		Vitoria, Brazil	Adjusting ballast-heavy weather
10/16/05	3:01	0.9	440				
10/16/05	7:01	43.3	444	16-Oct	1030 - 1050	Vitoria, Brazil	Ballast SW (365T)
10/18/05	23:31	39.6	448				
10/19/05	1:01	10.2	449	19-Oct	2000 - 2055	Vitoria, Brazil	Deballast
10/19/05	18:01	0.8	455				(in two steps)
				25-Oct		On voyage to Canada	
10/29/05	15:16	1.1	490			Mid-ocean	SW Flushing (Add 93T)
10/29/05	15:46	0.6	4	29-Oct			
11/10/05		End Deployment				Hamilton, Canada	

Identifying, Verifying, and Establishing Options for Best Management Practices for NOBOB Vessels

APPENDIX 3

Summary of microbial data from NOBOB ballast residuals.

Contributing PI:
Dr. Fred Dobbs, Old Dominion University

Note: The label ND designates samples for which ‘no data’ were collected. LH denotes the ship Lady Hamilton. Sediment and porewater samples are indicated in the Sample ID by the suffix ‘ws’. Residual-water samples are indicated by ‘wa’.

	Sample ID	Ship	Ballast Tank	Sampling date	Date Filtered	Salinity	Putative *V. cholerae* (cfu/L) rep1	Putative *V. cholerae* (cfu/L) rep2	*P. piscicida* (rep1/rep2)	*P. shumwayae* (rep1/rep2)	*E. coli*	*Enterococci*	*C. parvum* (rep1/rep2)	*G. lamblia* (rep1/rep2)	*E. bieneusi* (rep1/rep2)	*E. intestinalis* (rep1/rep2)	*E. cuniculi* (rep1/rep2)	*E. hellem* (rep1/rep2)
1	B-04211-01wa	Irma	5S	29-Jul-04	3-Aug-04	2.3	2300	3800	-/-	-/-	-	+	-/-	-/-	-/-	-/-	-/-	-/-
	B-04211-01ws	Irma	5S	29-Jul-04	3-Aug-04	ND	0	ND	-	-	ND	ND	-	-	-	-	-	-
	B-04211-02wa	Irma	4S	29-Jul-04	3-Aug-04	28	13300	7200	-/-	-/-	-	+	+/+	-/-	+/+	-/-	-/-	-/-
	B-04211-02ws	Irma	4S	29-Jul-04	3-Aug-04	ND	1350	ND	+	-	ND	ND	+	+	-	-	-	-
2	B-04334-01wa	Irma	4S	29-Nov-04	1-Dec-04	14	5000	14000	-/-	+/-	+	+	-/-	-/-	-/-	-/-	-/-	+/+
	B-04334-01ws	Irma	4S	29-Nov-04	1-Dec-04	31	13800	4300	-	-	ND	ND	+	-	-	-	-	-
	B-04334-02wa	Irma	5S	29-Nov-04	1-Dec-04	27	10000	6650	-/-	-/+	-	-	-/-	+/+	-/-	-/-	-/-	-/-
	B-04334-02ws	Irma	5S	29-Nov-04	1-Dec-04	42	8500	6200	-	-	ND	ND	-	-	-	-	-	-
3	B-05116-01wa	Irma	4S	26-Apr-05	28-Apr-05	1	170	30	-	+	-	+	-/-	-/-	+/+	-/-	-/-	-/-
	B-05116-01ws	Irma	4S	26-Apr-05	28-Apr-05	1	600	500	-	-	ND	ND	-	+	-	-	-	-
	B-05116-02wa	Irma	5S	26-Apr-05	28-Apr-05	7	0	40	-	-	-	+	+/+	+/+	-/-	-/-	-/-	-/-
	B-05116-02ws	Irma	5S	26-Apr-05	28-Apr-05	5	0	0	-	-	ND	ND	-	-	-	-	-	-
4	B-05230-03wa	LH	3S	17-Aug-05	18-Aug-05	15	13000	13800	-/-	-/-	ND	ND	-/-	-/-	-/-	-/-	-/-	-/-
	B-05230-03ws	LH	3S	17-Aug-05	18-Aug-05	ND	ND	ND	ND	ND	ND	ND	-	-	-	-	-	-
	B-05230-05wa	LH	5S	17-Aug-05	18-Aug-05	24	7045	0	+/-	+/-	ND	ND	+/+	-/-	-/-	-/-	-/-	-/-
	B-05230-05ws	LH	5S	17-Aug-05	18-Aug-05	ND	18100	11500	ND	ND	ND	ND	-	-	-	-	-	-
5	B-05246-01wa	LH	5S	3-Sep-05	8-Sep-05	3	340	0	+/-	+/+	ND	ND	-/-	-/-	+/+	+/+	-/-	+/+
	B-05246-01ws	LH	5S	3-Sep-05	8-Sep-05	ND	4100	ND	-	-	ND	ND	+	+	-	-	-	-
	B-05246-02wa	LH	3S	3-Sep-05	8-Sep-05	2	0	30	+/+	+/+	ND	ND	+/+	-/-	-/-	-/-	-/-	-/-
	B-05246-02ws	LH	3S	3-Sep-05	8-Sep-05	ND	100	0	-	-	ND	ND	-	+	-	-	-	-
6	B-05249-01wa	Irma	5S	6-Sep-05	8-Sep-05	30	3300	1000	-/-	-/-	ND	ND	-/-	+/+	-/-	-/-	-/-	-/-
	B-05249-01ws	Irma	5S	6-Sep-05	8-Sep-05	ND	4300	ND	-	-	ND	ND	-	+	-	-	-	-
	B-05249-02wa	Irma	4S	6-Sep-05	8-Sep-05	10	700	175	0	+/+	ND	ND	-/-	-/-	-/-	-/-	-/-	-/-
	B-05249-02ws	Irma	4S	6-Sep-05	8-Sep-05	ND	29700	ND	-	-	ND	ND	-	-	-	-	-	-
7	B-05314-01-wa	LH	3S	10-Nov-05	15-Nov-05	38	170	115	-/+	+/+	-	+	-/-	-/-	-/-	-/-	-/-	-/-
	B-05314-01-ws	LH	3S	10-Nov-05	15-Nov-05	ND	ND	ND	0	-	ND	ND	-	-	-	-	-	-
	B-05314-02-wa	LH	5S	10-Nov-05	15-Nov-05	40	690	385	+/-	+/+	-	+	+/+	-/-	-/-	-/-	-/-	+/+
	B-05314-02-ws	LH	5S	10-Nov-05	15-Nov-05	37	0	0	-	-	ND	ND	-	-	-	-	-	-
8	B-05336-01-wa	Irma	5S	2-Dec-05	6-Dec-05	19	2600	12850	-/-	-/-	+	+	-/-	-/-	-/-	-/-	-/-	-/-
	B-05336-01-ws	Irma	5S	2-Dec-05	6-Dec-05	19	2400	ND	pending	pending	ND	ND	-	+	-	-	-	-
	B-05336-02-wa	Irma	4S	2-Dec-05	6-Dec-05	19	5750	7700	-/-	-/-	+	-	-/-	-/-	-/-	-/-	-/-	-/-
	B-05336-02-ws	Irma	4S	2-Dec-05	6-Dec-05	15	3300	ND	pending	pending	ND	ND	-	+	-	-	-	-
9	B-06118-01-wa	Irma	4S	28-Apr-06	3-May-06	32.5	21000	15200	-/-	-/-	+	+	-/-	-/-	-/-	-/-	-/-	-/-
	B-06118-01-ws	Irma	4S	28-Apr-06	3-May-06	34	18000	29000	pending	pending	ND	ND	-	+	-	-	-	-
	B-06118-02-wa	Irma	5S	28-Apr-06	3-May-06	34.5	700	975	-/-	-/-	+	+	-/-	-/-	-/-	-/-	-/-	-/-
	B-06118-02-ws	Irma	5S	28-Apr-06	3-May-06	34	96000	38000	pending	pending	ND	ND	-	+	-	-	-	-
10	B-06195-01wa	Irma	5S	14-Jul-06	18-Jul-06	37	0	16000	-/-	-/-	+	+	+/+	-/-	+/+	-/-	-/-	+/+
	B-06195-01ws	Irma	5S	14-Jul-06	14-Jul-06	28	0	8000	pending	pending	ND	ND	-	+	-	-	-	-
	B-06195-02ws	Irma	4S	14-Jul-06	14-Jul-06	30	33200	65200	pending	pending	ND	ND	-	+	-	-	-	-

Identifying, Verifying, and Establishing Options for Best Management Practices for NOBOB Vessels

APPENDIX 4

A synthesis of literature-based information on the species composition, life-history characteristics, and reported environmental tolerances of zooplankton and invertebrates from northern European source ports.

Contributing PIs:

Dr. Greg Ruiz and Dr. Scott Santagata

Smithsonian Environmental Research Laboratory

A synthesis of literature-based information on the species composition, life-history characteristics, and reported environmental tolerances of zooplankton and invertebrates from northern European source ports.

One of our long-term goals is to track the source populations for species most likely to invade the Great Lakes and other low-salinity ports of the United States. For these reasons, we compiled a list of benthic and planktonic organisms from major ports in the North Sea and Baltic Sea that may best tolerate the environmental conditions of the Great Lakes (see Table 3).

Table 1: Species reported to occur in major ports of the North Sea and Baltic Sea. Some species from the Black-Azov Seas are included because of their potential overlap in the North Sea and Baltic Sea (Bij de Vaate et al., 2002; Grigorovich et al., 2005). Species that are considered indigenous (I) to the Great Lakes are in blue text, species considered exotic (E) to the Great Lakes are in red text, and species that could potentially be introduced (P) to the Great Lakes are in black text. Occurrence in a port region(s) does not necessarily indicate the source area for a particular exotic species to the Great Lakes Region. Observations for the salinity tolerance of particular species are listed where available. Port of Rotterdam (R), Antwerp and Ghent (A&G), Brake, Bremen, and Hamburg (B, B, &H), Kokta and Tallinn (K&T), St. Petersburg (St.P.), Klaipeda and the Gulf of Gdansk (K&GG), Stettin (S), and the Black and Azov Seas (B&AZ). ●= Present; **?**= Potential site

Taxonomy	**Species**	**I E P**	**R**	**A & G**	**B B & H**	**K & T**	**St P**	**K & G G**	**S**	**B & A Z**	**Salinity Tolerance and References**
Annelida-Oligochaeta	*Isochaetides michaelseni*	**P**					**?**			●	0.5-5[47]
Annelida-Oligochaeta	*Limnodrilus claparedeanus*	**P**		●	●					●	0.2-3[47, 67]
Annelida-Oligochaeta	*Limnodrilus hoffmeisteri*	**P**		●	●						[66, 67]
Annelida-Oligochaeta	*Marionina brevis*	**P**					**?**			●	0.5-5[47]
Annelida-Oligochaeta	*Marionina mica*	**P**					**?**			●	0.5-5[47]
Annelida-Oligochaeta	*Paranais frici*	**I**					●				0-30[21, 54]
Annelida-Oligochaeta	*Paranais littoralis*	**I**		●	●						[65, 67]
Annelida-Oligochaeta	*Potamothrix bedoti*	**I**				**?**	**?**			●	[23]
Annelida-Oligochaeta	*Potamothrix caspicus*	**P**					**?**			●	0.5-5[47]
Annelida-Oligochaeta	*Potamothrix hammoniensis*	**P**			●					●	0.5-5[47]
Annelida-Oligochaeta	*Potamothrix heukeri*	**P**					●				[54]
Annelida-Oligochaeta	*Potamothrix heuscheri*	**P**				**?**	**?**			●	[23]
Annelida-Oligochaeta	*Potamothrix moldaviensis*	**I**			●						0.3-3[47]

Taxonomy	**Species**	**I E P**	**R**	**A & G**	**B B & H**	**K & T**	**St P**	**K & G G**	**S**	**B & A Z**	**Salinity Tolerance and References**
Annelida-Oligochaeta	*Potamothrix vejdovskyi*	**I**					●				[54]
Annelida-Oligochaeta	*Psammoryctides barbatus*	**P**		**?**						●	0.5-5[47, 67]
Annelida-Oligochaeta	*Tubifex costatus*	**P**			●						[66]
Annelida-Oligochaeta	*Tubifex tubifex*	**P**		●	●						[66, 67]
Annelida-Oligochaeta	*Tubificoides pseudogaster*	**P**					●				[55]
Annelida-Polychaeta	*Hypania invalida*	**P**					●	●		●	0-2.8[49, 75]
Cnidaria-Hydrozoa	*Cordylophora caspia*	**E**			●		●	●	●		[13, 25]
Copepoda-Calanoida	*Arcartia bifilosa*	**P**	●	●	●	●	●	●	●		3 - 36[13, 20, 39]
Copepoda-Calanoida	*Arcartia clausii*	**P**						●			[58]
Copepoda-Calanoida	*Arcartia discaudata*	**P**	●								[226]
Copepoda-Calanoida	*Arcartia longiremis*	**P**						●	●		4.5-7.5 [13, 20]
Copepoda-Calanoida	*Arcartia tonsa*	**P**		●			●		●		0.7-33[13, 25, 38, 39, 70]
Copepoda-Calanoida	*Calanipeda aquae-dulcis*	**P**					**?**			●	[76]
Copepoda-Calanoida	*Centropages hamatus*	**P**		●	●			●	●		4.5-7.5[13, 25, 38, 39, 70]
Copepoda-Calanoida	*Eudiaptomus gracillis*	**P**						●			[58]
Copepoda-Calanoida	*Eudiaptomus graciloides*	**P**			●			●	●		0 - 7[13, 20]
Copepoda-Calanoida	*Eurytemora affinis (hirundoides)*	**E**	●	●	●	●	●	●	●	●	0 - 35+[13, 20, 40, 76]
Copepoda-Calanoida	*Eurytemora lacustris*	**I**	●								[76]
Copepoda-Calanoida	*Eurytemora velox*	**P**		●	●			●			[37]
Copepoda-Calanoida	*Heterocope appendiculata*	**P**					**?**			●	[71]
Copepoda-Calanoida	*Heterocope caspia*	**P**					**?**			●	[23]
Copepoda-Calanoida	*Pseudocalanus minutus*	**P**						●	●		4-7.5[13, 20]
Copepoda-Calanoida	*Temora longicornis*	**P**		●				●	●		4-7.5[13, 20, 39]
Copepoda-Cyclopoida	*Acanthocyclops robustus*	**I**	●	●	●				●		1-5[13, 70, 76]
Copepoda-Cyclopoida	*Acanthocyclops vernalis*	**P**			●						[37]
Copepoda-Cyclopoida	*Acanthocyclops viridis*	**P**						●			0-7[20, 70]
Copepoda-Cyclopoida	*Cyclops furcifer*	**P**			●						[37]

Taxonomy	Species	I E P	R	A & G	B B & H	K & T	St P	K & G G	S	B & A Z	Salinity Tolerance and References
Copepoda-Cyclopoida	*Cyclops kolensis*	**P**					**?**			●	^{23}
Copepoda-Cyclopoida	*Cyclops strenuus*	**I**		●				●			0-5$^{9, 20, 68, 70}$
Copepoda-Cyclopoida	*Cyclops vicinus*	**P**		●	●			●	●		^{13}
Copepoda-Cyclopoida	*Diacyclops bicuspidatus*	**P**		●	●				●		$^{13, 37}$
Copepoda-Cyclopoida	*Diacyclops bisetosus*	**P**			●						^{37}
Copepoda-Cyclopoida	*Diacyclops languidus*	**P**						●			^{58}
Copepoda-Cyclopoida	*Eucyclops macruroides*	**P**						●			^{58}
Copepoda-Cyclopoida	*Eucyclops serrulatus*	**P**		●	●			●	●		0-6$^{20, 37, 76}$
Copepoda-Cyclopoida	*Macrocyclops albidus*	**I**			●			●			$^{37, 58}$
Copepoda-Cyclopoida	*Macrocyclops fucus*	**I**						●			^{58}
Copepoda-Cyclopoida	*Megacyclops viridis*	**E**			●			●			0-?^{58}
Copepoda-Cyclopoida	*Merocyclops albidus*	**P**							●		^{13}
Copepoda-Cyclopoida	*Mesocyclops leuckarti*	**P**		●				●	●		0.7-10$^{13, 20}$
Copepoda-Cyclopoida	*Metacyclops gracilis*	**P**		●							^{70}
Copepoda-Cyclopoida	*Metacyclops problematicus*	**P**		●							^{70}
Copepoda-Cyclopoida	*Oithona* sp.	**P**	●								^{76}
Copepoda-Cyclopoida	*Paracyclops fimbriatus*	**P**						●			^{58}
Copepoda-Cyclopoida	*Paracyclops poppei*	**I**		●							^{70}
Copepoda-Cyclopoida	*Thermocyclops crassus*	**I**	●	●				●	●		0-0.7^{76}
Copepoda-Cyclopoida	*Thermocyclops dybowskii*	**P**	●					●			0-14$^{13, 70}$
Copepoda-Cyclopoida	*Thermocyclops oithonoides*	**P**		●				●	●		0-0.7$^{13, 58, 70, 76}$
Copepoda-Cyclopoida	*Tropocyclops prasinus*	**I**						●			^{58}
Copepoda-Harpacticoida	*Canthocamptus staphylinus*	**P**		●	●						$^{37, 76}$
Copepoda-Harpacticoida	*Ectinosoma abrau*	**P**					**?**			●	^{23}
Copepoda-Harpacticoida	*Ectinosoma edwardsi*	**P**						●			^{58}
Copepoda-Harpacticoida	*Heteropsyllus* nr. *nunni*	**E**					**?**			●	0-30$^{27, 78}$
Copepoda-Harpacticoida	*Ilocryptus agilis*	**P**		●							^{70}

Taxonomy	Species	IEP	R	A&G	BB&H	K&T	StP	K&GG	S	B&AZ	Salinity Tolerance and References
Copepoda-Harpacticoida	*Laophonte sp.*	**P**	●								[76]
Copepoda-Harpacticoida	*Mesochra lilljeborgi*	**P**			●						[37]
Copepoda-Harpacticoida	*Microarthridion littorale*	**P**		●							[70]
Copepoda-Harpacticoida	*Nitocra hibernica*	**E**						●			[58]
Copepoda-Harpacticoida	*Nitocra incerta*	**E**					**?**			●	[27]
Copepoda-Harpacticoida	*Nitocra lacustris*	**P**		●							10-40[62]
Copepoda-Harpacticoida	*Nitocra palustris*	**P**						●			[58]
Copepoda-Harpacticoida	*Onychocamptus mohammed*	**I**						●			[58]
Copepoda-Harpacticoida	*Paraleptastacus spinicaudata triseta*	**P**					**?**			●	[23]
Copepoda-Harpacticoida	*Psuedobradya sp.*	**P**		●							[70]
Copepoda-Harpacticoida	*Schizopera borutzkyi*	**E**								●	0 - 6[27, 44]
Copepoda-Harpacticoida	*Zaus sp.*	**P**	●								[76]
Crustacea-Amphipoda	*Bathyporeia elegans*	**P**		●							[81]
Crustacea-Amphipoda	*Bathyporeia pilosa*	**P**		●		●		●			[36, 60]
Crustacea-Amphipoda	*Chelicorophium curvispinum*	**P**			●			●		●	0 - 18[70]
Crustacea-Amphipoda	*Corophium curvispinum*	**P**						●	●	●	0-10[13, 25, 53, 76]
Crustacea-Amphipoda	*Corophium insidiosum*	**P**		●							[81]
Crustacea-Amphipoda	*Apocorophium lacustre*	**P**		●	●						0-16[6, 10, 15]
Crustacea-Amphipoda	*Corophium multisetosum*	**P**						●			[50]
Crustacea-Amphipoda	*Corophium volutator*	**P**		●	●	●		●			0.2-35[81]
Crustacea-Amphipoda	*Dikerogammarus haemobaphes*	**P**								●	0 - 16[24, 28, 81]
Crustacea-Amphipoda	*Dikerogammarus villosus*	**P**						●		●	0 - 20[11]
Crustacea-Amphipoda	*Echinogammarus berilloni*	**P**			●					●	0-35[8, 28, 81]
Crustacea-Amphipoda	*Echinogammarus ischnus*	**E**						●			0 - 18[16, 37, 53, 79]
Crustacea-Amphipoda	*Echinogammarus trichiatus*	**P**		●						●	[8, 16, 33, 15]
Crustacea-Amphipoda	*Echinogammarus (Chaetogammarus) warpachowskyi*	**P**						●		●	0- 17[53]
Crustacea-Amphipoda	*Gammarus duebeni*	**P**			●		●				0-85[37]

Taxonomy	Species	I E P	R	A & G	B B & H	K & T	St P	K & G G	S	B & A Z	Salinity Tolerance and References
Crustacea-Amphipoda	*Gammarus fossarum*	**P**		**?**							0-5[17, 57, 77]
Crustacea-Amphipoda	*Gammarus inaequicauda*	**P**				●			●		6.5-35[29, 30, 32, 73]
Crustacea-Amphipoda	*Gammarus lacustris*	**I**					●				0-5[17, 57, 77]
Crustacea-Amphipoda	*Gammarus locusta*	**P**						●			4-35[29, 30, 32]
Crustacea-Amphipoda	*Gammarus oceanicus*	**P**						●			1-41[48]
Crustacea-Amphipoda	*Gammarus pulex*	**P**			●			●			0-11[32, 56, 69, 77]
Crustacea-Amphipoda	*Gammarus roeselii*	**P**		**?**							0-9[17, 31, 77]
Crustacea-Amphipoda	*Gammarus salinus*	**P**		●				●			1-35[81]
Crustacea-Amphipoda	*Gammarus tigrinus*	**E**	●	●	●	●		●	●		0-34+[13, 25, 53]
Crustacea-Amphipoda	*Gammarus varsoviensis*	**P**							**?**		0-[31]
Crustacea-Amphipoda	*Gammarus zaddachi*	**P**		●	●			●			0-35[32, 37]
Crustacea-Amphipoda	*Gmelinoides fasciatus*	**P**					●	●		●	0-8[7, 8, 15, 53]
Crustacea-Amphipoda	*Iphigenella shablensis*	**P**					**?**			●	0-5[16, 23]
Crustacea-Amphipoda	*Monoporeia affinis*	**P**				●	●				0-20[11]
Crustacea-Amphipoda	*Obessogammarus crassus*	**P**						●		●	0-18[4, 53]
Crustacea-Amphipoda	*Pontogammarus maeoticus*	**P**								●	0-18[8, 16]
Crustacea-Amphipoda	*Pontogammarus obesus*	**P**					**?**			●	0-5[16, 23, 32]
Crustacea-Amphipoda	*Pontogammarus robustoides*	**P**					●	●	●	●	0 -18[13, 53]
Crustacea-Amphipoda	*Pontogammarus subnudus*	**P**					**?**			●	0-5[16, 23, 33]
Crustacea-Cladocera-Anomopoda-Bosminidae	*Bosmina longirostris*	**I**			●			●	●		0-10[13, 70, 76]
Crustacea-Cladocera-Anomopoda-Bosminidae	*Bosmina obtusirostris*	**P**					●			●	0-10[19, 33]
Crustacea-Cladocera-Anomopoda-Bosminidae	*Eubosmina coregoni*	**E**			●			●	●		0-8[19, 58]
Crustacea-Cladocera-Anomopoda-Bosminidae	*Eubosmina maritima*	**E**						●	●		0 - 12.5[19, 37, 58]
Crustacea-Cladocera-Anomopoda-Chydoridae	*Acroperus harpae*	**I**						●			<0.5[19.58]
Crustacea-Cladocera-Anomopoda-Chydoridae	*Alona affinis*	**I**			●			●			1-3[19, 37, 58]

Taxonomy	Species	I E P	R	A & G	B B & H	K & T	St P	K & G	S	B & A Z	Salinity Tolerance and References
Crustacea-Cladocera-Anomopoda-Chydoridae	*Alona quadrangularis*	**I**						●			^{58}
Crustacea-Cladocera-Anomopoda-Chydoridae	*Alonella excisa*	**I**			●			●			$^{37, 58}$
Crustacea-Cladocera-Anomopoda-Chydoridae	*Alonopsis elongata*	**I**						●			^{58}
Crustacea-Cladocera-Anomopoda-Chydoridae	*Camptocercus rectirostris*	**I**						●			0.5 -3$^{19, 58}$
Crustacea-Cladocera-Anomopoda-Chydoridae	*Chydorus globosus*	**I**						●			^{58}
Crustacea-Cladocera-Anomopoda-Chydoridae	*Chydorus piger*	**P**						●			^{58}
Crustacea-Cladocera-Anomopoda-Chydoridae	*Chydorus sphaericus*	**I**			●			●	●		0-12$^{9, 13, 20, 19, 70}$
Crustacea-Cladocera-Anomopoda-Chydoridae	*Disparalona rostrata*	**I**			●						^{37}
Crustacea-Cladocera-Anomopoda-Chydoridae	*Eurycercus lamellatus*	**I**			●			●			0-3$^{19, 58}$
Crustacea-Cladocera-Anomopoda-Chydoridae	*Graptoleberis testudinaria*	**I**						●			0-3$^{19, 58}$
Crustacea-Cladocera-Anomopoda-Chydoridae	*Leydigia acanthocercoides*	**I**		●							0 - 6$^{9, 70}$
Crustacea-Cladocera-Anomopoda-Chydoridae	*Leydigia quadrangularis*	**I**			●						1-3$^{19, 37}$
Crustacea-Cladocera-Anomopoda-Chydoridae	*Monospilus dispar*	**I**						●			^{58}
Crustacea-Cladocera-Anomopoda-Chydoridae	*Peracantha truncata*	**I**						●			^{58}
Crustacea-Cladocera-Anomopoda-Chydoridae	*Pleuroxus trigonellus*	**I**			●			●			$^{41, 58}$
Crustacea-Cladocera-Anomopoda-Chydoridae	*Pleuroxus trigonellus*	**I**						●			^{58}
Crustacea-Cladocera-Anomopoda-Chydoridae	*Rhynchotalona falcata*	**I**						●			^{58}

Taxonomy	Species	IEP	R	A & G	BB & H	K & T	St P	K & G G	S	B & A Z	Salinity Tolerance and References
Crustacea-Cladocera-Anomopoda-Chydoridae	*Rhynchotalona rostrata*	**P**						●			[58]
Crustacea-Cladocera-Anomopoda-Daphinidae	*Ceriodaphnia pulchella*	**I**						●			[58]
Crustacea-Cladocera-Anomopoda-Daphinidae	*Ceriodaphnia quadrangula*	**I**			●			●			0-6[9, 58, 70]
Crustacea-Cladocera-Anomopoda-Daphinidae	*Ceriodaphnia recticulata*	**I**		●				●			0-16[9, 58, 70]
Crustacea-Cladocera-Anomopoda- Daphinidae	*Daphnia ambigua*	**I**	●								0-8[76]
Crustacea-Cladocera-Anomopoda-Daphinidae	*Daphnia cristata*	**P**						●		●	[58]
Crustacea-Cladocera-Anomopoda-Daphinidae	*Daphnia cucullata*	**P**						●	●		0-5[13]
Crustacea-Cladocera-Anomopoda-Daphinidae	*Daphnia galeata galeata*	**E**	●								[76]
Crustacea-Cladocera-Anomopoda-Daphinidae	*Daphnia hyalina*	**P**						●			[58]
Crustacea-Cladocera-Anomopoda-Daphinidae	*Daphnia longispina*	**P**		●				●			0 -11.9[9, 70]
Crustacea-Cladocera-Anomopoda-Daphinidae	*Daphnia magna*	**P**		●	●			●			0-12.5[9, 72]
Crustacea-Cladocera-Anomopoda-Daphinidae	*Daphnia pulex*	**I**		●	●						0-13[37, 58, 70, 74]
Crustacea-Cladocera-Anomopoda-Daphinidae	*Scapholeberis kingi*	**I**			●						[37, 41]
Crustacea-Cladocera-Anomopoda-Daphinidae	*Scapholeberis mucrunata*	**P**						●			0-10[58]
Crustacea-Cladocera-Anomopoda-Daphinidae	*Simocephalus expinosus*	**I**			●						[66]
Crustacea-Cladocera-Anomopoda-Daphinidae	*Simocephalus vetulus*	**I**		●							0-15[3, 70]
Crustacea-Cladocera-Anomopoda-Macrothricidae	*Echinisca rosea*	**I**			●						[37]
Crustacea-Cladocera-Anomopoda-Macrothricidae	*Illiocryptus sordidus*	**I**			●						[66]

Taxonomy	Species	**I E P**	**R**	**A & G**	**B B & H**	**K & T**	**St P**	**K & G G**	**S**	**B & A Z**	**Salinity Tolerance and References**
Crustacea-Cladocera-Anomopoda-Macrothricidae	*Macrothrix hirsuticornis*	**P**					●				0-39[3]
Crustacea-Cladocera-Anomopoda-Macrothricidae	*Macrothrix laticornis*	**I**			●						1-3[3, 66]
Crustacea-Cladocera-Anomopoda- Moinidae	*Moina brachiata*	**P**		●	●						0-36[9]
Crustacea-Cladocera-Anomopoda-Moinidae	*Moina macrocopa*	**I**		●							0-22.2[3, 41]
Crustacea-Cladocera-Anomopoda-Moinidae	*Moina micura*	**I**			●						[66]
Crustacea-Cladocera-Anomopoda-Moinidae	*Moina rectirostris*	**P**							●		[13]
Crustacea-Cladocera-Ctenopoa-Sididae	*Diaphanosoma brachyurum*	**I**						●			0-5[9, 20]
Crustacea-Cladocera-Ctenopoa-Sididae	*Latona setifera*	**I**						●			[58]
Crustacea-Cladocera-Ctenopoa-Sididae	*Sida crystallina*	**I**		●		●	●	●			0-12[58]
Crustacea-Cladocera-Ctenopoda-Sididae	*Penilia avirostris*	**P**	●								10 to 49[3]
Crustacea-Cladocera-Haplopoda-Leptodoridae	*Leptodora kindtii*	**I**			●		●		●		0-8[13, 20, 144]
Crustacea-Cladocera-Onchypoda-Podonidae	*Cornigerius maeoticus maeoticus*	**P**					●			●	6-12.5[64, 70]
Crustacea-Cladocera-Onchypoda-Podonidae	*Evadne anonyx*	**P**					●				1-13[4, 44, 63, 64]
Crustacea-Cladocera-Onchypoda-Podonidae	*Evadne nordmanni*	**P**						●			1-35[20, 70]
Crustacea-Cladocera-Onchypoda-Podonidae	*Pleopis polyphemoides*	**P**						●		●	0-35[3, 12, 61]
Crustacea-Cladocera-Onchypoda-Podonidae	*Podon intermedius*	**P**						●			[58]
Crustacea-Cladocera-Onchypoda-Podonidae	*Podon leuckarti*	**P**	●						●		6.1-35[3, 4]
Crustacea-Cladocera-Onchypoda-Podonidae	*Podon polyphemoides*	**P**						●			[12, 20, 53]
Crustacea-Cladocera-Onchypoda-Podonidae	*Podonevadne trigona ovum*	**P**					**?**			●	0-22[1]

Taxonomy	**Species**	**IEP**	**R**	**A&G**	**BB&H**	**K&T**	**StP**	**K&GG**	**S**	**B&AZ**	**Salinity Tolerance and References**
Crustacea-Cladocera-Onchypoda-Polyphemidae	*Polyphemus pediculus*	**I**			●						0-8[2,3,121]
Crustacea-Cladocera-Onychopoda-Cercopagidae	*Bythotrephes longimanus*	**E**	●				●				0 to 8[24]
Crustacea-Cladocera-Onychopoda-Cercopagidae	*Cercopagis pengoi*	**E**					●	●			0-18[53, 63]
Crustacea-Cumacea	*Pseudocuma cercaroides*	**P**					**?**			●	[23]
Crustacea-Cumacea	*Pterocuma pectinata*	**P**					**?**			●	[23]
Crustacea-Decapoda	*Cancer pagurus*	**P**	●								[52]
Crustacea-Decapoda	*Carcinus maenas*	**P**	●								[52]
Crustacea-Decapoda	*Crangon crangon*	**P**	●		●						[52, 66]
Crustacea-Decapoda	*Eriocheir sinensis*	**P**			●		●	●	●		[13, 25, 50]
Crustacea-Decapoda	*Hemigrapsus penicillatus*	**P**	●								[52]
Crustacea-Decapoda	*Hemigrapsus sanguineus*	**P**	●								[52]
Crustacea-Decapoda	*Hippolyte varians*	**P**	●								[52]
Crustacea-Decapoda	*Liocarcinus holcenatus*	**P**	●								[52]
Crustacea-Decapoda	*Macropodia rostrata*	**P**	●								[52]
Crustacea-Decapoda	*Palaemon elegans*	**P**	●		●						0.9-7.5[22, 52]
Crustacea-Decapoda	*Palaemon longirostris*	**P**	●		●						[22, 52]
Crustacea-Decapoda	*Palaemon longirostris*	**P**	●								[52]
Crustacea-Decapoda	*Palaemon serratus*	**P**	●								[52]
Crustacea-Decapoda	*Palaemonetes varians*	**P**			●						[37]
Crustacea-Decapoda	*Pinnotheres pisum*	**P**	●								[52]
Crustacea-Decapoda	*Processa modica modica*	**P**	●								[52]
Crustacea-Decapoda	*Rhrithropanopeus harissii*	**P**	●								[52]
Crustacea-Isopoda	*Cyathura carinata*	**P**	●					●			[22, 32]
Crustacea-Isopoda	*Jaera albifrons*	**P**				●					[11]
Crustacea-Isopoda	*Jaera ischiosetosa*	**P**					●				[54]
Crustacea-Isopoda	*Jaera istri*	**P**			●					●	0-[23, 66]

Taxonomy	Species	I E P	R	A & G	B B & H	K & T	St P	K & G G	S	B & A Z	Salinity Tolerance and References
Crustacea-Isopoda	*Jaera praehirsuta*	**P**					●				[54]
Crustacea-Isopoda	*Jaera sarsi*	**P**					●			●	[23, 54]
Crustacea-Isopoda	*Jaera syei*	**P**					●				[54]
Crustacea-Isopoda	*Proasellus coxalis*	**P**			●						[66]
Crustacea-Isopoda	*Proasellus meridianus*	**P**		●							[8]
Crustacea-Mysidacea	*Hemimysis anomala*	**P**						●		●	0 to 19[53]
Crustacea-Mysidacea	*Limnomysis benedeni*	**P**	●	●	●	●	●	●			0-23[53]
Crustacea-Mysidacea	*Mesopodopsis slabberi*	**P**	●	●							0.5-35[8, 34, 49, 70]
Crustacea-Mysidacea	*Neomysis integer*	**P**	●	●	●						0.1 to 38[18]
Crustacea-Mysidacea	*Paramysis intermedia*	**P**					**?**			●	0-12[8, 22, 35, 49]
Crustacea-Mysidacea	*Paramysis lacustris*	**P**						●		●	0 – 18[53, 51]
Crustacea-Mysidacea	*Praunus flexuosus*	**P**						●			2-35[42]
Crustacea-Mysidacea	*Praunus inermis*	**P**							●		0.7-35[29, 42]
Mollusca-Bivalvia	*Congeria leucophaeata*	**P**			●						[66]
Mollusca-Bivalvia	*Corbicula fluminea*	**E**			●						[66]
Mollusca-Bivalvia	*Corbula gibba*	**P**		●							0-16[26, 33]
Mollusca-Bivalvia	*Dreissena bugensis*	**E**					●				0 to 2[43, 80]
Mollusca-Bivalvia	*Dreissena polymorpha*	**E**					●		●		0 to 4[25, 80]
Mollusca-Bivalvia	*Hypanis colorata*	**P**					**?**			●	[23]
Mollusca-Gastropoda	*Lithoglyphus naticoides*	**P**						●		●	0-1[13, 23]
Mollusca-Gastropoda	*Potamopyrgus antipodarum*	**E**			●		●		●		0 – 30[13, 25]
Mollusca-Gastropoda	*Potamopyrgus jenkinsi*	**P**			●	●					[11, 66]
Mollusca-Gastropoda	*Theodoxus fluviatilis*	**P**				●					[11]
Mollusca-Gastropoda	*Theodoxus pallasi*	**P**						●			[8, 13, 49]
Platyzoan-Trematoda	*Apophallus muehlingi*	**P**					**?**			●	[23]
Platyzoan-Trematoda	*Nicolla skrjabini*	**P**					**?**			●	[23]
Platyzoan-Trematoda	*Rossicotrema donicum*	**P**					**?**			●	[23]

Taxonomy	Species	I E P	R	A & G	B B & H	K & T	St P	K & G G	S	B & A Z	Salinity Tolerance and References
Platyzoan-Tricladia	*Dendrocoelum romanodanubiale*	**P**	●	●							[8]
Rotifer-Asplanidae	*Asplanchna herricki*	**I**						●			[58]
Rotifer-Asplanidae	*Asplanchna priodonta*	**I**			●				●		[58]
Rotifer-Asplanidae	*Asplanchna spp.*	**I**						●			[58]
Rotifer-Brachionidae	*Brachionus angularis*	**I**			●			●	●		[58, 66]
Rotifer-Brachionidae	*Brachionus calyciflorus*	**I**			●			●	●		0-8[13, 58, 66]
Rotifer-Brachionidae	*Brachionus diversicornis*	**I**						●			[58]
Rotifer-Brachionidae	*Brachionus quadridentatus*	**I**						●			[20, 58]
Rotifer-Brachionidae	*Colurella* sp.	**P**	●								[76]
Rotifer-Brachionidae	*Epiphanes* sp.	**P**	●								[76]
Rotifer-Brachionidae	*Euchlanis dilatata*	**I**						●			[20, 58]
Rotifer-Brachionidae	*Euchlanis lucksiana*	**P**						●			[58]
Rotifer-Brachionidae	*Euchlanis* sp.	**I**	●						●		[13, 76]
Rotifer-Brachionidae	*Kellichottia longispina*	**I**						●			[20, 58]
Rotifer-Brachionidae	*Keratella cochlearis*	**I**			●			●	●		[13, 58, 76]
Rotifer-Brachionidae	*Keratella cruciformis*	**P**						●			[58]
Rotifer-Brachionidae	*Keratella quadrata*	**I**			●			●	●		1-5[13, 58]
Rotifer-Brachionidae	*Keratella* sp.	**I**						●			[20]
Rotifer-Brachionidae	*Lepadella ovalis*	**I**	●								[76]
Rotifer-Brachionidae	*Lepadella* sp.	**I**	●								[76]
Rotifer-Brachionidae	*Mytilina* sp.	**I**	●								[76]
Rotifer-Brachionidae	*Notholca acuminata*	**I**						●			[20, 58]
Rotifer-Brachionidae	*Notholca squamula*	**I**						●			[20, 58]
Rotifer-Collothecidae	*Collotheca mutabilis*	**I**						●			[20, 58]
Rotifer-Colurellidae	*Paracolurella logima*	**P**						●			[20, 58]
Rotifer-Conochilidae	*Conochilus unicornis*	**I**						●			[20, 58]

Taxonomy	Species	I E P	R	A & G	B B & H	K & T	St P	K & G G	S	B & A Z	Salinity Tolerance and References
Rotifer-Lecanidae	*Lecane bulla*	**P**	●								[76]
Rotifer-Lecanidae	*Lecane luna*	**I**						●			[58]
Rotifer-Lecanidae	*Lecane* sp.	**I**	●								[76]
Rotifer-Notommatidae	*Cephalodella catellina*	**P**						●			[58]
Rotifer-Notommatidae	*Cephalodella* sp.	**I**	●								[76]
Rotifer-Philodinidae	*Rotaria neptunia*	**I**	●								[76]
Rotifer-Synchaetidae	*Polyarthra* spp.	**I**						●	●		[13, 58]
Rotifer-Synchaetidae	*Polyarthra vulgaris*	**I**						●			[58]
Rotifer-Synchaetidae	*Synchaeta baltica*	**P**						●			8-35[16, 58]
Rotifer-Synchaetidae	*Synchaeta monopus*	**P**						●			[58]
Rotifer-Synchaetidae	*Synchaeta* spp.	**I**						●	●		[13, 58]
Rotifer-Testudinellidae	*Filinia longiseta*	**I**						●	●		[13, 58]
Rotifer-Testudinellidae	*Pompholyx* sp.	**I**	●					●	●		[13, 58]
Rotifer-Testudinellidae	*Pompholyx sulcata*	**I**						●			[58]
Rotifer-Trichoceridae	*Trichocera capucina*	**I**	●					●			[58, 76]
Rotifer-Trichoceridae	*Trichocera pusilla*	**I**						●			[58]
Rotifer-Trichoceridae	*Trichocerca* sp.	**I**						●	●		[43, 58]

1. Aladin, N. V. (1982b). Salinity adaptation and osmoregulation abilities of the Cladocera. 2. Forms from Caspian and Aral seas . Zoologicheskii Zhurnal 61, 507-514.**2.** Aladin, N. V. (1982a). Salinity adaptations and osmoregulation abilities of the Cladocera . 1. Forms from open Seas and oceans. Zoologicheskii Zhurnal 61, 341-351.**3.** Aladin, N. V. and Potts, W. T. W. (1995). The Osmoregulatory Capacity of the Cladocera. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 164, 671-683.**4.** Alimov, A. F. (1997). Monitoring the Neva bay and the eastern Gulf of Finland. Technical report, 127 pp. Zoological Institute of the Russian Academy of Sciences, St. Petersburg. **5.** Bailey, S. A. , Nandakumar, K., and Macisaac, H. J. (2006). Does Saltwater Flushing Reduce Viability of Diapausing Eggs in Ship Ballast Sediment? Diversity and Distributions 12, 328-335.**6.** Barnes, R. S. K. (1994). The brackish-water fauna of northwestern Europe. Barnes, R. S. K. The brackish-water fauna of northwestern Europe. Cambridge (UK), Cambridge University Press.**7.** Berezina, N. A. (2005). Seasonal dynamics of structure and fecundity of the Baikalian amphipod (*Gmelinoides fasciatus,* Amphipoda, Crustacea) population in reedbeds of the Neva Bay. Zoologicheskii Zhurnal 84, 411-419.**8.** Bij de Vaate, A. *et al.* (2002). Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. Canadian Journal of Fisheries and Aquatic Science 59, 1159-1174.**9.** Boronat, L., Miracle, M. R., and Armengol, X. (2001). Cladoceran Assemblages in a Mineralization Gradient. Hydrobiologia 442, 75-

88.**10.** Bousfield, E. L. (1973). Shallow-water gammaridean Amphipoda of New England. Bousfield, E. L. Shallow-water gammaridean Amphipoda of New England. Ithaca, NY, Comstock Publishing Associates. **11.** Bruijs, M. C. M. *et al.* (2001). Oxygen Consumption, Temperature and Salinity Tolerance of the Invasive Amphipod *Dikerogammarus villosus*: Indicators of Further Dispersal Via Ballast Water Transport. Archiv Fur Hydrobiologie 152, 633-646.**12.** Bryan B. B. and Grant G. C. (1979). Parthenogenesis and the distribution of the Cladocera. Bulletin of the Biological Society of Washington 3, 54–59.**13.** Chojnacki, J. C. (1999). Description of Ecosystem of the Lower Odra and the Odra Estuary. Acta Hydrochimica Et Hydrobiologica 27, 257-267.**14.** Chubarenko, B., Chubarenko, I., and Baudler, H. (2005). Comparison of Dars-Zingst Bodden Chain and Vistula Lagoon (baltic Sea) in a view of hydrodynamic numerical modeling. Baltica 18, 56-67.**15.** Daunys D. and Zettler M.L. (2006). Invasion of the North American amphipod (*Gammarus tigrinus* Sexton, 1939) into the Curonian Lagoon, South Eastern Baltic Sea. Acta Zoologica Lituanica 16, 20-26.**16.** Dediu, I. I. (1980). Amphipods of fresh and brackish waters of the southwestern USSR. Dediu, I. I. Amphipods of fresh and brackish waters of the southwestern USSR. Shtiintsa, Kishinev, Mold. SSR. **17.** Dorgelo, J. (1974). Comparative ecophysiology of gammarids (Crustacea: Amphipoda) from marine, barckish, and fresh-water habitats, exposed to the influence of salinity-temperature combinations. 1. Effects on survival. Netherlands Journal of Aquatic Ecology 8, 90-109.**18.** Fockedey, N. *et al.* (2005). Temperature and Salinity Effects on Post-Marsupial Growth of Neomysis Integer (Crustacea : Mysidacea). Journal of Experimental Marine Biology and Ecology 326, 27-47.**19.** Frey, D. G. (1993). The Penetration of Cladocerans Into Saline Waters. Hydrobiologia 267, 233-248.**20.** Gasiunaite, Z. R. (2000). Coupling of the Limnetic and Brackishwater Plankton Crustaceans in the Curonian Lagoon (Baltic Sea). International Review of Hydrobiology 85, 653-661.**21.** Giere, O. W. (1978). Tolerance and Preference Reactions of Oligochaeta in Relation to Their Distribution. Giere, O. W. Tolerance and Preference Reactions of Oligochaeta in Relation to Their Distribution. New York and London, Plenum Press. **22.** Grabowski, M. (2006). Rapid colonization of the Polish Baltic coast by an Atlantic palaemonid shrimp *Palaemon elegans* Rathke, 1837. Aquatic Invasions 1, 116-123.**23.** Grigorovich, I. A. *et al.* (2003). Ballast-Mediated Animal Introductions in the Laurentian Great Lakes: Retrospective and Prospective Analyses. Canadian Journal of Fisheries and Aquatic Sciences 60, 740-756.**24.** Grigorovich, I. A. (1998). *Bythotrephes longimanus* in the Commonwealth of Independent States:variability, distribution and ecology. Hydrobiologia 739, 183-198.**25.** Gruszka P. (1999). The River Odra estuary as gateway for alien species immigration to the Baltic Sea basin. Acta Hydrochimica Hydrobiologica 27, 347-382.**26.** Holmes, S. P. and Miller, N. (2006). Aspects of the Ecology and Population Genetics of the Bivalve Corbula Gibba. Marine Ecology-Progress Series 315, 129-140.**27.** Horvath, T. G., Whitman, R. L., and Last, L. L. (2001). Establishment of Two Invasive Crustaceans (Copepoda : Harpacticoida) in the Nearshore Sands of Lake Michigan. Canadian Journal of Fisheries and Aquatic Sciences 58, 1261-1264.**28.** Ioffe, Ts. I., Salazkin, A. A., and Petrov, V. V. (1968). Biological foundations for enrichment of fish food resources in the Gorkyi, Kuibyshev and Volgograd reservoirs. Izv. Gos. Nauchno-Issled. Inst. Ozern. Rechn. Rybn. Khoz. 67, 30–80.**29.** Jazdzewski, K. (1970). Biology of Crustacea Malacostraca in the Bay of Puck, Polish Baltic Sea. Zoologica Poloniae 20, 423-479.**30.** Jazdzewski, K. (1973). Ecology of gammarids in the Bay of Puck. Oikos 15.**31.** Jazdzewski, K. (1980). Range extensions of some gammaridean species in European inland waters caused by human activity. Crustaceana Suppl. 6, 84-107.**32.** Jazdzewski, K., Konopacka, A., and Grabowski, M. (2005). Native and alien malacostracan Crustacea along the Polish Baltic Sea coast In the twentieth century. Oceanological and Hydrobiological Studies 34, 175-193.**33.** Karpevich, A. F. (1975). Theory and practice of acclimatization of aquatic organisms. Karpevich, A. F. Theory and practice of acclimatization of aquatic organisms. 75. Moscow, Pishchevaya Promyshlennost. **34.** Kelleher, B. *et al.* (1999). Current status of the freshwater Mysidae in the Netherlands, with the records of *Limnomysis benedeni* Czerniavsky, 1882, a Pontocaspian species in Dutch Rhine branches. Bulletin Zoölogisch Museum, Universiteit van Amsterdam 16, 89-94.**35.** Komarova, T. I. (1984). Mysids of the delta of the Dneipr and the Dneipr-Bug Lagoon. Vestnik Zoologii 35-38.**36.** Kotta, J. and Kotta, I. (1997). Do the towns of Helsinki and Tallinn oppress the zoobenthos in the adjacent sea? EMI Report Series 8, 55-71.**37.** Kukert K (1984). Die Crustaceen der Brackwassertumpel im Aubendeichsland zwischen Spieka-Neufeld und Arensch-Berensch/Cuxhaven und ihre Verteilung in Beziehung zum Salzgehalt (Crustacea: Cladocera, Copepoda, Amphipoda, Decapoda). Abhandlungen Naturwissenschaftlichen Verein zu Bremen 40, 115-136.**38.** Lance, J. (1963). The salinity toleranc of some estuarine planktonic copepods. Limnology and Oceanography 8, 440-449.**39.** Lance, J. (1964). The Salinity Tolerances of Some Estuarine Planktonic Crustaceans. Biological Bulletin 127, 108-118.**40.** Lee, C. E. (1999). Rapid and Repeated Invasions of Fresh Water by the Copepod Eurytemora Affinis. Evolution 53, 1423-1434.**41.** Lougheed, V. L. and Chow-Fraser, P. (2002). Development and use of a zooplankton index to monitor wetland quality in Canadian marshes of the Great Lakes basin. Ecological Applications 12 , 474-486.**42.** Mauchline, J. (1971). The biology of *Praunus flexuosus* and *P. neglectus* (Crustacea, Mysidacea). Journal of the Marine Biological Association of the United Kingdom 51, 641-652.**43.** McKenney, C. L. Jr. and Neff, J. M. (1979). Individual effects and interactions of salinity, temperature, and zinc on larval development of the grass shrimp *Palaemonetes pugio*. I. Survival and development duration through metamorphosis. Marine Biology 52, 177-188.**44.** Montschenko, V. (1967). Beitrag zur Kenntnis der Gattung Schizopera (Crustacea, Harpacticoida) im Schwarzen. Meeresforschung Zoologische Anzeiger 178, 367–374.**45.** Mordukhai-Boltovskoi (1968). Caspian fauna beyond the Caspian Sea. Internationale Revue der Gesamten Hydrobiologie 49, 139-170.**46.** Mordukhai-Boltovskoi, Ph. D. and Rivier, I. K. (1987). Predatory Cladocera of the world's fauna. Leningrad, Nauka. **47.** Moroz, T. G. (1994). Aquatic Oligochaeta of the Dnieper-Bug Estuary System. Hydrobiologia 278, 133-138.**48.** Normant, M. *et al.* (2005). Osmotic and Ionic Haemolymph Concentrations in the Baltic Sea Amphipod Gammarus Oceanicus in Relation to Water Salinity. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 141, 94-99.**49.** Ojaveer, Henn, Leppakoski, Erkki, Olenin, Sergej, and Ricciardi, Anthony. (2002). Ecological impact of Ponto-Caspian invaders in the Baltic Sea, European inland waters and the Great Lakes: an inter-ecosystem comparison. Leppakoski, E, Gollasch, S., and Olenin, S. Invasive Aquatic Species of Europe. 412-425. Dordrecht, Boston, London., Kluwer Academic Publishers. **50.** Olenin, S., Olenina, I., Daunys, D., and Gasiunaite, Z. (1999). The harbour profile of Klaipeda, Lithuania. The risk assessments of alien species in Nordic waters.**51.** Ovčarenko, I., Audzijonyte, A., and Rasuole Gasiunaite, Z. (2006). Tolerance of *Paramysis lacustris* and *Limnomysis benedeni* (Crustacea, Mysida) to sudden salinity changes: implications for ballast water treatment. Oceanologia 48, 231-242.**52.** Paalvast, P., Ledema, W., Ohm, M., and Posthoorn, R. (1998). MER Beheer Haringvlietsluizen, Deelrapport ecologies en landschap. RIZA rapport 98.051.**53.** Paavola, M., Olenin, S., and Leppakoski, E. (2005). Are Invasive Species Most Successful in Habitats of Low Native Species Richness Across European Brackish Water Seas? Estuarine Coastal and Shelf Science 64, 738-750.**54.** Panov, V. E. *et al.* (1997). Monitoring biodiversity in bottom and planktonic communities of the Neva Estuary. In: *Monitoring of biodiversity* p. 228–294. Moscow: Pensoft.**55.** Panov, V. E., Krylov, P. I., and Telesh, I. V. (1999). The St. Petersburg Harbour profile. Risk assessment of alien species in Nordic coastal waters.**56.** Pinkster, S. and Platvoet, D. (1983). Further observations on the distribution and biology of two alkien amphipods, *Gammarus tigrinus,* Sexton 1939 and *Crangonyx pseudogracilis*, Bousfield 1958, in the Netherlands (Crustacea, Amphipoda). Bulletin Zoologisch Museum Universiteit van Amsterdam 9, 154-164.**57.** Pinkster, S. *et al.* (1992). Drastic changes in the amphipod fauna (Crustacea) of Dutch inland waters during the last 25 years. Bijdragen tot de Dierkunde 61, 193-204.**58.** Pliüraitë, V. (2003). Species diversity of zooplankton in the Curonian Lagoon in 2001. Acta Zoologica Lithuanica 13, 106-113.**59.** Potts, W. T. W. and Durning (1980). Physiological evolution in the branchiopods. Comparative Biochemistry and Physiology B.- Comparative Biochemistry. 67, 475-484.**60.** Preece, G. S. (1971). The ecophysiological complex of Bathyporeia pilosa and B. pelagica (Crustacea: Amphipoda). II. Effects of exposure. Marine Biology 11, 28-34.**61.** Remane, A. and Schlieper, C. (1971). Die Binnengewasser. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart 25, 211-350.**62.** Rhodes, A. (2003). Methods for high density batch culture of *Nitokra lacustris*, a marine harpacticoid copepod. Howard I. Browman and Anne Berit Skiftesvik. The Big Fish Bang. Proceedings of the 26th Annual Larval Fish Conference. Bergen, Norway, Institute of Marine Research. **63.** Rivier, I. K. (1998). The predatory cladocera (Onychopoda: Podonudae, Polyphemidae, Cercopagidae) and Leptodorida of the world. Guides to the identification of the microinvertebrates of the continental waters of the world. Leiden, Backhuys. Guides to the identification of the microinvertebrates of the continental waters of the world. **64.** Rodionova, N. V., Krylov, P. I., and Panov, V. E. (2005). Invasion of the Ponto-Caspian predatory cladoceran *Cornigerius maeoticus maeoticus* into the Baltic Sea. Oceanology 45, 73-75.**65.** Schirmer, M. (2003). Limnologische funktionskontrolle der ausgleichs- und Ersatzmabnahme auf der Kleinensieler Plate. Bremen, Germany, University of Bremen. **66.** Schuchardt, B., Haesloop, U., and Schirmer, M. (1993). The tidal freshwater reach of the Weser Estuary: riverine or estuarine? The Netherlands journal of aquatic ecology 2, 215-226.**67.** Seys, J., Vincx, M., and Meire, P. (1999). Spatial Distribution of Oligochaetes (Clitellata) in the Tidal Freshwater and Brackish Parts of the Schelde Estuary (Belgium). Hydrobiologia 406, 119-132.**68.** Spittler, P. and Schiller, H. (1984). The Effect of Salinity on the Distribution and Population Growth of Chydorus Sphaericus (Cladocera). Limnologica 15, 507-512.**69.** Sutcliffe, D. W. (1967). Sodium regulation in the fresh-water amphipod, *Gammarus pulex* (L.). Journal of Experimental Biology 46, 499-S18.**70.** Tackx, M. L. M. *et al.* (2004). Zooplankton in the Schelde Estuary, Belgium and the Netherlands. Spatial and Temporal Patterns. Journal of Plankton Research 26, 133-141.**71.** Telesh, I. V. (1996). Species Composition of Planktonic Rotifera, Cladocera and Copepoda in the Littoral Zone of Lake Ladoga. Hydrobiologia 322, 181-185.**72.** Teschner, M. (1995). Effects of Salinity on the Life-History and Fitness of Daphnia-Magna - Variability Within and Between Populations. Hydrobiologia 307, 33-41.**73.** Vader, W. *et al.* (1984). *Gammarus inaequicauda*, in Norway (Crustacea, Amphipoda). Fauna Norvegica Ser. A. 5, 9-13.**74.** Van der Brink, F. W. B., Van der Velde, G., and Bij de Vaate, A. (1993). Ecological aspects, explosive range extension and impact of a mass invader, *Corophium curvispinum* Sars, 1895 (Crustacea: Amphipoda), in the Lower Rhine (The Netherlands). Oecologia 93, 224-232.**75.** Vanden Bossche, J. P. *et al.* (2001). First Record of the Pontocaspian Invader Hypania Invalida (Grube, 1860) (Polychaeta : Ampharetidae) in the River Meuse (Belgium). Belgian Journal of Zoology 131, 183-185.**76.** Wetsteyn, L. P. M. J. and Vink, M. (2001). An investigation into the presence of plankton organisms in the ballast water of ships arriving in Dutch Ports, and the survival of these organisms in Dutch surface and port waters. Ballast Water. Directorate-General of Public Works and Water Management. **77.** Wijnhoven, S., Van Riel, M. C., and van der Velde, G. (2003). Exotic and indigenous freshwater gammarid species: physiological tolerance to water temperature in relation to ionic content of the water. Aquatic Ecology 37, 151-158.**78.** Williams-Howze, J. (1996). Biology and Morphology of the Marine Harpacticoid Copepod Heteropsyllus Nunni Coull, During Encystment Diapause. Hydrobiologia 320, 179-189.**79.** Witt, J. D. S., Hebert, P. D. N., and Morton, W. B. (1997). *Echinogammarus ischnus:* another crustacean invader in the Laurentian Great Lakes system. Canadian Journal of Fisheries and Aquatic Sciences 54, 264-268.**80.** Wright, D. A. *et al.* (1996). Effect of Salinity and Temperature on Survival and Development of Young Zebra (Dreissena Polymorpha) and Quagga (Dreissena Bugensis) Mussels. Estuaries 19, 619-628.**81.** Ysebaert, T., De Neve, L., and Meire, P. (2000). The Subtidal Macrobenthos in the Mesohaline Part of the Schelde Estuary (Belgium): Influenced by Man? Journal of the Marine Biological Association of the United Kingdom 80, 587-597.

Table 2: Taxonomic breakdown of selected species pools shared among the ports of the North Sea, Baltic Sea, and the Great Lakes. Listed in blue text are shared species among the regions. Listed in red are the numbers of species that have been introduced to the Great Lakes. * *Mysis diluviana* is indigenous to the Great Lakes but does not occur in Northern Europe (see Dooh et al., 2006).

Taxon **Shared/Introduced/Total**	**Taxon** **Shared/Introduced/Total**	**Taxon** **Shared/Introduced/Total**
Hydrozoan 1/**1**/1	**Annelida** 5/**0**/19	**Cladocera** 40/**5**/63
Copepoda 15/**6**/59	**Amphipoda** 3/**2**/34	**Mysidacea** 1*/1/8
Cumacea 0/**0**/2	**Isopoda** 0/0/9	**Decapoda** 0/0/17
Platyzoans 0/**0**/4	**Mollusca** 4/**4**/11	**Rotifera** **32/**0/42

Table 3: Life history characteristics of selected species from the Ponto-Caspian, North Sea, and Baltic Sea Regions considered as potential invaders to the Great Lakes Region or low-salinity ports of the Northeastern United States. Shown for each species is the relative abundance reported for multiple source regions, reproductive capacity, salinity tolerance, and behavior. Blanks indicate no data available. *-Native to Gulf of St. Lawrence (GOSL); **Introduced to GOSL; ***Cryptogenic in GOSL, ^{1} Found in Great Lakes 11/06, A=Abundant, B=Baltic Sea Abundance, C=Common, DP=Demersal Planktonic, E=Epibenthic, H=Holoplanktonic, NA=not present, NFO=Non-tidal Freshwater Only, NSE=North Sea Estuaries Abundance, P=Present, P-C=Ponto-Caspian Abundance, R=Rare, S=Frequent Swimmer, TD=Tube Dwelling.

Species	**P-C**	**B**	**NSE**	**Length of Breeding Season, Site**	**Salinity Tolerance Range (ppt)**	**Brood size Average, Max, Site**	**Behavior**	**References**
Amphipoda								
Apocorophium lacustre	NA	R	R to A		0-16		TD-S	9, 15, 21
Chelicorophium curvispinum	A	A to R	A	April-October, Rhine	0-18	~16, 34, Rhine	TD-S	15, 19, 21, 23, 36, 53
*Corophium volutator***	NA	A	A	May-November, Netherlands; May-September , Poland, Finland	0.2-35	30, 66, Poland	TD-S	15, 19, 34, 67, 68
Dikerogammarus haemobaphes	NA	A			0-16		E-S	10, 23, 37, 59
Dikerogammarus villosus	A	A	A	April-September, Germany	0-20	52, 136.5, Germany	E-S	10, 17, 23, 40, 44, 51, 79
Echinogammarus berilloni	NA	NA	R		0-35	29, Netherlands	E-S	25, 77, 80
Echinogammarus trichiatus	C	NA	R				E-S	10, 23, 51
Echinogammarus (Chaetogammarus) warpachowskyi	A	C	NA		0-17		E-S	10, 23, 51, 53
*Gammarus duebeni**	NA	R	R	December-May, Sweden	0-85	36, 61, Poland	E-S	21, 25, 34, 35, 45, 67, 69
Gammarus fossarum	NA	P in NFO	P in NFO		0-5	11,_ , Netherlands	E-S	27, 25, 79
Gammarus inaequicauda	NA	R		May-September , Poland	6.5-35	27, 65, Poland	E-S	34, 35, 37, 76
*Gammarus locusta****	NA	R		May-November, Poland, Sweden	4-35	32.5, 93	E-S	25, 34, 38, 67

Species	**P-C**	**B**	**NSE**	**Length of Breeding Season, Site**	**Salinity Tolerance Range**	**Brood size** **Average, Max, Site**	**Behavior**	**References**
*Gammarus oceanicus**	NA	R		November-July, Poland, Finland; November-May, Sweden	4-35		E-S	21, 25, 34, 38,67
Gammarus pulex	A	R	R to A in NFO	March-July, Netherlands	0-11	15,_ Netherlands	E-S	37, 56, 72, 79
Gammarus roeselii	NA	R	R		0-9		E-S	27, 36, 79
Gammarus salinus	NA	A	A	March-November, Netherlands; March-September, Poland; February-October, Finland	1-35	46, 138, Poland	E-S	25, 34, 45, 67
Gammarus varsoviensis	NA	R	NA	May-August	0-		E-S	36
Gammarus zaddachi	NA	R to A	R to A	April-November, Poland; March-October, Sweden	0-35	33, 89 Poland	E-S	21, 26, 34, 71
Gmelinoides fasciatus	NA	C	NA	May-September	0-8	14, 46, Russia	E-S	10, 51, 53, 55
Iphigenella shablensis	R	NA	NA		0-5		E-S	23, 32
Monoporeia (*Pontoporeia*) *affinis*	NA	A	NA	October-April	0-7		E-S	30, 67
Obessogammarus crassus	C	A	R		0-18		E-S	5, 21, 23, 28, 53
Pontogammarus maeoticus	C	NA	NA		0-17		E-S	10, 23, 70
Pontogammarus obesus	A	NA	NA		0-5		E-S	23, 37
Pontogammarus robustoides	A	A	NA	March-October, Poland	0-18	44, 120, Poland	E-S	21, 23, 40
Pontogammarus subnudus	R	NA	NA		0-5		E-S	23, 40
Cladocera								
Bosmina obtusirostris		P	P		0-10		H	24
Daphnia cristata	C	C	C				H	57
Daphnia cucullata	C	C	C		0-5	1.5, 3.1 Polish Lake; 2, 5 Netherlands	H	11, 20, 48, 57
Daphnia longispina		C	C		0-11.9	23, 28, Finland	H	13, 33, 73, 81
Daphnia magna	NA	R	R		0-12.5	20, 80, Finland	H	1, 6, 13, 16, 22, 52, 75

Species	**P-C**	**B**	**NSE**	**Length of Breeding Season, Site**	**Salinity Tolerance Range**	**Brood size Average, Max, Site**	**Behavior**	**References**
Moina brachiata	P	P	C		0-36	17, 29, lab culture	H	3, 4, 60, 73
Macrothrix hirsuticornis	A	P	P		0-39		H	2, 4, 8, 73
Moina macrocopa	P	P	P		0-22.2	4, 16, lab culture	H	4, 16
Cornigerius maeoticus maeoticus	C	R	NA		6-12.5		H	2, 50, 62, 63
Evadne anonyx	A	R	NA	July-September, Russia	1-13	3.4, 6 Russia	H	2, 50, 64
Evadne nordmani	R	C	A	May-June, Baltic; March-October, Clyde Sea; April-October, Skaggerak, Sweden	1-35	4, 8 Germany; 5, 13 Narragansett Bay, R. I. , USA	H	3, 29, 33, 58, 62
Pleopis polyphemoides	A	A	C	June-September, Skaggerak, Sweden	0-35	5, 11, Narragansett Bay, R. I. , USA	H	2, 3, 18, 31
Podon leuckarti	R	C	C	May-June, Baltic; May-September, Skaggerak, Sweden	6.1-35	4, 6, Germany 4, 12, Narragansett Bay, R. I., USA	H	3, 4, 31, 58
Podonevadne trigona ovum	A	NA	NA		0-22		H	2, 62
Mysidacea								
Hemimysis anomala[1]	A	C	C		0-19	13, 28 Netherlands	DP	42, 47, 61, 65, 78
Limnomysis benedeni	A	A	A		0-23	26, 40 Netherlands	DP	10, 41, 42
Mesopodopsis slabberi	A	A			0.5-35	15, 25	DP	47, 74
Neomysis integer	NA	A	A	April-October, Netherlands, Poland	0.1-38	40, 98 Netherlands; 20, 42, Poland	DP	74
Paramysis intermedia	A	NA	NA	May-October	0-12	15, 45, Russia	DP	7, 10, 12, 46, 47, 51
Paramysis lacustris	A	A	NA	June-September	0-18	25, 60, Lithuania	DP	7, 21, 43, 46, 47
Praunus flexuosus	NA	A	A	Jan-October, U.K.; May-October, Poland	2-35	25, 42 U. K.	DP	34, 49, 74
Praunus inermis	NA	A	R	Jan-December, U. K.; April-October, Norway	0.7-35	20, 40 U. K.; 9-17, Poland	DP	34, 49, 74

1. Aladin, N. V. (1983). Displacement of the Critical Salinity Barrier in the Caspian and Aral Seas (the Branchiopoda and Ostracoda Taken as Examples). Zoologichesky Zhurnal 62 , 689-694.**2.** Aladin, N. V. (1982b). Salinity adaptation and osmoregulation abilities of the Cladocera. 2. Forms from Caspian and Aral seas . Zoologicheskii Zhurnal 61, 507-514.**3.** Aladin, N. V. (1982a). Salinity adaptations and osmoregulation abilities of the Cladocera . 1. Forms from open Seas and oceans. Zoologicheskii Zhurnal 61, 341-351.**4.** Aladin, N. V. and Potts, W. T. W. (1995). The Osmoregulatory Capacity of the Cladocera. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 164, 671-683.**5.** Alimov, A. F. (1997). Monitoring the Neva bay and the eastern Gulf of Finland. Technical report, 127 pp. Zoological Institute of the Russian Academy of Sciences, St. Petersburg. **6.** Arner, M. and Koivisto, S. (1993). Effects of salinity on metabolism and life history characteristics of *Daphnia magna*. Hydrobiolgia 259, 69-77.**7.** Audzijonyte, A., Daneliya, M. E., and Väinolä, R. (2005). Comparative phylogeography of Ponto-Caspian mysid crustaceans: isolation and exchange among dynamic inland sea basins. Molecular Ecology 15, 2969–2984.**8.** Bailey, S. A. , Nandakumar, K., and Macisaac, H. J. (2006). Does Saltwater Flushing Reduce Viability of Diapausing Eggs in Ship Ballast Sediment? Diversity and Distributions 12, 328-335.**9.** Barnes, R. S. K. (1994). The brackish-water fauna of northwestern Europe. Barnes, R. S. K. The brackish-water fauna of northwestern Europe. Cambridge (UK), Cambridge University Press.**10.** Bij de Vaate, A. *et al.* (2002). Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. Canadian Journal of Fisheries and Aquatic Science 59, 1159-1174.**11.** Boersma, M. and Vijverberg, J. (1994). Resource depression in *Daphnia galeatea, Daphnia cucullata*, and their interspecific hybrid: life history consequences. Journal of Plankton Research 16, 1741-1758.**12.** Borodich, N. D. and Havlena, F. K. (1972). The biology of mysids acclimatized in the reservoirs of the Volga River. Hydrobiologia 42, 527-539.**13.** Boronat, L., Miracle, M. R., and Armengol, X. (2001). Cladoceran Assemblages in a Mineralization Gradient. Hydrobiologia 442, 75-88.**14.** Bousfield, E. L. (1958). Freshwater amphipods of glaciated North America. Canadian Field-Naturalist 72, 55-113.**15.** Bousfield, E. L. (1973). Shallow-water gammaridean Amphipoda of New England. Bousfield, E. L. Shallow-water gammaridean Amphipoda of New England. Ithaca, NY, Comstock Publishing Associates. **16.** Brooks, J. L. (1957). The systematics of North American *Daphnia*. Memoirs of the Connecticut Academy of Arts and Sciences 13, 1-180.**17.** Bruijs, M. C. M. *et al.* (2001). Oxygen Consumption, Temperature and Salinity Tolerance of the Invasive Amphipod *Dikerogammarus villosus*: Indicators of Further Dispersal Via Ballast Water Transport. Archiv Fur Hydrobiologie 152, 633-646.**18.** Bryan B. B. and Grant G. C. (1979). Parthenogenesis and the distribution of the Cladocera. Bulletin of the Biological Society of Washington 3, 54–59.**19.** Buckley, P., Dussart, G., and Trigwell, J. (2004). Invasion and expansion of Corophiidae (Amphipoda) in the Stour estuary (Kernt, UK). Crustaceana 77, 425-433.**20.** Czeczuga, B. and Kozμowska, M. (2002). Fertility of *Eudiaptomus, Bosmina* and *Daphnia* (Crustacea) Representatives in lakes of varied trophic states in the Suwaμki District. Polish Journal of Environmental Studies 11, 23-32.**21.** Daunys D. and Zettler M.L. (2006). Invasion of the North American amphipod (*Gammarus tigrinus* Sexton, 1939) into the Curonian Lagoon, South Eastern Baltic Sea. Acta Zoologica Lituanica 16, 20-26.**22.** De Gelas, K. and De Meester, L. (2005). Phylogeography of *Daphnia magna* in Europe. Molecular Ecology 14, 753-764.**23.** Dediu, I. I. (1980). Amphipods of fresh and brackish waters of the southwestern USSR. Dediu, I. I. Amphipods of fresh and brackish waters of the southwestern USSR. Shtiintsa, Kishinev, Mold. SSR. **24.** Deevey, E. S. and Deevey, G. B. (1971). The American species of *Eubosmina* Seligo. (Crustacea, Cladocera). Limnology and Oceanography 16, 201-28.**25.** Den Hartog, C. (1964). The amphipods of the deltaic region of the rivers Rhine, Meuse and Scheldt, in relation to the hydrography of the sea. Part III. The Gammaridae. Netherlands Journal of Sea Research 2-3, 407-457.**26.** Den Hartog, C., van den Brink, F. W. B., and van der Velde, G. (1993). Why was the invasion of the river Rhine by *Corophium curvispinum* and *Corbicula* species so successful? Journal of Natural History 26, 1121-1129.**27.** Dorgelo, J. (1974). Comparative ecophysiology of gammarids (Crustacea: Amphipoda) from marine, barckish, and fresh-water habitats, exposed to the influence of salinity-temperature combinations. 1. Effects on survival. Netherlands Journal of Aquatic Ecology 8, 90-109.**28.** Eggers, T. O. and Anlauf, A. (2003). *Obesogammarus crassus* (G.O. Sars 1894, Crustacea, Amphipoda) erreichte die Elbe. Lauterbornia 55, 125-128.**29.** Eriksson, S. (1974). The occurrence of marine Cladocera on the west coast of Sweden. Marine Biology 26, 319-327.**30.** Filippov, A. A. (2006). Adaptability of the amphipod *Pontoporeia affinis* (Crustacea: Amphipoda) to salinity changes. Russian Journal of Marine Biology 32, 198–200.**31.** Fofonoff, P. W. (1994). Marine Cladocerans in Narragansett Bay. Kingston. University of Rhode Island.**32.** Grigorovich, I. A. *et al.* (2003). Ballast-Mediated Animal Introductions in the Laurentian Great Lakes: Retrospective and Prospective Analyses. Canadian Journal of Fisheries and Aquatic Sciences

60, 740-756.**33.** Hanski, I. and Ranta, E. (1983). Coexistence in a patchy environment: Three species of *Daphnia* in rock pools. Journal of Animal Ecology 52, 263-279.**34.** Jazdzewski, K. (1970). Biology of Crustacea Malacostraca in the Bay of Puck, Polish Baltic Sea. Zoologica Poloniae 20, 423-479.**35.** Jazdzewski, K. (1973). Ecology of gammarids in the Bay of Puck. Oikos 15, 121-126.**36.** Jazdzewski, K. (1980). Range extensions of some gammaridean species in European inland waters caused by human activity. Crustaceana Suppl. 6, 84-107.**37.** Jazdzewski, K., Konopacka, A., and Grabowski, M. (2005). Native and alien malacostracan Crustacea along the Polish Baltic Sea coast In the twentieth century. Oceanological and Hydrobiological Studies 34, 175-193.**38.** Jeczmien, W. and Szaniawska, A. (2000). Changes in species composition of the genus *Gammarus Fabricius* in Puck Bay. Oceanologia 42, 71-87.**39.** Jorgensen, O. M. (1933). On the marine Cladocera from the Northumbrian plankton. Journal of the Marine Biological Association of the United Kingdom 19, 179-226.**40.** Karpevich, A. F. (1975). Theory and practice of acclimatization of aquatic organisms. Karpevich, A. F. Theory and practice of acclimatization of aquatic organisms. Moscow, Pishchevaya Promyshlennost. **41.** Kelleher, B. *et al.* (1999). Current status of the freshwater Mysidae in the Netherlands, with the records of *Limnomysis benedeni* Czerniavsky, 1882, a Pontocaspian species in Dutch Rhine branches. Bulletin Zoölogisch Museum, Universiteit van Amsterdam 16, 89-94.**42.** Ketelaars, H. A. M. *et al.* (1999). Ecological effects of the mass occurrence of the Ponto-Caspian invader, *Hemimysis anomala* G.O. Sars, 1907 (Crustacea: Mysidacea), in a freshwater storage reservoir in the Netherlands, with notes on its autoecology and new records. Hydrobiologia 394, 233-248.**43.** Khemeleva, N. N. and Baicharov, V. M. (1987). Patterns of reproduction of Pontocaspian relict *Paramysis lacustris* with distribtuion area. International Revue de Gesamten Hydrobiologie.**44.** Kley, A. and Maier, G. (2003). Life history characteristics of the invasive freshwater gammarids *Dikerogammarus villosus* and *Echinogammarus ischnus* in the river Main and the Main-Donau canal. Archiv für Hydrobiolgie 156, 457–469.**45.** Kolding, S. (1973). Habitat selection and life cycle characteristics of five species of the amphipod genus *Gammarus* in the Baltic. Oikos 173-178.**46.** Komarova, T. I. (1991). Mysidaceans (Mysidacea). Vol. 26. Malacostracans. Kiev, Ukraine., Naukova Dumka. Fauna of Ukraine. **47.** Komarova, T. I. (1984). Mysids of the delta of the Dneipr and the Dneipr-Bug Lagoon. Vestnik Zoologii 35-38.**48.** Koski, M., Viitasalo, M., and Kuosa, H. (1999). Seasonal development of mesozooplankton biomass and production on the SW coast of Finland. Ophelia 50, 69-91.**49.** Mauchline, J. (1965). Breeding and fecundity of Praunus inermis (Crustacea, Mysidacea). Journal of the Marine Biological Association of the United Kingdom 45, 663-671.**50.** Mordukhai-Boltovskoi, Ph. D. and Rivier, I. K. (1987). Predatory Cladocera of the world's fauna. Leningrad, Nauka. **51.** Ojaveer, Henn, Leppakoski, Erkki, Olenin, Sergej, and Ricciardi, Anthony. (2002). Ecological impact of Ponto-Caspian invaders in the Baltic Sea, European inland waters and the Great Lakes: an inter-ecosystem comparison. Leppakoski, E, Gollasch, S., and Olenin, S. Invasive Aquatic Species of Europe. 412-425. Dordrecht, Boston, London., Kluwer Academic Publishers. **52.** Ortells, R., Reusch, T. B. H., and Lampert, W. (2005). Salinity Tolerance in Daphnia Magna: Characteristics of Genotypes Hatching From Mixed Sediments. Oecologia 143, 509-516.**53.** Paavola, M., Olenin, S., and Leppakoski, E. (2005). Are Invasive Species Most Successful in Habitats of Low Native Species Richness Across European Brackish Water Seas? Estuarine Coastal and Shelf Science 64, 738-750.**54.** Panov, V. Ye. (1986). Growth and production of *Gammarus lacustris* in the Neva Inlet. Hydrobiological Journal 22, 37-42.**55.** Panov, V. E. and Berizina, N. A. (2002). Invasion history, biology, and impacts of the Baikalian amphipod *Gmelinoides fasciatus*. In: *Invasive Aquatic Species of Europe* (eds. Leppakoski, E., Gollasch, S., and Olenin, S.), pp. 96-103. Dordrecht, Boston, London.: Kluwer Academic Publishers.**56.** Pinkster, S. and Platvoet, D. (1983). Further observations on the distribution and biology of two alkien amphipods, *Gammarus tigrinus,* Sexton 1939 and *Crangonyx pseudogracilis*, Bousfield 1958, in the Netherlands (Crustacea, Amphipoda). Bulletin Zoologisch Museum Universiteit van Amsterdam 9, 154-164.**57.** Pliūraitē, V. (2003). Species diversity of zooplankton in the Curonian Lagoon in 2001. Acta Zoologica Lithuanica 13, 106-113.**58.** Poggensee, P. a. L. J. (1981). On the population dynamics of two brackish-water cladocera *Podon leuckarti* and *Evadne nordmanni* in Kiel Fjord. Kieler Meeresforschung. Sonderheft 5, 268-273.**59.** Ponomareva, S. A. (1975). Effect of salinity on the fresh-water shrimp *Dikerogammarus haemobaphes* (Eichwald) from the mouth of the Dnepr. Hydrobiological Journal 11, 67-69.**60.** Potts, W. T. W. and Durning (1980). Physiological evolution in the branchiopods. Comparative Biochemistry and Physiology B.- Comparative Biochemistry. 67, 475-484.**61.** Reznichenko, O. G. (1959). On the ecology and morphology of the mysidaceans of the genus *Hemimysis* (Crustacea, Malacostraca). Trudy Vsesoyuznye Gidrobiolichesckovo Obshchestva 9, 320-343.**62.** Rivier, I. K. (1998). The predatory cladocera (Onychopoda: Podonudae, Polyphemidae, Cercopagidae) and Leptodorida of the world. Leiden, Backhuys. Guides to the identification of the microinvertebrates of the continental waters of the world. **63.** Rodionova, N. V., Krylov, P. I., and Panov, V. E. (2005). Invasion of the Ponto-Caspian predatory cladoceran *Cornigerius maeoticus maeoticus* into the Baltic Sea. Oceanology 45, 73-75.**64.** Rodionova, Natalie V. and Panov, Vadim E. (2006). Establishment of the Ponto-Caspian predatory cladoceran *Evadne anonyx* in the eastern Gulf of Finland, Baltic Sea. Aquatic Invasions 1, 7-12.**65.** Salemaa, H. and Hietalahti, V. (1994). *Hemimysis anomala* G.O. Sars (Crustacea: Mysidacea) - Immigration of a Pontocaspian mysid into the Baltic Sea. Annales Zoologici Fennici 30, 271-276.**66.** Schwenk, K., Posada, D., and HebertPaul D. N. (2000). Molecular systematics of European *Hyalodaphnia*: the role of contemporary hybridization in ancient species. Proceedings of the Royal Society of London B. 267, 1822-1842.**67.** Segerstråle, S. G. (1950). The amphipods on the coasts of Finland- Some facts and problems. Societas Scientarum Fennica Commentationes Biologicae 10, 2-27.**68.** Shoemaker, C. R. (1947). Further notes on the amphipod genus *Corophium*, from the east coast of North America. Journal of the Washington Academy of Sciences 37, 47-63.**69.** Simm, M. and Ojaveer, H. (2006). Taxonomic status and reproduction dynamics of the non-indigenous *Cercopagis* in the Gulf of Riga (Baltic Sea). Hydrobiologia 554, 147–154.**70.** Soldatova, I. N. (1986). Eco-physiological properties of *Pontogammarus maeoticus* (Amphipoda) in a salinity gradient . Marine Biology 92, 115-123.**71.** Sutcliffe, D. W. (1968). Sodium Regulation and Adaptation to Fresh Water in Gammarid Crustaceans. Journal of Experimental Biology 48, 359-380.**72.** Sutcliffe, D. W. (1967). Sodium regulation in the fresh-water amphipod, *Gammarus pulex* (L.). Journal of Experimental Biology 46, 499-S18.**73.** Tackx, M. L. M. *et al.* (2004). Zooplankton in the Schelde Estuary, Belgium and the Netherlands. Spatial and Temporal Patterns. Journal of Plankton Research 26, 133-141.**74.** Tattersall, W. M. and Tattersall, Olive M. (1951). The British Mysidacea. Tattersall, W. M. and Tattersall, Olive M. The British Mysidacea. London, Ray Society. **75.** Teschner, M. (1995). Effects of Salinity on the Life-History and Fitness of Daphnia-Magna - Variability Within and Between Populations. Hydrobiologia 307, 33-41.**76.** Vader, W. *et al.* (1984). *Gammarus inaequicauda*, in Norway (Crustacea, Amphipoda). Fauna Norvegica Ser. A. 5, 9-13.**77.** Van Maren, M. J. Some notes on the intertidal gammarids Crustacea: Amphipoda from the Atlantic Coast of the Iberian Penisula Spain, Portugal. Beaufortia 23(305), 153-168. 75. **78.** Verslycke, T. *et al.* (2005). First occurrence of the Ponto-Caspian invader *Hemimysis anomala* (Sars, 1907) in Belgium (Crustacea: Mysidacea). Belgian Journal of Zoology 130, 157-158.**79.** Wijnhoven, S., Van Riel, M. C., and van der Velde, G. (2003). Exotic and indigenous freshwater gammarid species: physiological tolerance to water temperature in relation to ionic content of the water. Aquatic Ecology 37, 151-158.**80.** Wouters, K. (2002). On the distribution of alien non-marine and estuarine macro-crustaceans in Belgium. Bulletin Van Het Koninklin Instituut Voor Naturwetenschappen, Biologie. 72, 119-129.**81.** Zinovyeh, A. N. (1931). Salt and brackish water bodies of the Troitskiy district, Ural region. Isv. Permskago bio., nauchno-issled. in-ta 9, 271-295.

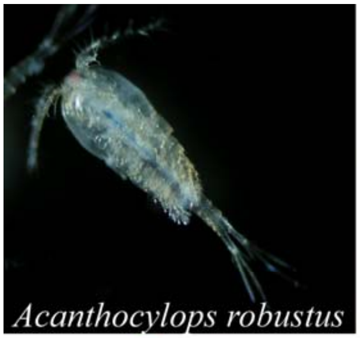
Identifying, Verifying, and Establishing Options for Best Management Practices for NOBOB Vessels

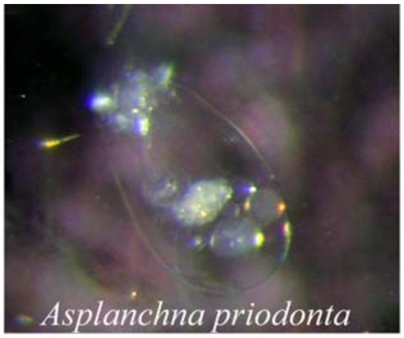
APPENDIX 5

Photographs of Inverts Used in Salinity Tolerance Experiments

Contributing PIs:
Dr. Scott Santagata
Smithsonian Environmental Research Center

Rotterdam Zooplankton Photographs (Scott Santagata)


Acanthocylops robustus


Asplanchna priodonta


Bythotrephes longimanus


Bythotrephes longimanus

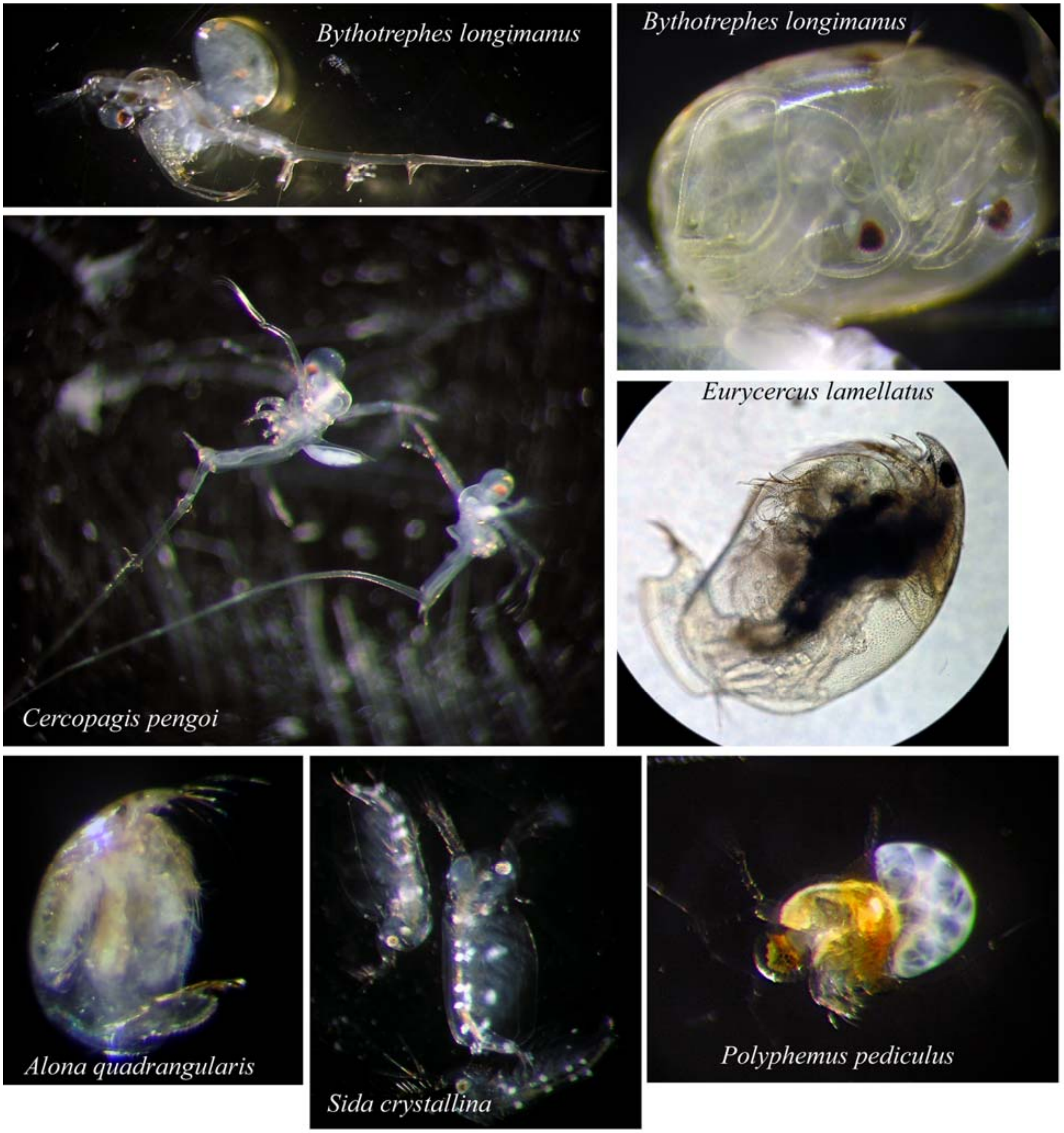

Cyathura carinata


Daphnia galeata galeata


Neomysis integer

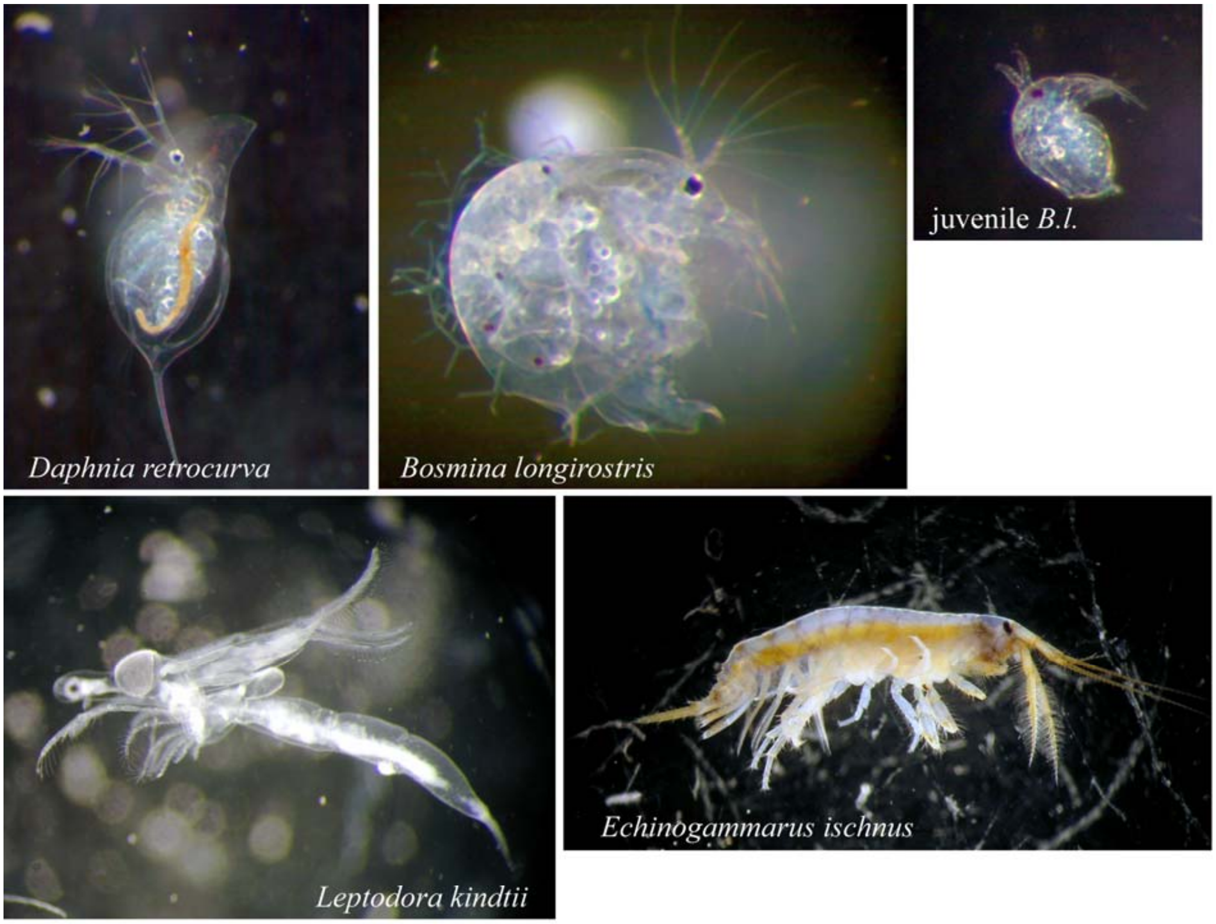

Rhithropanopeus harissii

Lake Michigan Zooplankton Photographs (Scott Santagata)



Lake Michigan Zooplankton and Invertebrates

Lake Erie Zooplankton Photographs



Lake Erie Zooplankton and Invertebrates

Identifying, Verifying, and Establishing Options for Best Management Practices for NOBOB Vessels

APPENDIX 6

Salinity Tolerance Experimental Design
of the
Smithsonian Environmental Research Laboratory

Contributing PIs:
Dr. Greg Ruiz and Dr. Scott Santagatta
Smithsonian Environmental Research Laboratory

Appendix 1: Salinity Tolerance Protocol

Species:___________________________

Today's Date:____________________________

Collection Date:___________________ **Date Exp Started:**_______________

Collection Site:____________________ **Exp. Run No.:**__________________

Ambient Sal:______________________ **Analysed by:**___________________

Ambient Temp:____________________ **Collection Gear:**________________

Timepoint	Treatment	Rep.	Time		Trans Sal. (ppt)	No. organisms			Comments
			Observed	Transferred		Live	Dead	Mori.	
T-0	CONTROL Sal:	1							
		2							
		3							
		4							
	F-T Sal:	1			Amb to 14				
		2			Amb to 14				
		3			Amb to 14				
		4			Amb to 14				
	E-R Sal:	1			Amb to 34				
		2			Amb to 34				
		3			Amb to 34				
		4			Amb to 34				

T-1 1 hour	CONTROL Sal:	1							
		2							
		3							
		4							
	F-T Sal:	1			14 to 24				
		2			14 to 24				
		3			14 to 24				
		4			14 to 24				
	E-R Sal:	1			34				
		2			34				
		3			34				
		4			34				

T-2 2 hours	CONTROL Sal:	1							
		2							
		3							
		4							
	F-T Sal:	1			24 to 34				
		2			24 to 34				
		3			24 to 34				
		4			24 to 34				
	E-R Sal:	1			34				
		2			34				
		3			34				
		4			34				

Species:__________________________

Today's Date:__________________________

Collection Date:___________________ **Date Exp Started:**_______________

Collection Site:____________________ **Exp. Run No.:**__________________

Ambient Sal:______________________ **Analysed by:**___________________

Ambient Temp:____________________ **Collection Gear:**________________

Timepoint	Treatment	Rep.	Time		Trans Sal. (ppt)	No. organisms			Comments
			Observed	Transferred		Live	Dead	Mori.	
T-3 3 hours	**CONTROL Sal:**	1							
		2							
		3							
		4							
	F-T Sal:	1			34				
		2			34				
		3			34				
		4			34				
	E-R Sal:	1			34				
		2			34				
		3			34				
		4			34				
T-4 24 h Change Media	**CONTROL Sal:**	1							
		2							
		3							
		4							
	F-T Sal:	1			34				
		2			34				
		3			34				
		4			34				
	E-R Sal:	1			34				
		2			34				
		3			34				
		4			34				
T-5 48 h	**CONTROL Sal:**	1							
		2							
		3							
		4							
	F-T Sal:	1			34				
		2			34				
		3			34				
		4			34				
	E-R Sal:	1			34				
		2			34				
		3			34				
		4			34				

Identifying, Verifying, and Establishing Options for Best Management Practices for NOBOB Vessels

APPENDIX 7

Products and Presentations for Disseminating Project Results

Products and Presentation for Disseminating Project Results

I. Publications

Gray, D.K., T.H. Johengen, D.F. Reid and H.J. MacIsaac (In press). Efficacy of open-ocean ballast water exchange as a means of preventing invertebrate invasions between freshwater ports. Submitted to Limnology and Oceanography

Heinemann, S., and F.C. Dobbs. 2006. Microbiological 'bottle effects' are not to be ignored (a comment on Mimura et al. 2005). Mar. Pollut. Bull. 52:1310.

Doblin, M.A., and F.C. Dobbs. 2006. Setting a size-exclusion limit to remove toxic dinoflagellate cysts in ships' ballast water. Mar. Pollut. Bull. 52:259-263.

Drake, L.A., A.E. Meyer, R.L. Forsberg, R.E. Baier, M.A. Doblin, S. Heinemann, W.P. Johnson, M. Koch, P.A. Rublee, and F.C. Dobbs. 2005. Potential invasion of microorganisms and pathogens via 'interior hull fouling': Biofilms inside ballast-water tanks. Biol. Invas. 7:969-982.

Dobbs, F.C., and A. Rogerson. Ridding ships' ballast water of microorganisms. 2005. Environ. Sci. Technol. 39 (12):259A-264A.

II. Presentations and Published Abstracts

Drake, L.A., M.A. Doblin, and F.C Dobbs. 2007. Ecology and prevention of potential aquatic microbial bioinvasions via ships' ballast discharge. Abstract submitted to the Rhode Island Natural History Survey 12th Annual Ecology of Rhode Island Conference: Invasive Species: A Threat to Rhode Island's Biodiversity. Narragansett, Rhode Island, March 2007.

Santagata et al. 2007. The efficacy of ballast water exchange for preventing the spread of nonindigenous species among freshwater and estuarine ports of the United States and Europe. Accepted Abstract for the IAGLR 2007: "50th Great Lakes Research Conference - Past, Present, Future".

Santagata, S. 2007. Ballast water exchange and the spread of nonindigenous species among the aquatic habitats of North America and Europe. Invited seminar at Longwood University (Farmville, VA).

Drake, L.A., M.A. Doblin, and F.C. Dobbs. 2006. Potential microbial bioinvasions via ships' ballast and proposed international legislation to reduce introductions. New England Estuarine Research Society, New London, CT.

Dobbs, F.C., M.A. Doblin, and L.A. Drake. 2006. Microorganisms in discharged ballast water: What to do about D-2? 3rd International Conference and Exhibition on Ballast Water Management, Singapore.

Dobbs, F.C. 2006. Can free-living aquatic microorganisms be "invasive species?" International Society for Microbial Ecology, Vienna, Austria.

Dobbs, F.C., M.A. Doblin, and L.A. Drake. 2006. Pathogens in ships' ballast tanks. Ocean Sciences Meeting, Honolulu.

Gray, D.K., van Overdijk, C.D.A., Johengen, T.H., Reid, D.F. & MacIsaac, H.J. 2006. Does open-ocean ballast exchange prevent the transfer of invertebrates between freshwater ports? 14th International Conference on Aquatic Invasive Species. Key Biscayne, Florida, U.S.A.

Gray, D.K., van Overdijk, C.D.A., Johengen, T.H., Reid, D.F. & MacIsaac, H.J. 2006. Does open-ocean ballast exchange reduce the risk of future Great Lakes' introductions from transoceanic vessels? 49th Annual Conference on Great Lakes Research. Windsor, ON, Canada.

Johengen, T., D. Reid, P. Jenkins, H. MacIsaac, G. Ruiz, and F. Dobbs 2006. Instrumented Ballast Tank Studies to Examine Ballast Management Practices. U.S. Coast Guard 2006 Ballast Water Conference, Cleveland, OH.

Johengen, T. 2006. The Role of Shipping in Invasive Species Introductions in the Great Lakes. Inland Seas Education Association. Traverse City. MI.

Reid, D.F., P.T. Jenkins, and T.H. Johengen. 2006. Ballast Water Best Management Practices for Transoceanic Ships: Theory and Practicability. 14th International Conference on Aquatic Invasive Species. Key Biscayne, Florida, U.S.A.

Santagata, S. 2006. Evolutionary and Ecological implications of zooplankton life histories. Invited seminar at Texas A&M University (Galveston and College Station, TX).

Thomson, F.K., S.A. Heinemann, W.L. Hynes, and F.C. Dobbs. 2005. Characterization of antibiotic resistance genes in *Vibrio cholerae* isolated from ships' ballast tanks. American Society of Microbiology, Virginia Branch, Annual Meeting, Norfolk.

Heinemann, S.A., F.K.Thomson, W.L. Hynes, and F.C. Dobbs. 2005. Assessing the potential for horizontal gene transfer of plasmid-borne antibiotic resistance in *Vibrio cholerae* isolated from ships' ballast. American Society of Microbiology, Virginia Branch, Annual Meeting, Norfolk.

Thomson, F., III, S.A. Heinemann, W.L. Hynes, and F.C. Dobbs. 2005. Ships' ballast as a potential vector for the transfer of antibiotic-resistance genes of *Vibrio cholerae*. Vibrio 2005, Gent, Belgium.

Dobbs, F.C. 2005. Can microorganisms be invasive and if so, what are their ecological impacts? 18th Biennial Conference of the Estuarine Research Federation, Norfolk, Virginia.

Heinemann, S.A., F.K. Thomson III, W.L. Hynes, and F.C. Dobbs. 2005. Plasmid-borne antibiotic resistance in *Vibrio cholerae* isolates from ships' ballast. Atlantic Estuarine Research Society, Solomons, Maryland.

Dobbs, F.C. 2005. Can microorganisms be invasive and if so, what are their ecological impacts? Fourth International Conference on Marine Bioinvasions, Wellington, New Zealand.