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

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Revisions

This manuscript is subject in the short-term to minor technical corrections, although none are expected to substantively change the results and outcomes. Revisions will be documented on the cover page by number and date, and documented here.

Revision 1, May 20, 2005: corrected list of Principal Investigators and expanded Acknowledgements section.

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Executive Summary

Over the last decade, much attention has been focused on ballast water as a vector for nonindigenous species introductions to the Great Lakes and marine coastal ecosystems, and on open-ocean ballast exchange as a defense against new introductions. However the issue of NOBOB (no-ballast-on-board) vessel operations in the Great Lakes has risen from a position of relative obscurity to become a major concern in the Great Lakes basin today. On average, less than 20% of ocean vessels entering the Great Lakes in recent years contained declarable ballast water on board (U.S. Coast Guard, pers. comm..; Grigorovich et al., 2003) and many of those vessels with declarable ballast had some empty tanks as well. NOBOB vessels and individual tanks with unpumpable and therefore undeclarable ballast escape scrutiny under existing U.S. and Canadian federal, state, and provincial laws, yet the residual volumes of unpumpable ballast water and sediment may contain live aquatic organisms and resting stages - eggs, spores, and cysts - accumulated over numerous previous ballasting operations.

This multidisciplinary research program was designed to directly assess the potential invasion vectors represented by overseas vessels operating in the Great Lakes. It provides the beginning of a scientific foundation for developing new policies and for identifying effective preventive measures and treatments. The goals of this study were to (1) greatly expand the biological and physical characterization of NOBOB tanks beyond that presently available, (2) assess the invasion risk associated with "clean" (i.e., little sediment accumulation) vs. "dirty" (i.e., significant sediment accumulation) ballast tanks, (3) measure the relationship between ship management practices and invasion risk to determine if certain management practices appear to reduce the risk posed by NOBOB vessels, and (4) quantify the effectiveness of open-ocean exchange in decreasing the diversity and concentration of nonindigenous species that enter the Great Lakes in "exchanged" ballast water. Project activities were organized around three interrelated Tasks that were designed to help accomplish these goals and serve as the organizational structure for presenting our project results.

Task 1: <u>Assessment of NOBOB Vessels</u>

The goal of Task 1 was to **c**haracterize biological communities (invertebrates, phytoplankton, and microorganisms) present in NOBOB tanks and correlate these findings with ballast management practices and ballasting history.

Ballast Management Survey

The ballast management survey conducted between December 2000 and December 2002 involved boarding 103 foreign flag ships with ballast capacities ranging between 1,459 and 25,533 metric tonnes, and was carried out in the ports of Toronto and Hamilton on Lake Ontario, Thorold on the Welland Canal, Cleveland and Toledo on Lake Erie and Detroit and Windsor on the Detroit River. These vessels were considered a representative cross section of those trading into the Great Lakes. In order to examine their practices and procedure for all aspects of ballast water and sediment management, interviews were conducted with Masters and senior officers, and various documents were examined including procedure manuals and operating and reporting records. Interviews and document examinations were augmented by entry and sampling or

visual assessment of residuals in random ballast tanks. Cooperation from ships staff was excellent and access to ballast tanks was readily provided.

With very few exceptions there was awareness by ship staff of ANS issues, and that whether for environmental or commercial reasons there were conscientious efforts being made to minimize the amounts of total residuals and especially of sediment being carried in water ballast tanks. Residual (or ”unpumpable”) ballast is as much a factor of commercial shipping as ballast itself, but has little relationship to the overall ballast capacity or the total number of tanks on a ship. The survey found total residuals ranging from negligible to 200 tonnes (t), and sediment accumulation ranging from negligible to 100 t, with sixty percent of ships estimated to have less than 10 t. Factors such as design and outfitting inadequacies, the vagaries of the cargo or parcels of cargo being loaded to replace the ballast, and loading port rotation all contribute to the ships inability to completely evacuate its tanks, and ant residuals and the biota they contain will commingle with subsequent ballast taken on board.

The survey found that the sources of residual ballast being carried into the Great Lakes by these vessels came from around the globe, but the most frequent source was Western Europe (38%), followed next by the Great Lakes (18%). The survey also confirmed that numerous NOBOB ships, predominantly those employed on the North Europe - Great Lakes trade, often ballasted on both continents in fresh or brackish water prior to taking on the full load of cargo for the transoceanic passage. With the transoceanic passage occurring both ways in loaded condition they often did not have the option of flushing their tanks with saltwater, which would provide a salinity barrier to the freshwater biota carried in the residuals, similar to that expected from open ocean exchange. Yet it was found that the most effective method of minimizing sediment accumulation was flushing of ballast tanks with clean ocean water as soon as voyage circumstances permitted. This reduced both sediment deposition and consolidation, particularly if ballasting had been under conditions of high turbidity. When load line or other draft conditions permitted, flushing could be and was undertaken on loaded passages.

The survey found that of the 49 NOBOB ships involved in the survey that entered the lakes after the last ballast carried was either fresh or brackish water, 31 entered with freshwater residuals, having been constrained from flushing the tanks in mid-ocean. Ships in this condition can reasonably be considered to present the most serious threat of inoculation. These findings clearly indicate a need for development of either ship management or, failing that, treatment processes that ensure that fresh or brackish water residuals from offshore are not commingled with freshwater ballast discharged within the Great Lakes.

Vessel Traffic Survey

Our studies confirm earlier analyses that NOBOBs dominate Great Lakes saltwater vessel entries; however, we discovered significant discrepancies in the details reported by Colautti et al (2004) and the U.S. Coast Guard. For the period of comparison the average annual NOBOB entry as a percentage of total entries was 92% ± 5% according to the Colautti et al. data, but 78% ± 5% based on the Coast Guard records. In most cases (>93%) the disagreement involved a Colautti et al. designation of NOBOB vs. a Coast Guard designation of BOB. St. Lawrence Seaway data for the 2000 season indicates that 89% of the vessels entered as NOBOBs. However, further analysis of the Seaway data reveal that only ~7% of the vessels entering that

year would have legally been subject to the deep-water ballast exchange and salinity verification requirements in effect at that time, the remainder having entered the system as NOBOBs, but ballasted at freshwater ports between Quebec City and Montreal, and were thus counted as in a ballast condition by the Seaway. Such vessels would have been counted as being in a ballasted condition, but compliant with entry regulations, by the U.S. Coast Guard. These numbers are illustrative of a pattern that has been developing since the early 1990s as a result of the economic realities of the deep-sea trade into the Great Lakes, and lead us to conclude that in general, the best estimate is that over 90% of the vessels entering the Great Lakes do so as NOBOBs.

Sampling Summary

From December 2000 – December 2002 we entered and collected residual materials from 82 individual empty ballast tanks on 42 vessels. In most cases we were able to collect both water and sediment residuals from each tank for a total of 75 residual water samples, 73 wet and 4 dry residual sediment samples, and 65 plankton (net-filtered) samples (Table 3.3). The cooperation and responsiveness of the shipping industry was excellent throughout the study.

Chemistry

Salinity of residual water samples ranged from 0 – 70 ppt, with about 50 percent of the samples falling in a fresh or brackish (less than 10 ppt) category. This finding is significant – if we assume this is a representative sample, then it is quite probable that a significant number of NOBOBs could contain organisms that are adapted to and may be able to survive in freshwater ecosystems like the Great Lakes.

Dissolved nutrient concentrations varied significantly and often reached extremely high values compared to normal environmental samples. Concentrations were not strongly correlated to salinity, which indicates significant alteration or contamination occurred within the tanks, hence they are not a good indicator of ballast source origin or water quality.

Sediment nutrient concentrations were also highly variable and higher than most natural aquatic sediments. There were at least 6 samples that showed significant contamination, most likely associated with materials involved in ship operations rather than the result of contaminated source material.

Microbiology

Unlike many of their invertebrate counterparts, microbial invaders cannot be seen without a compound microscope and their presence might only be noticed in spectacular cases, e.g., red tides or outbreaks of illness. Thus, there is a bias inherent in the detection of nonindigenous microorganisms. Nonetheless, it would be simplistic and possibly very wrong to consider that aquatic microbial invasions do not occur or could not be mediated by ballast water.

In residual water, most virus-like-particle (VLP) concentrations ranged between 10^7 and 10^9 ml^{-1} and all but one sample had between 10^5 and 10^9 bacteria ml^{-1} (Figs. 3.9, 3.10). In sediment pore water, VLP concentrations varied between 10^7 and 10^{11} ml^{-1} and bacteria concentrations ranged from 10^4 to 10^8 ml^{-1} (Figs. 3.9, 3.10).

Concentrations of VLPs and bacteria had no apparent relationship to ambient air temperature, the time period since tanks were last cleaned, the total sediment residual, or the % residual of the total ballast capacity. Thus, there appears to be no predictability with respect to biological residuals and these ballast-management-related parameters.

As measured by our investigators at Old Dominion University (ODU), total dinoflagellate cyst abundance in residual sediments varied over an order of magnitude, from approximately 80 to 850 cysts per gram of sediment. Germination occurred in 33-44 % of the cysts examined, but differences in time to germination, salinity at which germination occurred, and growth of different organisms in individual plates together serve to highlight the variability among samples. Dinoflagellates and other unicellular algae found in sediments germinated in laboratory studies, even a year after their collection, emphasizing the importance of biological "resting stages" in consideration of ballast practices and management.

Summary results of our analyses on microbial pathogens include:

- We found microbial pathogens in residuals, including *Vibrio cholerae, Cryptosporidium parvum, Giardia lamblia, Encephalitozoon intenstinalis, Pfiesteria piscicida, P. shumwayae*, and *Aureococcus anophagefferens* (see Fig. 11).
- We did not detect *E. coli* or enterococci in any of the samples tested.
- Overall, 26 of 42 (62%) ships sampled tested “positive” for one or more pathogens.
- Overall, 40 of 82 (49%) ballast tanks sampled tested “positive” for one or more pathogens.
- There were few incidences of pathogen co-occurrence: 1 tank in 2001 and 2 tanks in 2002 tested positive for three pathogens. Four tanks in 2001 and 8 tanks in 2002 tested positive for two pathogens.
- There was no consistent temporal pattern in pathogen presence. In 2001, pathogens were detected throughout the sampling season (May to November), but more frequently in summer (June-July) than in fall (October-November). In 2002, pathogens were detected from June to November, but not in September or December (see Fig. 12).
- Data suggest ballasting operations in Antwerp (Belgium) are most associated with ships carrying pathogens into the Great Lakes. Table 2 lists the location of ballasting operations of ships sampled in this study, prior to their most recent entry into the Great Lakes, ranked by the number of “positive” tanks. Tanks with water from Antwerp have the greatest pathogen frequency, with other European ports and the Port of Matanzas (Cuba) and Maracaibo (Venezuela) having moderate pathogen frequency.

While we do not dismiss the potential health concern of these pathogens in arriving ships, it is relevant to consider that no outbreaks or epidemics of cholera, crytosporidiosis, or giardiasis have been associated with NOBOB ship traffic, or for that matter, with ballasting operations of ships in the Great Lakes. The high numbers of bacteria and viruses found within ballast residuals do not imply a high propensity for human disease. The overwhelming majority of these

bacteria are natural, nonpathogenic forms, and their constancy of number is a balance between nutrient supply and grazing by their predators.

Cryptosporidium and *Giardia* certainly are pathogenic to humans, and when encysted, can survive for long times in a dormant state. There are many sources of these protozoans to Great Lakes waters, however, and we do not know the proportion contributed (if any) by NOBOB ships. We suggest further study of these organisms, especially with respect to their genetic variation, as a means to assessing their potential human-health implications.

Harmful algal bloom (HAB) species that produce resistant resting stages (e.g. *Pfiesteria piscicida* or *shumwayae*) or those that don't (e.g. brown tide organism, *Aureococcus anophagefferens*) were detected in 3-10% of residual water and sediment samples. These HAB species tolerate a wide range of salinities, but are unlikely to become established in the freshwater Great Lakes (no demonstrated growth at zero salinity). However, as noted for our phytoplankton results, there is a precedent for the establishment of exotic phytoplankton species in the freshwater Great Lakes, despite their being more commonly found in brackish or marine waters.

In the case of cholera, at least, there likely is little chance for ingestion by humans of the "minimum infective dose", which for cholera is approximately 10,000 to 100,000 cells. Although we do not know the concentrations of cholera bacteria sampled in this Great Lakes NOBOB study (we know only that they were present), we assume they were about the same as a related study performed in the Chesapeake Bay (Ruiz et al., 2000). If so, then a healthy adult would need to drink between one and ten liters of ballast residuals to become ill.

In summary, we have demonstrated the presence (and in some cases, the concentrations) of microorganisms in the ballast residuals of NOBOB ships. Whether these microorganisms are entrained in ballasting operations and are discharged from the ship into the Great Lakes is not known. This uncertainty is a key point to address in the future. Even if we assume microorganisms are discharged, their fate in receiving waters, including their potential to cause disease is not known.

Phytoplankton

Dinoflagellates were also examined by CILER and GLERL investigators. In their analyses, the number of dinoflagellate species appeared to be negatively correlated with ship's age, salinity of the residual water, and whether or not the tank had been flushed with seawater.

These investigators were able to identify cysts of several harmful dinoflagellate species, including cysts belonging to potentially toxic species of the genus *Alexandrium* known to cause paralytic shellfish poisoning. Based upon morphological descriptions in the literature, five *Alexandrium* species were identified. In contrast to results at ODU, no germination was noted for any of the marine dinoflagellates when cultured in five different growth media including: two common freshwater media, one standard seawater media, filtered Grand River water, and filtered Lake Michigan water.

Every ballast sample produced significant phytoplankton growth (evidenced as increased fluorescence) in at least one culture treatment (Table 3.11).

- In 2001, both of the common freshwater media we used produced germination and growth in at least 80% of the samples (Table 3.11). Grand River water produced growth in at least 75% of the samples. The lowest response was found for standard saltwater media, which produced positive growth in only 41 % of the experiments.
- In 2002, both of the common freshwater media we used produced growth in 100% and 63% of the samples respectively, whereas the saltwater media produced growth in 53% of the samples. Filtered Grand River water produced growth in 95% of the samples, but filtered Lake Michigan water produced the lowest response at 21%.

Diatoms were the dominant species that grew in all of our experiments, with lesser amounts of green algae, small flagellates and dinoflagellates. A total of 154 phytoplankton species were found in our experimental treatments, among them were 41 taxa (30 identified species) of non-indigenous diatoms (Table 3.12). All of these non-indigenous diatoms found in the experimental treatments were marine in origin (i.e., described from a marine environment). Nine of these nonindigenous species (NIS) have been reportedly found in the Great Lakes (*Actinocyclus normanii, Actinocyclus normanii fo. subsalsa, Coscinodiscus radiatus, Cyclotella distinguenda, Navicula pelliculosa, Pleurosira laevis, Skeletonema costatum, Skeletonema subsalsum, Surirella ovata v. crumena*; Stoermer et al. 1999).

Although we identified 41 NIS taxa in total from all samples, the actual abundance (i.e., number of cells) of NIS in each sample was relatively low. Specifically, NIS constituted <5% of the total phytoplankton abundance for treatments where positive growth was noted. Almost 30% of our experimental treatments did not have any nonindigenous species present, and only 18% of the experiments had more than 4 nonindigenous species present in the same sample. Only a few taxa were found in a number of samples. Among the 41 non-indigenous taxa, ten appeared in more than 10% of the samples (Table 3.13). These taxa included: *Odontella aurita, Thalassoisira* sp.*, Thalassiosira ecentrica, Actinophycus undulates, Skeletonema costatum, Paralia sulcata, Raphoneis amphiceros, Actinocyclus normanii, Actinocyclus normanii* fo *subsalsa,* and *Coscinodiscus* sp.

Live Invertebrates

Residual Sediment

Thirty-six of the 42 ships sampled were analyzed for invertebrates within residual sediments. Three of these 36 ships had no live invertebrate taxa present within the collected sediment. Nematodes dominated the overall relative abundances (91%), followed by harpacticoid (5%) and cyclopoid copepods (3%). Nematodes occurred in 91% of ships entering the Great Lakes, harpacticoids 46%, and cyclopoids 49%. Based on our samples, these taxa contribute almost 99% of all organisms entering the Great Lakes associated with ballast sediment.

A total of 35 copepod species were identified from the remaining 33 ships, including twenty harpacticoid species. Three of the harpacticoid species were nonindigenous but already established in the Great Lakes. Two other nonindigenous freshwater species were identified that do not have a known population in the Great Lakes. Three species were freshwater taxa which

are native to the Great Lakes. Four species were classified as brackish water fauna and the remaining eight were marine.

Eleven cyclopoid species were identified, ten of which are freshwater species. Six of these freshwater species are known from the Great Lakes, including *Cyclops strenuus*, a probable earlier invader that was recorded from two ships. Four other nonindigenous freshwater species were identified that do not have established populations in the Great Lakes. Also, one species of marine calanoid copepod, and two species of marine poecilostomatoid copepods were recorded.

Residual Water

The taxonomic composition of water fauna differed greatly from that of sediments. Copepods comprised the most abundant group in residual waters (97.3% of abundance per ship, consisting of (on average) 66.0% nauplii, 20.4% cyclopoids, and 10.8% harpacticoids, with calanoids and poecilostomatoids comprising the remainder). Rotifers were the next most abundant taxon at 1.2% of total abundance. Remaining taxa collectively comprised <1.5% of total abundance.

Copepods were also the most species rich group, with five calanoid, twelve cyclopoid and ten harpacticoid taxa recognized. This total includes thirteen species already recorded from the Great Lakes, including three that are already established. Ten of the remaining fourteen species are marine taxa, which presumably would not survive if introduced to the Great Lakes, leaving four freshwater or brackish-water species that could potentially tolerate conditions in the lakes.

At least eight cladoceran species were recorded, of which three are not established in the Great Lakes; one of these, *Daphnia magna*, is a North American species, while the other two, *D. cristata* and *D. atkinsoni*, are European natives.

Seven rotifer species were identified, all of which are native to the Great Lakes. At least three *Gammarus* species (Amphipoda) were identified, all of which are from European estuarine brackish waters. Small bivalves were recorded on several ships, including *Driessena* veligers. However, these were typically low in abundance and frequency overall.

A statistically significant relationship was found between pore water salinity and total animal abundance in sediments, with lower abundances at higher salinities, although the explained variance was low. None of the other variables assessed were important in determining animal abundances. Similar results were found for residual water data, with a significant inverse relationship between salinity and total invertebrate abundance.

No clear relationship existed between total numbers of animals and region of ballast origin, except that areas with medium to high salinities (> 20‰) had lower median abundances than those with salinities < 20‰. However, examination of fresh and brackish water animals showed a clear affinity for ships that had taken their last ballast from low salinity ports, with those on the North Sea, Great Lakes and Baltic Sea having the highest abundances as compared to other regions. This may indicate that fresh and brackish water taxa are relatively transient, and dependent on the last ballast source.

The average number of NOBOB ships entering the Great Lakes between 1994 and 2000 was 484, of which 249 subsequently loaded and then discharged mixed Great Lakes ballast water into the Great Lakes (Colautti et al. 2004). Ships sampled as part our Task 1 invertebrate analyses averaged 15 t (= 15000 kg) of ballast sediment and 46.8 t (= 46800 l) of residual water. Thus, at average animal densities of 1322.5 $ind \cdot kg^{-1}$ in ballast sediment, NOBOB ships carried

approximately 49.5 x 10^8 individuals into the Great Lakes basin in sediment each year between 1994 and 2000 (Table 3.15). Similarly, the average number of propagules carried annually in residual water is 12.7 x 10^7. Thus, on average a total of 50.7 x 10^8 sediment and water-borne animals may have the opportunity for introduction to the Great Lakes each year via NOBOB vessels. However, only 22.6 x 10^7 propagules were freshwater or brackish rotifers, cladocerans and copepods that may pose a risk of invasion. Some of these taxa may already exist in the Great Lakes, and may have originated from previous ballasting in the Great Lakes. Thus, the average propagule supply of nonindigenous freshwater and brackish copepods and cladocerans potentially entering in residual sediments (see Table 3.16), excluding those having already invaded, was 25.9 x 10^6 individuals per year. From residual water, the average propagule supply of nonindigenous organisms (excluding those having already invaded) was 20.5 x 10^4 individuals per year. Thus, NIS that have already invaded comprised more of the potential nonindigenous propagule supply in the water fraction, while NIS that have not yet invaded comprised much more of the potential propagule supply in the sediment fraction.

Invertebrate Resting Stages

The density of invertebrate resting stages in ship sediments had a lognormal distribution, ranging from 4.0 x 10^4 to 9.1 x 10^7 resting stages·t^{-1} (median and mean values of 7.2 x 10^5 t^{-1} and 3.6 x 10^6 t^{-1}, respectively). Taxonomic identity based on resting stage morphology was made for 12 groups from the sediment collected.

We hatched 76 distinct taxa from resting eggs separated from sediment residuals collected from 36 ships. Twenty-one NIS were identified, consisting of 14 rotifers and seven cladocerans (Table 3.17). One of the NIS identified, *Bosmina maritima*, is already established in the Great Lakes. However, both the total abundance and frequency of occurrence of NIS was low in comparison to species considered native to the Great Lakes.

Analyses indicated that higher salinity and lower temperature each suppressed total abundance and species richness of hatched taxa independently, and there was no interaction effect for salinity+temperature on either variable.

In whole-sediment experiments, 21 taxa were hatched, although six sediments had no animals emerge under any treatment regime. Burial in sediment significantly decreased both total abundance and species richness of hatched taxa, with only 0-43% of the individuals successfully hatched from isolated resting stages emerging from buried resting stages.

Resting stage density was weakly correlated to the salinity of residual ballast water. All other ballast history variables were found to be insignificant in relation to resting stage density.

Incorporation of experimental values for resting stage density, viability and sediment tonnage into our propagule pressure model (Eqn. 1) revealed that NOBOB ships in this study carry up to 1.2 x 10^8 viable resting stages·$ship^{-1}$, with a mean density of 1.0 x 10^7 (Fig. 5). However, resting stages from sediments of 13% of ships sampled could not be induced to hatch in the laboratory under any conditions, and were apparently non-viable in freshwater. Thirty-two percent of the ships sampled carried resting stages of NIS, at densities up to 4.5 x 10^6 resting stages·$ship^{-1}$ (Eqn. 2).

The mean density of active animals transported in residual sediments sampled in this study, 49.5 x 10^8 $year^{-1}$, is higher than that of dormant stages from the same set of sediments, 24.5 x 10^8

year^{-1}. However, many of the active and dormant sediment taxa are buried or have adaptations to ensure they remain in association with sediments, even during flow or turbulent conditions, and will thus have little chance for discharge from ballast tanks. As such, only a small proportion of sediment taxa (approximately 8% or less) are likely to have the potential to enter the lakes with discharged ballast at their final Great Lakes port-of-call. Risk is likely to vary by taxon, however. For example, nematodes will occur within the sediment and are less likely to be discharged, while more epibenthic taxa (e.g., harpacticoids) may be discharged more readily. This may reflect why most of the common epibenthic nonindigenous organisms found in this study have already invaded the system.

In contrast, active invertebrates in residual water are available for discharge at a mean density of 12.7×10^7 year^{-1}. Despite the density of active and dormant taxa in sediments being greater than the number of invertebrates in residual water, planktonic animals likely have greater opportunities for discharge with ballast water (see MacIsaac et al. 2002). Evidence for this comes from the difference between the numbers of freshwater and brackish NIS which have and have not invaded the system to date; a large proportion of the nonindigenous propagule supply in the water fraction is comprised of taxa that have already invaded (87%), while only 11% of the nonindigenous propagule supply in sediments have invaded to date. Thus, despite sediments containing higher densities of nonindigenous propagules overall, only the most frequently occurring epibenthic species may be able to invade the Great Lakes system. Further, *in situ* hatching studies suggest that less than 1% of invertebrate diapausing eggs will hatch and be available for introduction (see Task 2 section). Therefore, fresh and brackish residual ballast water may pose the greatest risk for introduction of invertebrates.

Task 2: Filled Ballast Tank Experiments

The objective of Task 2 was to measure the effect of adding Great Lakes water as ballast to NOBOB tanks on germination and growth of nonindigenous species present in ballast residuals and on their potential release from ballast tanks. .

Microbiology

In general, VLP and bacteria abundance declined by about a factor of 2 during ballast transit within the Great Lakes. Provided more water wasn't added to tanks, chlorophyll-*a* concentration also declined by greater than 97%.

Pathogens were detected intermittently during most ballast transits through the GL, but there was large variability between experiments.

Phytoplankton

In general, phytoplankton species diversity declined during vessel transit (Fig. 4.9) and there tended to be a shift in species dominance, with the potentially harmful blue-green alga, *Microcystis*, being the favored competitor in ballast tanks (Fig. 4.11). .

Invertebrate Resting Stages

In all, five Task 2 experiments were completed during the project, all aboard bulk carriers (Table 4.1). Resting egg hatching experiments using Emergence Traps (IETraps) were conducted during the last four voyages between October 2002 and September 2003.

IETraps remained submerged for 6 to 11 days, depending on ship schedule. In total, 19 individuals were hatched from 41 experimental replicates, producing an average hatching abundance of 0.5 individuals per 500g replicate (Table 4.8). All live control animals were recovered alive, indicating that environmental conditions within traps could support life for the duration of each voyage.

Diapausing eggs were not as likely to hatch *in situ* as under laboratory conditions. Both total abundance and species richness of organisms hatched was significantly lower *in situ* than in laboratory characterization trials. In addition, the effect of burial appeared to have a significant impact on the number of eggs that hatched.

Despite the fact that each NOBOB vessel may carry 15 t of sediment, the probability that NIS will be present and receive hatching cues is small, and the calculated inoculum size is estimated to be 87-375 individuals per taxa per ship. Approximately 250 NOBOB vessels conduct multi-port operations on the Great Lakes each year that may provide conditions for hatching and introduction of resting stages (Colautti et al. 2004). We estimate that approximately 32% of these vessels will carry resting stages of NIS (Bailey et al. in press), providing a frequency of ~80 inoculations per year. This translates to approximately 5.7×10^3 to 3.0×10^4 nonindigenous individuals being introduced via the residual sediment vector per year.

Instrumented Emergence Traps

On one occasion we conducted an *in situ* IETrap experiment that included one water quality sonde imbedded in the trap and one mounted adjacent to and outside the trap during the ballasted deployment. The environment inside the IETrap went hypoxic over the first two days, but a number of re-oxygenation events were recorded during the voyage that coincided with periods when the ship was in transit. In spite of strong evidence that the traps likely go hypoxic or anoxic due to biological and/or chemical oxygen demand associated with sediment, hatching of diapausing eggs did occur inside IETraps during shipboard experiments, albeit at a very low rate (see above). Our live-animal control results also suggested that conditions were sufficient to maintain both *L. variegatus*, which can survive under low oxygen conditions, and *H. azteca*, which is known to be particularly sensitive to poor ambient conditions. If oxygen demand associated with sediment inside IETraps during *in situ* experiments is causing hypoxia or anoxia, hatching results from trap experiments should be viewed with caution and may underestimate the hatching potential of diapausing eggs in ballast tanks. Redesign and further testing of the IETraps is necessary if they are to be routinely used for *in situ* hatching experiments that include sediment.

Live Invertebrates in Filled Ballast Tanks

Zooplankton densities for all of the taxa across all voyages tended to decrease as voyage length increased. However, during voyages 1, 3 and 5, Rotifera species increased in abundance as the voyage progressed to the upper Lakes.

Several NIS were detected in the Great Lakes water loaded in the lower lakes as ballast at the start of experiment voyages 1, 2, 3 and 4, including: the calanoid copepod *Eurytemora affinis*, the fishhook waterflea, *Cercopagis pengoi*; and the amphipod, *Echinogammarus ischnus*. Although these organisms are already present in the lower Great Lakes, and thus their presence in the filled NOBOB tanks is not a surprise, ballasting in the lower lakes by NOBOBs presents a risk of spreading such species to the upper lakes, as was the case with *Cercopagis*, which was first introduced in Lake Ontario.

Two NIS rotifers that are currently not found in the Great Lakes were detected in ballast water samples; *Brachionus diversicornis* (voyage 4) and *B. leydigi* (voyage 3). The former was also detected in harbor samples collected during the same voyage and may constitute a new invasion by this species. *B. leydigi* was detected in the tank 10 days after ballasting and may have hatched in tank, so we cannot deduce a possible new invasion from its presence in our samples.

Task 3: Ballast Water Exchange Experiments

The goal of Task 3 was to test the effectiveness of open-ocean exchange for vessels arriving to the Great Lakes from fresh and brackish water European ports. We conducted three successful on-board ballast exchange experiments under this Task.

For all three experimental voyages, we initially targeted ships making repeat voyages from low salinity ports in the Baltic/northern Europe region to the U.S. east coast or Great Lakes region. However, it proved exceedingly difficult to identify suitable candidate vessels. Some of the most common difficulties included the fact that vessels were: (1) from high-salinity source ports or berths; (2) traveling fully loaded with cargo and carrying no ballast between Europe and the U.S.; (3) too small to accommodate the research team; and/or (4) fitted with ballast tanks whose physical design (e.g., depths or configurations) prohibited access or were incompatible with required sampling methods. For these reasons, we found it necessary to shift our efforts to locating vessels originating from any low salinity port and engaging in a voyage of at least 5 days length, regardless of the destination port. Locating vessels was quite difficult even using these broadened search criteria.

The final result of our ship search was three voyages originating from ports in three different geographic areas (Rotterdam, The Netherlands; La Baie, Canada; and Benicia, California). For each of the three ports selected, advance information indicated that it would be a suitable low-salinity port. However, the salinity was higher than anticipated upon the research team's arrival in each location. The discrepancy was the most extreme at the Port of Rotterdam, where source ballast salinity averaged ~30 ppt. The research team chose to continue with this experiment (*Berge Nord*), the results of which provided a measure of comparison between exchange efficacy with high salinity water and exchange efficacy with low salinity water. The second and third voyages (*Federal Progress* and *Kenai*), while starting at higher than optimum lower salinities, were comparable to each other because they had intersecting ranges of starting salinities (*Federal Progress*: 11-19 ppt; *Kenai*: 15.4-15.8 ppt). Although not in the preferred range of 5

ppt or less, the initial salinities for these two voyages were still sufficiently below those typical of the mid-ocean to allow analyses based on changes in salinity.

Physical Tracer Estimates of BWE Efficacy

Changes in the concentration of the physical tracers, salinity and rhodamine dye, were used to calculate ballast water exchange efficacy with regards to removing the original water mass from the tanks. Overall, exchange efficacy with regard to the water mass was high among all three vessels. Exchange efficacy based on salinity measurements ranged from 80.0% (*Federal Progress*) to 100.1% (*Berge Nord)***.** Exchange efficacy based on rhodamine-dye ranged from 86.4% (*Berge Nord*) to 98.5%, (*Federal Progress*).

There was no noticeable difference in calculated exchange efficacies when comparing type of exchange [flow-through (*Berge Nord*) vs. empty-refill (*Federal Progress* and *Kenai*)] or starting salinity [high (*Berge Nord*) vs. low (*Federal Progress* and *Kenai*)].

Biological Results

The efficacy of exchange in removing biological specimens was more variable, both among and within vessels. This variability may be attributed to a number of different factors. In the case of the *Federal Progress,* both of the zooplankton target taxa (*Eurytemora* sp. and Rotifera) declined by 99.9% from their original abundance in the control tanks. This result could be due to a number of factors.

(1) After boarding the *Federal Progress* and discussing which tanks were available to us, we realized that the #3 tank pair was the only pair that could work as our control tanks. Later we were informed that this tank pair had been recently treated with an anti-rust treatment. The treatment was greasy to the touch and left a slippery film on equipment used in the tank. It also had a strong petroleum odor that was noticeable before and after the tanks were filled. This chemical treatment may have had a negative effect on the survivorship of organisms entrained in these tanks and accelerated any natural attrition that took place.

(2) All tanks on this voyage underwent an extreme temperature change between initial sampling at T0 and final sampling at T1. The control tanks experienced an approximate 15˚C increase in temperature in this timeframe. Temperature stress may have played a role in the attrition rate in these tanks (and possibly in the exchange tanks, as well).

(3) There was significant seiching (i.e. “sloshing”) in all the tanks of this vessel. Organisms in these tanks may have been subjected to a heavy battering as a result.

In cases such as this, a very large decline in the control tank makes it difficult to accurately assess the effect of exchange. Since exchange efficacy is assessed as a change in concentration that occurs only as a result of the exchange process, one must account for what would happen “naturally” in the tank without the exchange (i.e. what occurs in the control tank).

A total of 16 zooplankton taxa met the criteria for use as target experimental taxa across all three voyages to estimate exchange efficacy on the basis of changes in organism density. No single taxon qualified as a target on more than one vessel. The majority of these taxa experienced

changes in density between -85% and -100% in the exchange tanks. Two taxa, Polychaeta and Gastropoda, actually increased in one of two exchange tanks following exchange on the *Kenai*. Changes in the control tanks were more variable than those in the exchange tanks, with the majority of targets changing in abundance between -30% to +15%.

Comparison across target taxa indicates that in most cases, ballast water exchange efficacy was >90% (Table 5.20). For five of the targets, exchange efficacy was between 95% and 100% in both tank pairs of their respective vessels. Of the targets that were present in more than one tank pair, exchange efficacy values were generally within ± 3% of each other between the tanks of a vessel.

Overall, the empty-refill treatment more consistently had a negative effect on survivorship than did the flow-through treatment. Four taxa experienced 100% mortality in both the flow-through and the exchange treatments (Rotifera, Cladocera, *Acartia* spp. and *Eurytemora* spp.); however, these 4 taxa also experienced higher mortality in the control dishes than the majority of taxa tested. It is possible these taxa are more sensitive to handling in the laboratory than others, as well as being sensitive to increased salinity. Groups that exhibited high survivorship in both of the exchange treatments *and* the controls are the ones that warrant close scrutiny with regard to invasion potential.

Only four taxa qualified as phytoplankton target taxa across the three voyages (Table 5.20). As with the zooplankton, no single taxon qualified as a target on more than one vessel. All targets declined between 60-100% in the exchange tanks and, unlike the zooplankton, all phytoplankton targets decreased in concentration in the control tanks (Fig. 5.28). As a result, exchange efficacies for phytoplankton were considerably more variable than for zooplankton, ranging from –1.6% to 100% (Table 5.20).

While we didn't identify specific bacterial or viral targets for calculating exchange efficacy, we calculated the percentage change in the total abundance of bacteria and viruses to see if there were any differences as a result of exchange. Table 5.21 shows that for 2 of 3 experiments, bacteria abundance declined in tanks as a result of exchange—only on the *Berge Nord* did it increase. Further, VLP abundance declined in tanks as a result of exchange in 2 of 3 experiments—only on the *Kenai* did it increase.

Thus, the three ballast water exchange experiments conducted for Task 3 resulted in exchange efficacies of 80% to 100% for the majority of parameters measured. Exchange efficacies based on the removal of the original water mass (as measured by changes in physical tracers) fell consistently within this range. Exchange efficacies for individual biological tracers were somewhat more variable, both among and within the ships tested, but the majority were exchanged at 80% or greater efficacy. Exchange efficacy was not noticeably different between high-salinity ballast water and low-salinity ballast water or between flow-through and empty-refill methods of exchange for the ships compared in this study.

Salinity Tolerance

Given the limited availability of organisms suitable for shipboard experiments, it was necessary to expand the scope of this project to include laboratory-based salinity tolerance experiments to address the question of whether "salinity shock" improves the effectiveness of ballast water exchange with respect to killing organisms from low-salinity environments that remain in the

tank following mid-ocean exchange. Experimental trials run on 14 different zooplankton taxa from low-salinity and freshwater habitats in the Upper Chesapeake Bay watershed showed a variable response to high salinity exposure across taxa, with empty-refill exchange having the most significant negative effect on survival (Fig. 5.24).

Ballast Water Exchange Summary

The combined results of our ballast water exchange and salinity tolerance experiments make it evident that while exchange is highly effective for reducing the concentration of organisms (i.e. zoo-, phyto-, bacterio- and virio-plankton) entrained at a source port (regardless of salinity), the range of tolerance to high salinity exposure that exists across low-salinity taxa makes it difficult to generalize about the frequency with which species from low-salinity environments are killed by "salinity shock" via mid-ocean ballast water exchange. Variability in tolerance to salinity changes is well known among coastal organisms from low-salinity environments; however the range of tolerance is poorly documented for the majority of species. Further studies are needed to close this gap in knowledge. To address this issue we are conducting a follow-up study, also funded by the Great Lakes Protection Fund, in which we are collaborating with European colleagues on an extensive series of laboratory experiments to characterize the effects of salinity exposure on a wide range of zooplankton species found in Northern European low-salinity source ports. These experiments are modeled upon the Chesapeake Bay laboratory experiments conducted during this study, and will place a priority on species that are considered "high risk" as potential Great Lakes invaders.

Chapter 1: Introduction

Over the last decade, much attention has been focused on ballast water as a vector for nonindigenous species introductions to the Great Lakes and marine coastal ecosystems, and on open-ocean ballast exchange as a defense against new introductions. However the issue of NOBOB (no-ballast-on-board) vessel operations in the Great Lakes has risen from a position of relative obscurity to become a major concern in the Great Lakes basin today. On average, less than 20% of ocean vessels entering the Great Lakes in recent years contained declarable ballast water on board (U.S. Coast Guard, pers. comm..; Grigorovich et al., 2003) and many of those vessels with declarable ballast had some empty tanks as well. NOBOB vessels and individual tanks with unpumpable and therefore undeclarable ballast escape scrutiny under existing U.S. and Canadian federal, state, and provincial laws, yet the residual volumes of unpumpable ballast water and sediment may contain live aquatic organisms and resting stages - eggs, spores, and cysts - accumulated over numerous previous ballasting operations.

This multidisciplinary research program was designed to directly assess the potential invasion vectors represented by overseas vessels operating in the Great Lakes. It provides the beginning of a scientific foundation for developing new policies and for identifying effective preventive measures and treatments. The goals of our study were to (1) greatly expand the biological and physical characterization of NOBOB tanks beyond that presently available, (2) assess the invasion risk in proportion to the amount of sediment and water residuals accumulated within the 'empty' ballast tanks, (3) measure the relationship between ship management practices and invasion risk to determine if certain management practices appear to reduce the risk posed by NOBOB vessels, and (4) quantify the effectiveness of open-ocean exchange in decreasing the diversity and concentration of nonindigenous species that enter the Great Lakes in "exchanged" ballast water.

1.1. Background

Global shipping moves roughly 80 percent of the world's commodities and is fundamental to world trade (National Research Council, 1996). As an unintended result of global trade activities, numerous cases of nonindigenous species introductions have occurred worldwide. In total, over 180 nonindigenous species are established in the Great Lakes (Holeck et al., 2004, Ricciardi, pers. comm.), and approx. 40% of all documented invasions in the Great Lakes are thought to be attributable to shipping activities (A. Ricciardi, unpubl. data). Furthermore, the apparent rate of introductions, as reflected in published reports of new species, has continued to increase during the past few decades (Carlton 1985, Jones 1991, Mills 1991, Ricciardi 2001).

Recognition of the potential impacts of nonindigenous species focused government, public, and scientific attention on the role of shipping as a dispersal vector. In 1990, the U.S. Congress enacted P.L. 101-646 (the Nonindigenous Aquatic Nuisance Prevention and Control Act) to help prevent future introductions. In particular, the discharge of ballast water from transoceanic vessels was targeted as a principal vector for nonindigenous aquatic species. As a result, regulations were promulgated in 1993 by the U. S. Coast Guard requiring ships carrying ballast water inbound to the Great Lakes to use an approved ballast-water management scheme that

would be biologically protective of the Great Lakes. In practice, open-ocean ballast water exchange (BWE), with a target of 95% volumetric exchange, is the only strategy currently available for commercial ships to reduce the quantities of nonindigenous coastal species in ballast water (National Research Council 1996). However, the efficacy of open-ocean BWE with respect to minimizing species introductions has been unclear, and these regulations did not address the residual unpumpable ballast in NOBOB vessels.

Some scientists regard BWE as a semi-permeable filter at best (e.g. Locke et al. 1993). Ships sampled during 1995 at the entrance to the Great Lakes carried an assortment of live marine, brackish and freshwater fauna, despite having reported that they fully exchanged ballast water on the open-ocean (Harvey et al. 1999). Locke et al. (1993) determined that up to one-third of ships that declared mid-ocean exchange still contained live, freshwater-tolerant zooplankton. Furthermore, Dickman and Zhang (1999) found only a 48% difference in the densities of diatoms and dinoflagellates between exchanged and unexchanged ballast tanks. The euryhaline fishhook waterflea, *Cercopagis pengoi*, almost certainly invaded Lake Ontario since 1993, well after implementation of mandated open-ocean exchange (MacIsaac et al. 1999). These examples, together with perceived problems regarding BWE as an effective strategy, have resulted in significant efforts focused on developing and testing alternative ballast-water treatment technologies, such as the multi-million dollar "*Great Lakes Ballast Technology Demonstration Project"* supported by the Great Lakes Protection Fund. However, even at its highest theoretical effectiveness, BWE can never be a completely effective barrier to all species introductions, because of "NOBOB" (no-ballast-on-board) vessels.

NOBOBs are ships that have pumped out their ballast tanks as much as possible, until the ballast pump draws air. Design, maintenance, and/or operational considerations invariably preclude discharge of 100% of the water in a ballast tank, and tanks are thus rarely completely dry. Ballast tanks are also rarely free of residual sediment and the presence and accumulation of sediment entrained into tanks during ballasting operations is especially problematic and significantly reduces the effectiveness of the ballast-exchange treatment approach. Furthermore, sediment may reduce the effectiveness of other treatment approaches applied directly to the tanks or to incoming ballast water. Specifically, sediment in suspension will seriously denigrate the effectiveness of treatments such as UV radiation and ultrasonic waves, and requires the addition of significantly higher dosages of chlorine, ozone, hydrogen peroxide, and possibly some of the organic acid biocides currently under consideration for treatment approaches (Sano et al., 2003, 2004). Early analysis (Reeves, 1997) of vessel traffic entering the Great Lakes indicated that more than 85% of the oceanic vessels entering the Great Lakes are NOBOBs and thus not subject to the ballast water management regulations of 1993.

The potential for NOBOB-associated invasive-species introductions lies within their ballast residues, which can contain not only a wide assortment of viable larval and mature plants, animals, and microorganisms, but also "resting stages" (Locke et al., 1991; Hallegraeff and Bolch 1992; Locke et al. 1993; Galil and Hülsmann 1997; Dickman and Zhang 1999; Hamer et al. 2000). Life cycles of many invertebrate, phytoplankton (including toxic dinoflagellates), protozoan and bacterial species include the capability of producing resting stages (variously called cysts, ephippia, resting eggs, or spores according to taxon). The ability to produce resting stages ensures the long-term viability of a population because resting stages are extremely resistant to adverse conditions, including anoxia, noxious chemicals, freezing, and passage

through digestive tracts of fish and waterfowl. Resting eggs of invertebrates and cysts of dinoflagellates are usually negatively buoyant and sink. Resting stages may remain viable in sediments in a virtual suspended metabolic state for decades or even centuries (Hairston et al. 1995) and can germinate when exposed to favorable light, temperature and/or other environmental queues. The importance of invertebrate, phytoplankton, and microbial resting stages to invasion potential in the Great Lakes had not been determined prior to this (Great Lakes NOBOB Assessment) project.

Sediment accumulation in ballast tanks can be appreciable, depending on elapsed time since the ship was last drydocked (Hamer et al. 2000). A synopsis of 13 separate European studies recorded a total of 990 different species in a combination of ballast water and sediment samples (Gollasch et al. 2000). Furthermore, Kelly (1993) reported that Japanese ships visiting the USA carried viable cysts and spores of nonindigenous species after 11-15 days' voyage. In this regard, tank sediments serve as a repository for particles, living or otherwise, that settle from water within the tank. Therefore, sediment and water residuals within a NOBOB tank will contain an integrated assortment of organisms found in the source water of multiple ballasting operations that have occurred over time scales ranging from days to possibly years in the case of resting stages.

It is these issues that make it critically important to better understand NOBOB vessel operations, to conduct an assessment of the biological conditions in NOBOB tanks, and to better understand and document the effectiveness and limitations associated with BWE. These are the drivers of the research we report here.

1.2. Study Rationale

1.2.1. NOBOBs

Considering that NOBOB ships constitute the bulk of commercial ship traffic entering the Great Lakes, we undertook this study to examine the potential risk of invasions associated with their residual ballast water and sediment, and to assist in developing recommendations with respect to treatment strategies.

Reeves (1997) appears to have been the first to describe the general pattern of operations of NOBOB vessels while in the Great Lakes, especially the potential for their ballast residuals to mix with and be discharged with new ballast water added in the Great Lakes. Great Lakes water taken on as ballast by a NOBOB vessel to maintain trim and stability during operations mixes with residual ballast water, sediment, and any associated nonindigenous organisms, and is later discharged into the Great Lakes as the vessel moves between a succession of ports. Thus, ballast-water operations of NOBOB vessels present a hypothetical risk of invasion, but the magnitude of such risk had not been examined scientifically. Furthermore, little was known about the relationship between accumulation of sediment, biological content, and ballast-water management practices of ocean vessels, and their patterns of operation in the Great Lakes. More specifically, there were few scientifically credible data to characterize or assess (1) the composition and abundance of biological communities in NOBOB ballast tanks; (2) the effect of current ballast

management practices on the accumulation of sediment and organisms in ballast tanks; (3) the significance of filling and discharging NOBOB ballast tanks to the potential introduction of nonindigenous organisms to the Great Lakes; and (4) the operational patterns of NOBOB vessels within the Lakes. Reliable, systematically collected information was needed to provide a basis for decisions about the need for regulation, and for identifying management approaches and/or treatment strategies that have the highest likelihood for success in preventing and controlling new introductions via the NOBOB vector.

1.2.2. Ballast Water Exchange

Despite popular perception, there exist few published quantitative studies of the biological effects of ballast water exchange. Furthermore, most of these previous studies ignored microorganisms and those taxa that form cysts. These limitations represent fundamental gaps in understanding the performance of ballast water exchange as a barrier to future invasions.

In particular, a crucial consideration for the Great Lakes is the effectiveness of open-ocean ballast exchange when the original ballast is fresh or low salinity water, which differs in density and biota from open-ocean saline water. The freshwater regions of Europe and especially the coastal regions of the Baltic and Black Seas have been implicated as source regions for most of the Great Lakes invaders found since 1985 (zebra mussel, quagga mussel, round goby, tubenose goby, amphipod *Echinogammarus ischnus*, the fishhook waterflea, *Cercopagis pengoi*, and the diatom, *Thalassiosira baltica*, see Ricciardi and MacIsaac 2000). Many of the aquatic organisms found in these regions (a) are euryhaline and may survive exposure to higher salinities and (b) form resting stages that accumulate in bottom sediments and are difficult to remove with exchange. Therefore, the effectiveness of exchanging freshwater from these regions for open-ocean saltwater is an important, largely unresolved question to consider when evaluating how well ballast exchange protects the Great Lakes from new invasions.

1.3. Work Plan

In order to meet these project goals, we pursued the following three interrelated objectives:

- Task 1: Characterize biological communities (invertebrates, phytoplankton, and microorganisms) present in NOBOB tanks and correlate these findings with ballast management practices and ballasting history.
- Task 2: Measure the effect of adding Great Lakes water as ballast to NOBOB tanks on germination and growth of nonindigenous species present in ballast residuals and on their potential release from ballast tanks. .
- Task 3: Test the effectiveness of open-ocean exchange for vessels arriving to the Great Lakes from fresh and brackish water European ports.

The presentation of our findings in this Final Report is organized around these three tasks, with an initial chapter devoted to the results of the ballast management practices and ballasting history survey, followed by chapters describing the biological and chemical results associated with each task.

1.4. Communications and Collaborations

The major stakeholders and client communities for the issue of nonindigenous species introduction via ballast water include the ship-owners, management agencies, policy-makers, the scientific community, the news media, and the public at large. Throughout the project we actively pursued communication with major stakeholders concerned with the introduction of aquatic nuisance species in the Great Lakes and disseminated interim project results through a variety of scientific and industry based meetings, publications, interviews, and interim reports. A list of all our presentations and products is provided at the end of this report.

Chapter 2. Water Ballast and Sediment Management in NOBOB Ships

2.1. Ballast Management – a Brief History

"Ballast management" has been the term used to describe one of the fundamental tasks required to safely operate a ship, a practice of basic good seamanship that has existed as long as ships have.

A commercial ship is a self-propelled container for the carriage of people and/or goods. The specific type of goods, or range of commodities that it is intended to carry as commercial cargo will effect its design, whether liquids or solids in bulk or in packaged form, or even vehicles and passengers. There is always a need to replace and properly distribute weight throughout the hull when cargo is removed to maintain stability, balance structural strength, and submerge the hull sufficiently that the rudder and propeller are effective so that directional stability and momentum can be maintained despite the effect of wind or wave. The material that is used as the counterbalance is termed ballast.

In the early days of seafaring when ships were built of wood and propelled by wind and sails, ballast took the form of slate or stones laboriously loaded manually into the holds, to replace the cargo being delivered. Such ballast ultimately ended up in the dockside cobbled roads of the world's major sea ports. But with the advent of steel construction at the end of the nineteenth century, ship owners and designers were able to contemplate integrating compartments into the hull which would carry water as ballast, which could be pumped in as the cargo was being off-loaded and pumped out as the next cargo was being loaded. It represented a major breakthrough in commercial ship operation, significantly reducing time in port and reducing voyage costs. Unlike the original stone ballast, there was minimal labor involvement, and the ballast was both abundant and free.

Inherent with the intake of large quantities of water from alongside of a ship however is the intake of matter floating on or suspended in that water, and many of the ports at which water ballast would need to be taken are situated on the major rivers of the world or in tidal estuaries where natural debris, sediment and mud is constantly in motion and where biota flourish. These are also areas through which the many byproducts of large concentrations of human population drain, particularly those which cause and spread disease.

While it was possible to eliminate the larger constituents by screening the water intakes, it soon became apparent to the shipping community that, particularly on ships engaged in short sea voyages, it was possible to accumulate significant amounts of permanent ballast in the form of mud, which was counter-productive to their commercial interests. This in turn engendered good seamanship practices to minimize such accumulation, the most demanding of which being the manual cleaning of the tanks if all else failed.

While ship owners managed their own ships, set the standards of operation and maintenance, recruited and trained their own officers and crews and established a company culture generally high standards of seamanship were maintained throughout the international shipping industry.

This was the norm in international shipping until the early nineteen seventies. The advent of flags of convenience, offshore registries, and investor ship owners who had no interest in managing their assets, or even in the standards to which those assets were managed, had by then started a downward spiral that, through competitive pressures, slowly enveloped the entire international shipping industry. Management of many ships was outsourced to third party managers, who often outsourced management components themselves, particularly the employment of officers and crew. The experienced, well trained but expensive ship staff from the established seafaring nations were rapidly replaced by cheaper alternatives, crews that often had questionable training and qualifications, and little understanding of the basic tenets of good seamanship.

The era of the substandard ship had arrived, and it would take a mounting toll of ship losses primarily resulting from neglect, a series of catastrophic oil spills and major loss of life from two high profile ferry disasters resulting from poor seamanship, to awaken the national and international agencies responsible for the administration of the industry to the fact that standards had become unacceptable.

Conventions covering Safety of Life at Sea, Standards of Training and Certification for Watch Keepers and Prevention of Pollution from Ships were all negotiated and implemented during the 1970s by the International Maritime Organization (IMO), and became the basis for recovery. This international body, established under a United Nations Convention in 1948, and first convened in 1959, is mandated to establish standards for international shipping, and works through technical committees composed of experts from each of the major seafaring nations. While it has no direct regulatory authority, and while the standards developed by these committees are initially promulgated as non-binding resolutions, when ultimately adopted under specific Conventions it becomes obligatory for all members to develop national regulations pertaining to ships that fly their flag or operate within their waters.

Nevertheless it would take more than twenty years before the industry could really be considered to have recovered to an acceptable level of management quality. During that time period inspection regimes underwent a major overhaul, and new inspection regimes administered by port states were put in place to deal with unscrupulous ship owners who would still try to circumvent the law for financial gain. Documented management systems governing both safety and environmental protection were legislated for both shore and ship staff and are now subject to third party verification and certification on an on-going basis. Failure to achieve or maintain the required standards can cause a ship to be withdrawn from service either temporarily until the deficiencies are rectified to the satisfaction of the inspection body, or in the worst case withdrawn permanently.

From the perspective of ballast management, from a regime that started in North America in 1989 with the introduction of voluntary guidelines for deep ocean ballast exchange to protect the Great Lakes from aquatic nuisance species invasions, the process has developed through national regulations for reporting and management in countries where aquatic species invasions have been considered particularly harmful, such as Argentina, Australia, Brazil, Chile, Israel, New Zealand and the United States, to the point where IMO adopted the International Convention for

the Control and Management of Ships Ballast Water and Sediment in 2004. The cornerstone with respect to the operation of individual ships is a documented ballast management system, with procedures for all aspects of the management of both ballast and residual sediment, the recording of all ballast operations and reporting as required by port state authorities. While the Ballast Water Convention introduces comprehensive measures to prevent, minimize and ultimately eliminate the transfer of harmful aquatic organisms and pathogens through the control and management of ships ballast water and sediments, it allows the participating parties, either individually or collectively to implement more restrictive measures than those stipulated if circumstances warrant, providing that those measures are consistent with international law.

2.2. Ballast and Sediment Management Surveys

2.2.1. Analyses of Vessel Traffic to the Great Lakes

With a major focus of the overall project being the role of NOBOB ships in the transoceanic migration of, and more specifically the introduction of, aquatic nuisance species into the Great Lakes, it is essential to first understand the significance of the issue. The St. Lawrence Seaway opened in 1959. Grigorovich et al (2003) compiled and analyzed records related to saltwater vessel traffic entering the Great Lakes for the period 1959-2000. Colautti et al (2004) focused on a subset of the same records, covering 1978-2000. Both documented the overwhelming predominance of NOBOBs over ballasted vessel entries, especially since the mid-1980s.

We obtained U.S. Coast Guard vessel ballast entry summary statistics for the years 1995-1997 for comparison with the Colautti records for the same period (Table 2.1). In addition, we obtained St. Lawrence Seaway Management Corporation's records for the year 2000. Although the most significant finding, that NOBOBs dominated Great Lakes vessel entries in all years holds across all data sources, there are significant discrepancies in the details reported by each source. For example, for the period of comparison (Table 2.1), the annual average of NOBOB

Table 2.1: Foreign Vessel Traffic Entering the Great Lakes: a Comparison of U.S. Coast Guard Records vs. Colautti et al, 2004 for 1994-2000. U.S. Coast Guard summary statistics provided by U.S. Coast Guard 9th District.

	Seaway	**Colautti**	USCG	**Colautti**	USCG	USCG	**Colautti**	USCG	**Colautti**	USCG
Year	# Salties	**# Salties**	# Salties	**# in ballast**	# in ballast	% in ballast	**# NOBOB**	# NOBOB	**% NOBOB**	% NOBOB
1995		**439**	455	**67**	120	26%	**372**	335	**85%**	74%
1996		**513**	529	**24**	92	17%	**489**	437	**95%**	83%
1997		**476**	574	**29**	93	16%	**448**	481	**94%**	84%
1998		**622**	579	**39**	103	18%	**583**	476	**94%**	82%
1999		**497**	549	**62**	134	24%	**435**	415	**88%**	76%
2000	662*	**554**	577	**64**	159	28%	**490**	418	**88%**	72%
AVG		**527**	544	**43**	117	22%	**484**	427	**92%**	78%
SD		**64**	48	**21**	26	5%	**75**	53	**5%**	5%

* 75 in or with ballast.

entries as a percentage of total entries was 92% ± 5% according to Colautti et al., but 78% ± 5% based on Coast Guard records. The St. Lawrence Seaway Management Corporation data for 2000 indicated that 662 ocean ships entered the Seaway, with 75 (~11%) of those ships in a ballast condition. If we assume the Seaway record is the most correct, then both the Colautti and the Coast Guard records for 2000 severely underreported the total (554 and 557 respectively, vs. 662) by about 17%.

In spite of this the Colautti distribution between BOB and NOBOB entries for 2000 was quite close to the Seaway's (88% vs 89% as NOBOBs). We also obtained U.S. Coast Guard Vessel Inspection and Vessel Ballast Reports for the period 1994-1997 (Table 2.2) and performed a one-on-one comparison of against the Colautti records. For the four comparison years there was a 28-58% range in overlap between the Coast Guard and Colautti records. The Coast Guard records, when they exist for a ship entry, reflect actual ballast condition data reported to and verified (in the case of BOB vessels) by the Coast Guard. However, it should be noted that the Coast Guard assigned a designation of "in ballast" if a ship was carrying any pumpable ballast, even if only in the fore or after peak tank(s). Colautti et al. (2004) did not have records of the actual ballast status of each vessel and designated the ballast status based on whether the records

Table 2.2: Summary Statistics for Comparison of Colautti et al (2004) and U.S. Coast Guard Vessel Inspection Records, 1994 – 1997.

Year	Total # Records Colautti	Total # Records USCG	USCG as % of Colautti	Overlapping Records	Records with Disagree	Records with NOBOB
1994	587	164	28%	157	29	18%
1995	440	172	39%	166	47	28%
1996	508	196	39%	191	58	30%
1997	475	274	58%	260	37	14%

indicated that a ship loaded (BOB) or discharged (NOBOB) cargo at its first port of call. The error rate in the Colautti records that matched the Coast Guard records ranged from 14-30%, averaging 23%, i.e., Colautti had a BOB/NOBOB designation that disagreed with Coast Guard records on average, 23% of the time. In most cases (>92.5%) the disagreement involved a Colautti designation as "NOBOB" when the Coast Guard records indicted "BOB".

A further analysis of the year 2000 Seaway data (see Table 2.1) shows that of the 75 entries designated as in a ballast condition, 28 *had actually entered Canadian waters as NOBOB ships*, but discharged their cargo in freshwater ports between Quebec City and Montreal, where they then took ballast on board. These 28 ships would not have been subject to the deep-water ballast exchange requirements before entering Canadian waters, or to the United States reporting and salinity verification requirements in effect at that time, and therefore, only 47 of the ships that entered the Great Lakes in 2000 would have legally come under the ballast water exchange protection regime. These numbers are illustrative of a pattern that has been developing since the early 1990s as a result of the economic realities of the deep-sea trade into the Great Lakes, where it is imperative to carry an inbound cargo to guarantee the commercial success of the voyage.

Thus during the 2000 international shipping season it can be reasonably assumed that over 90% of the ocean ships entering the Great Lakes would have taken freshwater ballast into tanks which probably contained residuals from offshore, coastal or inland waters and the resulting mixture would ultimately have been discharged at one or more of the Great Lakes loading facilities.

2.2.2. Survey of Vessel Ballast Management Practices and Ballast Residuals

At the start of this research program the exact nature of the residuals on NOBOB ships was unknown, as was the current state of the industry with respect to ballast management particularly with respect to limiting the retention of residuals and the accumulation of sediment and mud in the ballast tanks. For ships employed on regular trades, such as between North Europe and the Great Lakes, the possibility existed that the movement in both directions could be with freshwater residuals, with inoculation thus occurring at either end of the passage, but again these were strictly assumptions.

In order to clarify these issues we carried out a survey of a representative number and cross section of the ocean ship entries into the Great Lakes over the two-year period from December 2000 to December 2002. The success of the survey was necessarily dependent on the cooperation of the trade to permit boarding of ships at their first inbound discharge port to carry out interviews with ship staff based on a standard survey form (Appendix 1) and examine pertinent documentation in order to establish a ballast history for five prior voyages or to the last dry-docking, whichever was the shorter period. In addition, entry into either or both peak and double bottom ballast tanks was undertaken where circumstances permitted to verify data provided and to visually assess the accumulation of both water and sediment accumulation in order to try and gain a better understanding of how mud and sediment accumulates within the tanks, and the effectiveness of management practices used in limiting such accumulation. During this latter process the scientific team took samples of both liquid and solid residuals separately, or of water-sediment slurry, and in cases where ships had retained ballast which had not been subject to exchange, samples of that ballast water extracted through either sounding pipes or through access hatches.

Solicitation was made through the two principal bodies representing the international fleet entering the Great Lakes (Shipping Federation of Canada and the United States Great Lakes Shipping Association), directly to the major participants who are either domiciled or have direct representation in Canada or the United States (Fednav and Canfornav in Montreal, and Polsteam in New York), and through local agents. In all cases the co-operation was excellent and access to vessels was readily provided.

During the survey period between December 2000 and December 2002, a total of 103 surveys were carried out on board ships in Toronto and Hamilton on Lake Ontario, Thorold on the Welland Canal, Cleveland and Toledo on Lake Erie and Detroit and Windsor on the Detroit River. The ships surveyed were considered to reasonably represent the ocean trade into the Great Lakes. Bulk carriers, chemical tankers, and general/project cargo carriers form the nucleus of the fleet, with dry bulk carriers forming almost ninety percent of the entry tonnage. They were operated by 55 individual owners or managers, and registered in 26 different Flag

States. The oldest ship surveyed was built in 1977, the newest delivered in 2002. Ballast capacities ranged between 1485 m^3 and 25533 m^3, with over fifty percent greater than 10000 m^3.

The findings contained in this section of the report are a result of that survey which is summarized in Appendix 2, and observations are based both on the survey, and the experience of the principle investigator (Captain Philip T. Jenkins) in the operation and management of these ships. While they are directly reflective of the current fleet trading into the Great Lakes, they may well be pertinent to the larger shipping community.

2.2.3. Ballast History

During each survey we collected a history of up to the last five ballasting operations from each of the NOBOB ships. From these surveys we were able to establish the location of 160 ballasting operations. The sources of residual ballast being carried into the Great Lakes by the vessels we surveyed were literally from around the world. Western Europe was the region from which the most number of ballasting operations had occurred (Fig. 2.1). The Great Lakes themselves were the second most predominant region where ballasting had occurred.

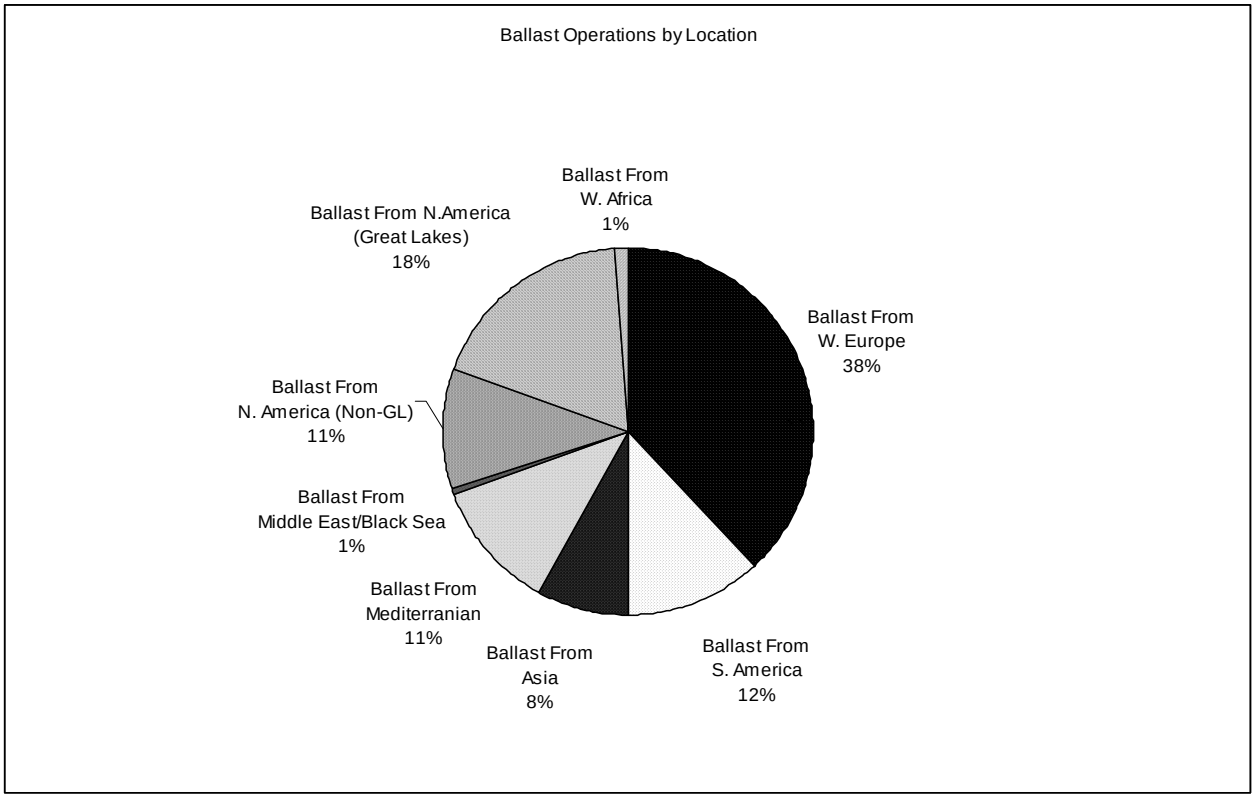


Fig. 2.1: History of up to the last five locations where the NOBOB ship had ballasted prior to the survey.

These survey results are generally consistent with those of Colautti et al (2004). They found that arrivals during the period 1986-1998 were dominated by vessels whose last ports of call were in Europe, especially Western Europe. However, they note that their data may not reflect where the ships in their survey were actually last ballasted, since it is likely that they deballasted and took

cargo, rather than ballast water, aboard at their last ports of call before coming to the Great Lakes. By comparison, the data we present here reflect actual ballast uptake locations compiled directly from ships' ballast logs.

One major difference between our results and those of Colautti et al (2004) is the identification of the Great Lakes as a significant ballasting source contributor. However, given the multiple port cargo operations typical of the Great Lakes salties, this comes as no surprise, but does fill a gap in the otherwise excellent analysis by Colautti et al. (2004).

2.2.4. Ballast Tank Residuals

On the ships surveyed, we found that total ballast residuals ranged from negligible up to 200 tonnes. We also found that ship design and outfitting, technical and operational ship management, and the vagaries of a particular cargo, or combination of parcels of cargo and/or loading ports, can all be contributing factors, and in the final analysis it comes down to the skill of the responsible sea staff on any given ship as to the quantity that remains on board when the cargo loading is complete.

The oldest ship in the survey was built in 1977, the newest delivered in 2002. Sediment accumulations ranged from negligible to 100 tonnes, with sixty percent of the ships estimated to be carrying less than 10 tonnes. *The amount of total residuals (water + sediment, Fig 2.2), and the amount of sediment residuals (Fig. 2.3) were neither closely correlated to the ballast capacity of the ship nor the age of the ship; rather that it is directly related to both the quality of maintenance and management received during it's lifetime, and to the trade in which it is employed.*

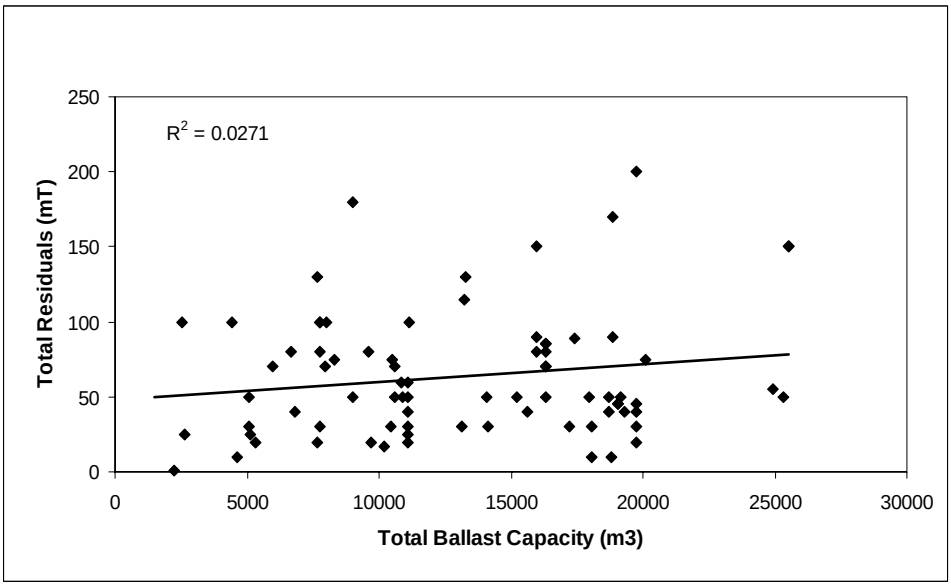


Fig. 2.2: Estimated amounts of total residuals present in the empty ballast tanks of NOBOB vessels against the total ballast capacity of the vessels (n=79).

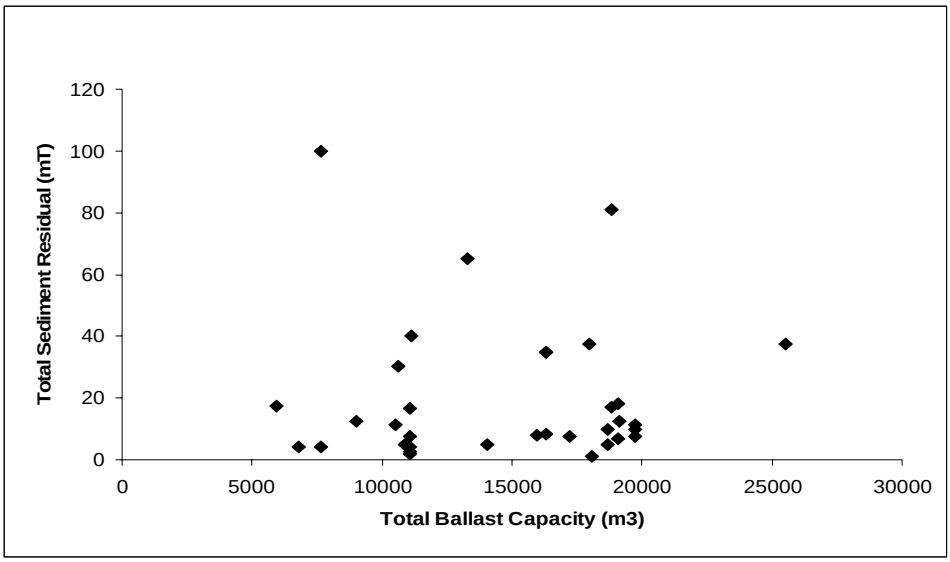


Fig. 2.3: Estimated amounts of sediment residuals present in the empty ballast tanks of NOBOB vessels against the total ballast capacity of the vessels (n=34).

2.3. Structural Considerations

To properly appreciate the issue of aquatic nuisance species inoculation by NOBOB ships it is first necessary to have a basic understanding of the structure of a ship, and of some of the intricacies of the operation of ballasting a ship. The following figures are illustrative of the structure and ballast tank layout on typical dry bulk carriers, which represent the vast majority of the international fleet servicing the Great Lakes. Whether on these, or much larger carriers, residual ballast water, or "unpumpable ballast" is as much a factor of commercial marine transportation as ballast water itself, and there are few ships, particularly in the dry bulk cargo trade, that can completely evacuate their ballast during the course of the deballasting/cargo loading cycle.

Much of the structure that reinforces the box girder that is a ship's hull is contained within the ballast tanks (Figs 2.4, and 2.5), or conversely in modern ship design, those areas of the ship where the structure makes the stowage or handling of cargo impractical are used in some cases as fuel tanks (Fig 2.6), or ballast spaces (Fig 2.7), to enhance either the ship's operational range or and improve it's sea keeping characteristics.

The successful carriage of cargo is the ships prime function. By concentrating the members that reinforce the ships outer shell from both dynamic stress and external forces at the bottom and top of the hull, and at strategic intervals throughout the length of the hull, the designers provide large unencumbered compartments for the carriage of cargo, compartments that are relatively easy to clean between cargoes and which lend readily to the expeditious handling of cargo.

While the transverse web frames in the topside tanks and in the hopper side tank each provide strength to the box girder, the sloping plates they support within the cargo hold function to allow

grain cargoes to self trim and fill all the available space at the top of the hold when loading, and all bulk cargoes to flow to the area directly below the cargo hatch for ease of handling in the latter stages of discharge (Figure 2.6, top).

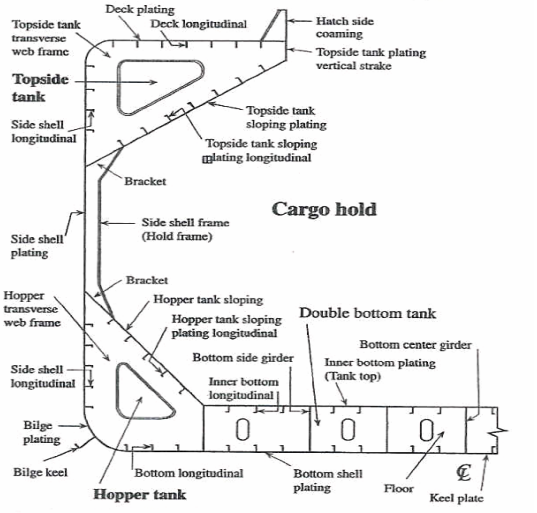


Fig. 2.4: Standard terminology for structural members of a typical bulk carrier, transverse cross section of the cargo hold and adjacent ballast tanks. (Figure reproduced by kind permission of the International Association of Classification Societies – IACS).

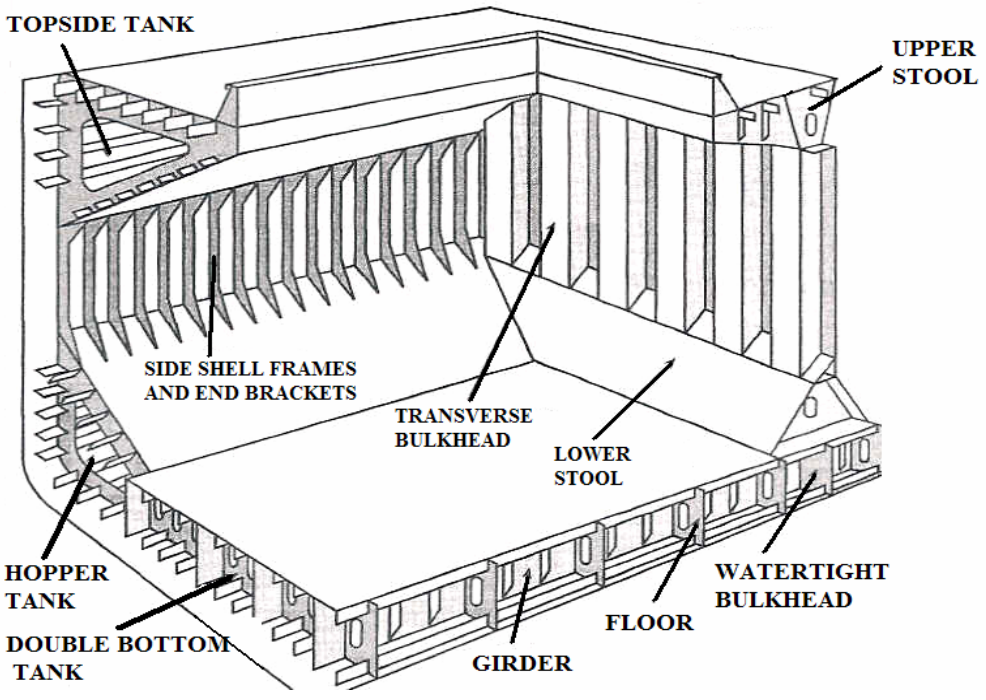


Fig 2.5: Standard terminology for compartments and structural members, a three dimensional view of a typical bulk carrier midsection. (Figure reproduced by kind permission of the International Association of Classification Societies – IACS).

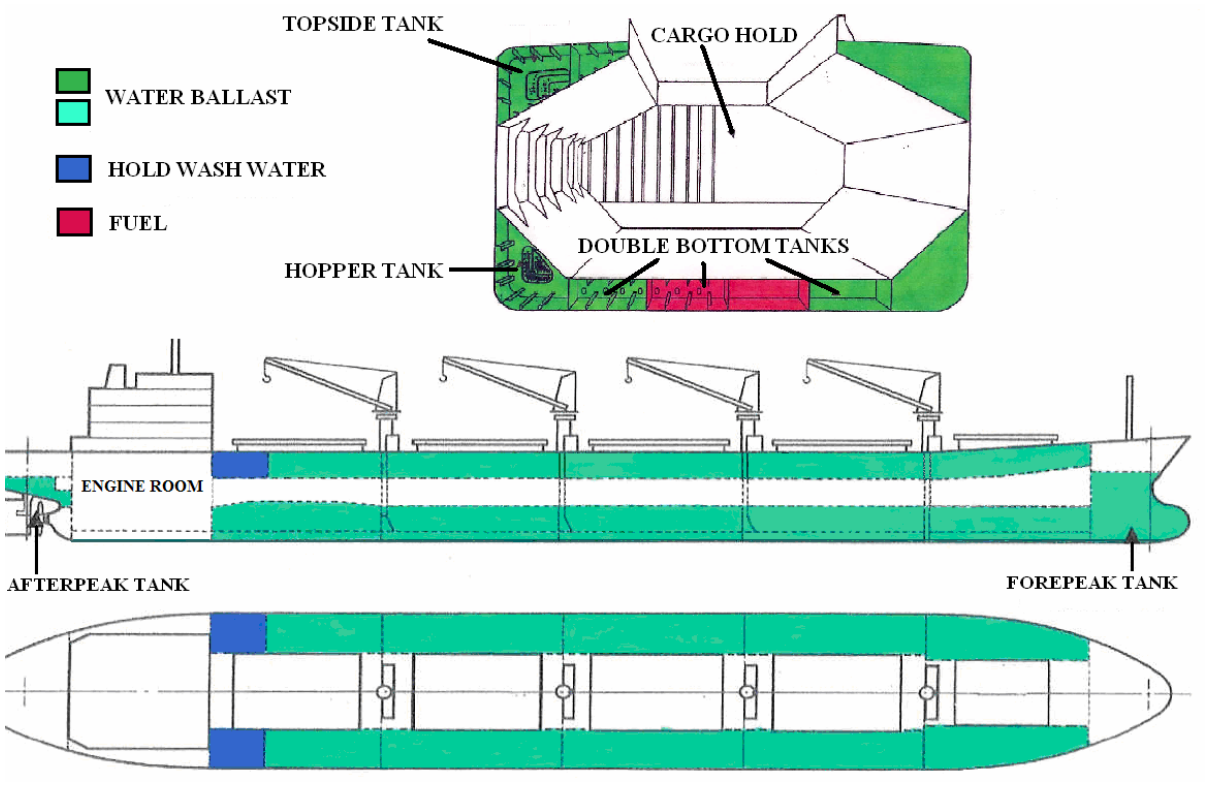


Fig 2.6: This is the most common of ocean going bulk carrier designs currently operating into the Great Lakes. Hopper and double bottom tanks are contiguous. The topside tanks may be completely independent with their own pumping system, or connected by ducts to the hopper, and thus double bottom tanks directly below.

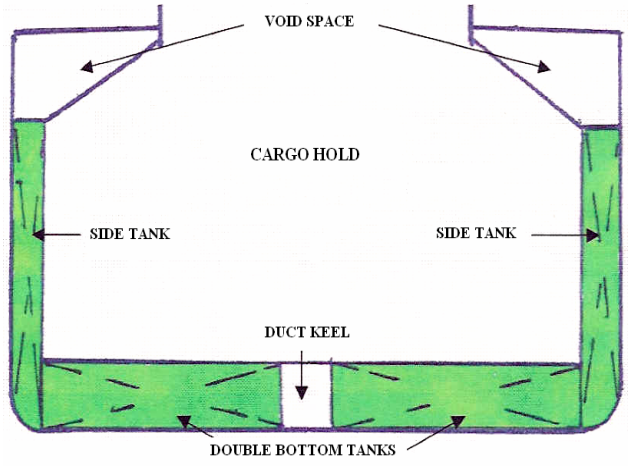


Fig 2.7: This design is intended to increase the ship's versatility with the cargo space more compatible with the carriage of containers and break bulk cargoes of structural and slab steel. Side and double bottom tanks are separated and each has its own bellmouth. Pairs of double bottom tanks are either designated for the carriage of ballast or fuel oil. All side tanks are ballast tanks and are omitted from the figure for clarity, ballast can be carried in upper bulkhead stool tanks running transversely across the ship at the end of each cargo hold (see Fig. 2.5).

As can be seen from the foregoing series of illustrations, the structure within the double bottom areas of a bulk carrier is particularly complex, as it also provides the additional strength necessary to permit cargo concentration in the cargo hold immediately above.

The structure within the forepeak and after peak tanks (Photos 2.1 and 2.2), while differing from the double bottoms, can be equally as complex, the fore peak to resist the forces engendered as the ship pushes through waves, and in some cases ice, the after peak as it generally provides the main support for the rudder or steering nozzle.

Photo 2.1: Typical connections of internal members and side shell plating in forward part of fore peak tank.

Photo 2.2: Deep transverse floors vertically stiffened in after peak tank. Note ballast line leading to bellmouth.

Strength and continuity of strength without excessive structural weight are the key considerations in design and in subsequent construction. Drainage (Photo 2.3) is a secondary consideration and it is doubtful whether the entrapment of sediment and mud within these compartments, and the maintenance and cleaning of those same compartments has, until recently, been given more than a passing thought in either the design or construction phase of the ship's development.

Photo 2.3: Typical connection of transverse floor, longitudinal bottom shell frame and vertical floor stiffener. Note restricted drainage on this side of longitudinal frame, as evidenced by the accumulation of scale and mud.

Because topside tanks (Photos 2.4 and 2.5) generally have a lighter internal structure, and have a significant taper toward the bottom of the tank they tend to drain completely with little or no sediment accumulation. The area in these tanks where the water column is highest and sediment precipitation consequently greatest is generally in the same fore and aft plane as the drainage, ensuring a good velocity and more than adequate scouring throughout deballasting. Generally accessible from the main deck, topside tanks are also relatively easy to maintain.

Photo 2.4: Transverse and longitudinal framing within a topside tank.

Photo 2.5: An alternative that improves both drainage and cleanliness, longitudinal framing for the sloping plate is in the cargo hold. This permits the tank to also be used for carrying grain.

2.4. Ballast System Considerations

Ballast water is in most cases, introduced and evacuated through a single source, a bellmouth (Photos 2.6 and 2.7), located at the after end of each tank towards the centerline of the ship in the case of fore peak and double bottom tanks, close to the side shell in the case of topside tanks, and in the aft peak located in the deepest point. The exceptions are designs where topside tanks drain directly overboard, or are flooded and drain through the double bottom tank directly below.

Photo 2.6: Typical bellmouth installation in a forepeak tank. See also Photo 2.2.

Photo 2.7: Typical bellmouth installation in a double bottom tank.

There are numerous piping, pump and valve configurations that can form a ballast system, varying from individual lines of piping (Photo 2.8) running to each tank from a valve manifold subdivided port and starboard that is situated in the engine room, to two main ballast lines, one running down each side of the ship, or alternatively through the duct keel or void space, with branch lines running off into each tank separately. There will be individual valves (Photo 2.9) to control the flow into each tank, generally remotely operated with hydraulic lifters. In the former case these would be situated in the engine room manifold, while in the latter case there would be main line valves in the engine room, with individual tank line valves located either immediately adjacent to the tank in a central duct keel, void space or bulkhead stool.

Photo 2.8: Ballast line running through hopper tank showing connection at watertight terminal bulkhead.

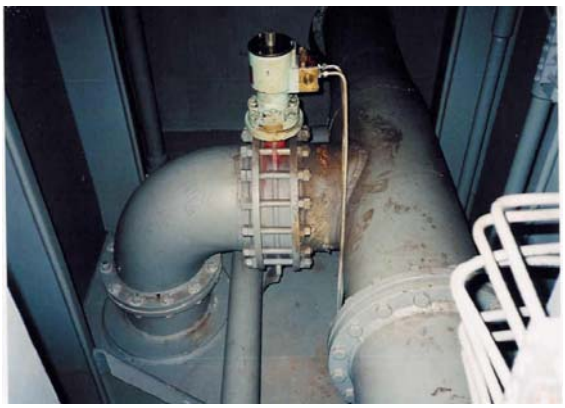


Photo 2.9: Hydraulic lifter and valve to double bottom tank located in lower bulkhead stool. The bellmouth is directly below, 2 meters from the valve.

The choice of ballast system design can itself contribute to the amount of ballast residuals carried. For example, the elevation of the ballast line above the bellmouth and the distance of

the individual tank valve from the bellmouth can contribute to the volume of water that may drop back into the tank once pumping stops and valves are shut.

In an effort to simplify the ballast system it is not uncommon in modern bulk carrier design for the topside tanks to be connected to the hopper side tank and thus to the double bottom tank below by ducts at the forward and aft end, through which they are both filled and drained, eliminating the need for a separate piping system to the top tanks. In some cases, these ducts also serve as an access way to the double bottom, thus making the double bottom tanks accessible irrespective of whether the adjacent cargo hold is full or not. While there are both capital cost and maintenance cost savings in such a design, there are drawbacks in operation due to the crew's inability to utilize the topside tanks individually for stability and heel purposes, and the latter can be particularly detrimental to their ability to strip the tanks during the latter stages of the deballasting operation by creating a list in order to pool water for more complete pump-out (Fig. 2.8).

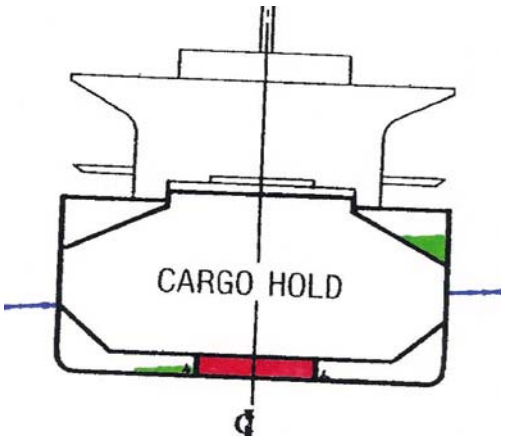


Fig. 2.8: Ballast placed in topside tank to provide the ship with a port or starboard list for pooling water in opposite side double bottoms.

In more conventional tank arrangements the double bottom tanks are completely separate, and can only be accessed through manholes within the cargo hold. Thus entry for inspection, maintenance or cleaning is only possible when the hold, or the area of the hold where the manhole is located, is clear of cargo.

Most modern ships have at least a pair of high volume centrifugal pumps as their main ballast pumps, which can strip the ballast out efficiently until it reaches the level of the internal framing, at which point the restricted drainage through longitudinal frames, floors and intercostals severely reduces the flow to the pump.

To compensate for this, many ships have either low volume stripping pumps or eductors to evacuate the residuals, however not all have separate stripping lines(Photo 2.10), and stripping efficiency is somewhat degraded using the main ballast lines.

Photo 2.10: Main ballast line and stripping line bellmouths separated by longitudinal shell frame in an existing ocean bulk carrier. Note relatively poor transverse drainage.

Eductors, which work on a venturi system using high-pressure water to create the suction, are the most efficient devices for stripping. They are able to maintain suction irrespective of the flow volume from the ballast tank, and can be run continuously without constant attention and adjustment by the engineers, which is the case with pumps. In addition there is no requirement for strainers to eliminate debris and abrasive materials which can damage a pump's impeller, which themselves need constant attention to avoid clogging. Nevertheless neither are particularly efficient through a main ballast line bellmouth unless the ship is trimmed well by the stern (Fig. 2.9), and heeled when possible to suit the configuration of the particular tank being drained, the purpose of which is to optimize drainage toward the ballast system bell mouth and to create a pool around it until the final stages of evacuation.

2.5. Ballast Management

2.5.1. Deballasting

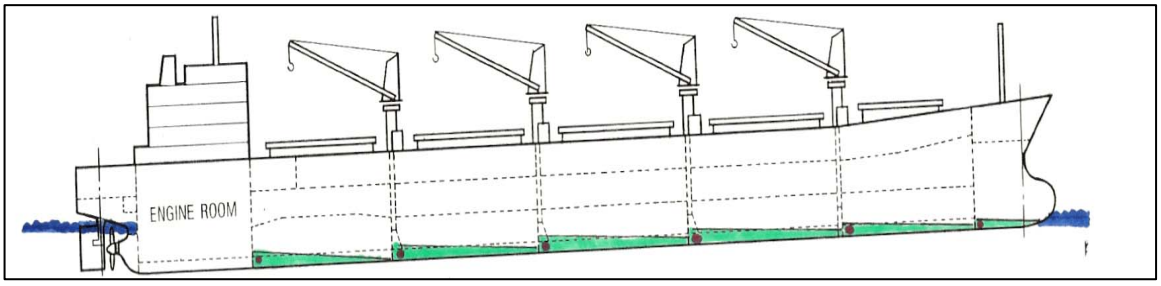


Fig. 2.9: Ship trimmed well by the stern to facilitate ballast evacuation. Stress conditions permitting, tanks will be drained in sequence starting forward in the late stages of deballasting.

When deballasting to load a homogenous bulk cargo this trim condition is generally easy to achieve with careful planning of both the cargo loading and deballasting sequence, and without unduly stressing the hull. It becomes a far more complex problem with multiple parcel or multiple port loadings or with specific cargoes such as steel coils, and even with the best planning it may not be possible to produce a sufficient stern trim for the final stages of deballasting. Depth of water at the berth can be another critical factor, limiting the stern draft permissible.

The bellmouth in each tank, which is the terminal point of the ballast pipe, has to be installed with sufficient clearance above the bottom shell to permit the water to flow in and out of the tank at a rate compatible with the rest of the ballast system, which means a clearance generally between three and five centimeters. The closer the trim of the ship becomes to even keel, the greater the volume of water left across the bottom of an individual tank, or across the bottom of the ship, which the system will be unable to evacuate.

In the type of ocean going bulk carriers built to trade specifically into the Great Lakes, and most common in this study, one centimeter of water across the double bottom ballast spaces can represent between twenty and thirty five tonnes of ballast water depending on whether it has double bottom fuel tanks or not, from which it can be easily seen how critical good management of the deballasting operation is to reducing the amount of ballast residual carried in even the cleanest of tanks.

Realistically, without a dedicated stripping system even the most skilful staff on one of these ships are unlikely to be able to have less than twenty tonnes of residual ballast when concurrent cargo loading and deballasting operations are undertaken.

2.5.2. Tank and System Maintenance

Management and upkeep of the ballast spaces and ballast system components are equally important as management of ballasting and deballasting. Leaking pipes or leaking valves, drainage blocked by mud or loose scale (Photos 2.11-2.14), or particularly a combination of the two, can further reduce deballasting efficiency. Regular inspections of ballast tanks to assess the condition of the tank coatings, structure, ballast system components and general cleanliness with respect to sediment and mud accumulation are a sound ballast management practice, as is dealing with deficiencies expeditiously and effectively, and these are all areas for constant improvement under any good management system.

The study found that all the owners/managers involved had requirements for regular inspections of the ballast tanks normally at intervals not exceeding six months. On sixty of the ships surveyed there were documented procedures for this process, for reporting findings to the owner or manager and for follow up action. On the remainder of the ships it was found that the process of developing Ballast Management Programs was underway, with procedures being developed either by the senior officers on instruction by the owner/manager or by the owner/manager in consultation with sea staff.

Photo 2.11: Dresser couplings provide watertight connections between pipe sections within the tank. Regular inspections ensure tightness to prevent leakage between tanks and loss of suction to pump from air ingress.

Photo 2.12: Ballast trapped at the forward end of a topside tank by blocked drainage through a bracket at the shell connection.

Photo 2.13: Scale, shale and sediment starting to restrict drainage where longitudinal frames pass through a bilge floor. Sediment will accumulate rapidly forward of this without remediation.

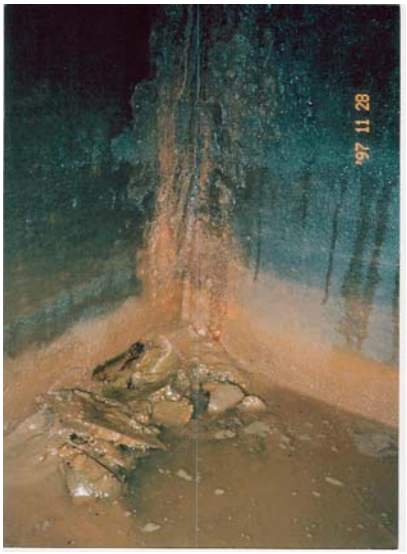


Photo 2.14: Scale and mud blocking drainage at the connection between a longitudinal side girder and transverse floor (see also Photo 2.16), resulting in water accumulation on the forward side that is unpumpable.

But no matter how well managed a fleet, or individual ship may be, many are handicapped by manpower availability, trade conditions and the vagaries of individual ship design. The study found that such is the case for many of the ships employed in the Great Lakes trade, where the brief periods available between one cargo or another, whether on foreign coasts, particularly European, or in the Great Lakes are often barely sufficient for the limited crew to be able to clean and dry the cargo compartments in preparation for loading the outbound cargo while dealing with the already added work load of confined waters navigation or canal and lock transits. However it was also found that it was not uncommon for owners/managers to put additional riding crew on board at suitable periods or on long ocean passages to augment the regular crew for maintenance such as this, particularly if the ship is imminently to undergo survey on dry dock.

Ballast tank cleaning is time consuming and arduous work, particularly cleaning the double bottom areas where the tank height on a typical bulk carrier or chemical tanker trading into the

Great Lakes is between one and two meters, and compartments between internal girders and floors can be less than three meters square.

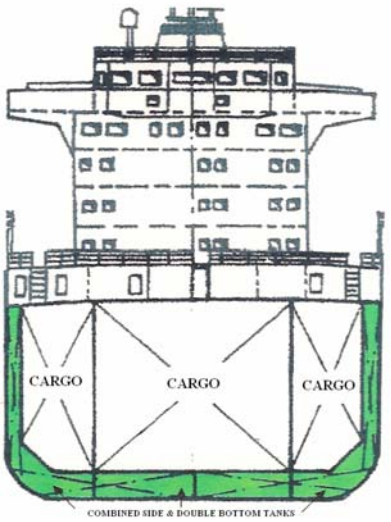


Fig. 2.10: IMO Type II tanker with combined side and double bottom tanks. With side tanks accessible from the main deck, inspection, cleaning and maintenance are easier for the crew to deal with regularly than on most bulk carriers. There are numerous tank arrangement variations with chemical tankers, which are constructed in three distinct IMO (International Maritime Organization) types, depending on the hazard presented by the chemical(s) they are designed to carry. The IMO Type II tankers most commonly in the Great Lakes trade have completely segregated ballast tanks, and side and double bottom tanks may be combined, or separated (Fig. 2.7), or may form one complete tank surrounding the adjacent cargo spaces.

On both chemical tankers and dry bulk carriers where the double bottom and side tank on each side are common and are accessible from the main deck, it is easier to clean sequentially, washing down from the top to remove sediment deposits from both horizontal and vertical surfaces, and working back from the forward end towards the bellmouth. Providing the deposits have not been allowed to collect and compact to any great degree, pressure hoses from the ship's deck water/fire line will accomplish the job, but it is still difficult work dragging a fully charged hose through the limited spaces and lighting holes.

In conventionally designed bulk carriers, where topside tanks and double bottom tanks are separate compartments even if joined by a drainage duct, the crew can reasonably be expected to clean a pair of topside tanks in a day. But with the rigging of hoses first through the cargo compartment, and then through manholes in the tank top or hopper plates to clean the double bottom tanks, it can take twice as long to wash through a double bottom tank, and it may still be necessary to remove some solids by hand, shoveling into drums to be hauled on to deck for disposal. Given that the average bulk carrier in the trade has between twenty and thirty ballast tanks, comprising two peak tanks, the remaining divided equally between top or side tanks and double bottom tanks, it is easy to envision the difficulties surrounding crew cleaning.

The study found that to minimize or avoid the necessity for major manual cleaning, the most common ballast management practice used in cases where taking high turbidity ballast is unavoidable, is to limit the amount of ballast taken aboard to the minimum quantity necessary to allow the ship to proceed safely to deep and cleaner water. The number of tanks used in such circumstances may also be minimized, and those tanks then drained and flushed with clean water as soon as circumstances permit, in order to avoid the sediment settling out and compacting. However, the study also found that settling can occur in a relatively short period of time, and

significant sediment accumulation can occur after only a single ballasting (Photos 2.15 & 2.16), so where a long river passage is involved this practice may be of limited benefit.

Photos 2.15 and 2.16: Sediment deposits on horizontal stringer plates within a forepeak after a single ballasting

The study also found that flushing of double bottom tanks either as part of the aforementioned exchange process, or on a loaded passage where the ship is not constrained by draft considerations, is the next most common practice for limiting sediment accumulation.

Flushing of double bottom tanks means that after discharging the dirty ballast completely, several tons of clean water are pumped in and allowed to slosh around for a time in response to the rolling and pitching movement of the ship in a seaway, before being pumped out and the tank refilled with clean water. If conditions permit, the flushing can be done several times before the tank is completely refilled. Similarly, whenever circumstances permit on a loaded passage the practice can be used to loosen up and remove sediment that may have settled out from the previous ballasting. In this case it has to be very carefully planned to ensure that the ship does not go down by the head (bow), in which case, due to the location of the bellmouth at the after end, it would be impossible to pump the water back out.

Unless the ship has a very efficient stripping system with minimal clearance between the bellmouth and bottom shell, this process will likely increase the amount of unpumpable ballast on board a loaded ship, and care must be taken not to submerge the load line, or to exceed draft limitations at a discharging berth or for transiting rivers and canals (Photo 2.17 & 2.18). This is a process where the sea staff must "know their ship" well, or where there must have been good record keeping by previous sea staff so that the results can be predictable.

Photos 2.17 and 2.18: When ships are at even keel draft for entering the St. Lawrence Seaway, residual water is below the limber holes and is unpumpable.

While intended to restrict sediment accumulation, this type of tank flushing appears to have an equally important function from the perspective of Great Lakes ecosystem protection, as it can provide an immediate method of mitigating the NOBOB threat by transmitting a salinity shock to any freshwater biota in the residuals during the flushing process, and leaving predominantly saline residuals when completed.

While the complex arrangement of closely placed and deep transverse floors, longitudinal girders and wash plates in the lower forepeak tank is not conducive to cleaning by this type of flushing; the forepeak tank is always accessible when the ship is loaded with cargo, and thus available for manual cleaning. The lower area where sediment will accumulate in the greatest quantity can also still be subjected to the same saline shock by introducing a limited amount of seawater.

However flushing is not an option for ships that have been loaded by owners or charterers to arrive at the entrance to the St. Lawrence Seaway exactly at the maximum permitted draft, because the risk for the Master of exceeding that draft and being refused entry are too great.

This is a situation that needs to be addressed by the industry, where a balance would need to be achieved between operational necessity and commercial ambition, as numerous ships trading between North Europe and the Great Lakes are repeatedly ballasting in freshwater at both ends of the passage. Without being subject to the ballast exchange regime, they carry only freshwater residuals, and the problems that they contain, in both directions.

The study determined that there were forty-nine NOBOB ships in our survey group of 103 that entered the Great Lakes after their last offshore ballast carried was either fresh or brackish water, arriving in the following condition:

2 ships had chlorinated residuals after mandatory treatment in Brazil/Argentina;

16 ships had flushed double bottom tanks in mid ocean during loaded passage resulting in high salinity residual;

31 ships had the original offshore fresh/brackish water residuals.

The tonnage required to be loaded under the commercial contract if the Master is to be able to provide a saline flush of the tanks during the transoceanic passage would have to reflect the operational capability of the ship based on its history rather than it's design parameters, with detailed procedures for conduct of the process laid out in the ship specific Ballast Management Plan.

2.6. Design and Construction Considerations - Ballast Tank Drainage

There are numerous areas where improvements in drainage through internal members, most particularly in forepeak and double bottom tanks would be beneficial to both residual reduction and sediment removal, but unlike most other technological improvements, it is impractical to consider them being carried out retroactively unless the ship is to undergo significant life extension steel renewals. Changes in sizing and distribution of drainage holes in structural members is something that needs to be done at the design and construction stages of any ship to ensure that the structural integrity is not compromised. A deeper or thicker structural member may be required or a different welding procedure necessary, but neither can be realistically entertained for retroactive installation.

When surveying the internal arrangement within the tank of an existing ship, it is impossible to judge whether the design of openings where internal members are connected to the shell plating and to other internals has been a result of continuity considerations for structural strength, for the ease and economy of construction and/or unit erections, or any combination of these

Photo 2.19: Limber holes in longitudinal bilge framing where prefabricated hull units have been joined together. The absence of a continuous frame/shell connection reduces potential accumulation. However the same type of connection to the shell has not been utilized in other locations or members.

factors. The best indication that there have been changes made from the original design during either the translation to construction drawings or subsequently for ease of construction is when the drainage holes and method of internal attachment vary in close proximity to one another (Photo 2.19).

With longitudinal strength and continuity of longitudinal members paramount considerations in design, drainage in the longitudinal plane is currently always better than in the transverse plane once the water level is below the level of the longitudinal shell frames (Photos 2. 20-2.26). Thus, unless blockages of the slots or limber holes has occurred from debris or scale, the area

between longitudinal girders running forward from the bellmouth appears to drain more quickly than the others in the late stages of deballasting, encouraging the water in the outer bays to flow transversely into that drained area, rather than longitudinally in response to trim and pump action. With the restricted drainage through these longitudinal members, sediment precipitation and accumulation thus increases outboard to the upper turn of bilge.

Photo 2.20: Obvious disparity between longitudinal & transverse drainage where longitudinals pass through transverse floor slots.

Photo 2.21: Sediment accumulation starting forward of a transverse floor and between the plane of transverse drainage through longitudinal frames.

Photo 2.22: Scouring in sediment on the bottom shell is an indicator of flow intensity in the transverse plane in the outboard bays of the double bottom and hopper tanks during the final stages of deballasting, leaving large areas of accumulating sediment between the limber holes in longitudinal shell frames.

In numerous cases that transverse drainage is not only inhibited by the paucity of limber holes in the longitudinal members, but also by the method of attachment of those members to the shell. Where the design calls for a continuous weld attachment, the limber hole is often cut further above the attachment edge of the frame than need would dictate, which can compound residual retention, and in particular sediment accumulation.

Photo 2.23: An example of poor transverse drainage, a relatively small limber hole with the bottom more than 2cm above the bottom shell.

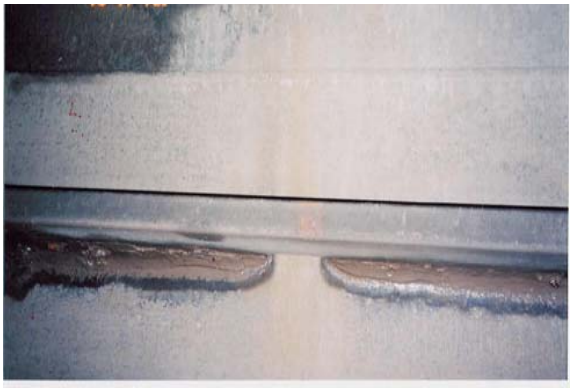


Photo 2.24: By contrast, use of wrap-around welding allows this limber hole through a bottom longitudinal to be open all the way to the shell.

Photos 2.25 & 2.26: These sediments have accumulated and compacted outboard of bilge longitudinal and can no longer be removed by flushing

Standardization in design, particularly with respect to economy of construction also dictates that the limber holes in longitudinals most commonly lie in the same transverse plane, while flow back to the bellmouth, particularly in the after half of the tank, would logically tend to be diagonally.

Increasing the drainage area in the longitudinal members along the bottom shell and arranging the limber holes to enhance the flow directly toward the bellmouth are two of the considerations for future double bottom tank design if sediment accumulation is to be eliminated.

Design changes that minimize ballast residuals should always be attractive to the ship owner, for every tonne of residual carried represents a tonne less cargo, thus impacting directly on the ship's

revenue earning capability. This is a factor that is particularly critical in trades, where draft restrictions prevent the ship from operating to its full potential.

As owners replace ships in their fleet, which may occur in fifteen to twenty year cycles in this trade, they are always looking for improvements in operating efficiency, and this is one of the key elements. Since the late 1990s there have been more than 30 new bulk carriers introduced into the trade, replacing tonnage built in the late 70s and early 80s, and these ship's are generally all more efficient in stripping out ballast than their predecessors, but this is not necessarily so when it comes to reducing or eliminating sediment.

In comparing observations from recent introductions to the fleet trading into the Great Lakes, and using figures 2.6 (top) and 2.7 as illustrations, when all other conditions are equal the latter is more efficient that the former in stripping residual ballast, by virtue of the fact that there are twice as many bellmouths servicing the same cross sectional area of the double bottom.

However the latter design is far more likely to accumulate sediment than the former, for while drainage through the bottom shell longitudinal framing is relatively similar, longitudinal strength within the side tanks has been achieved by a series of stringer plates with vertical drainage only through central lightening holes. This leads to accumulation on the horizontal stringer surfaces and around attachments, similar to the conditions that exist in forepeak tanks.

Photo 2.27: Within the side tanks other horizontal surfaces have been largely eliminated, both side shell and hold longitudinal bulkheads are framed vertically.

Photo 2.28: Side tank stringer plate. The absence of drainage around the perimeter, and particularly along the connection to the shell plating results in sediment accumulation over a relatively large area of plating.

While there are features in individual designs which can be beneficial (Photo 2.27) in preventing sediment accumulation, those benefits can be offset by other features (Photo 2.28). Until there is at least an equal emphasis placed on the elimination of sediment as there is on residual water, design or construction anomalies like this are likely to persist, and for that to occur it will take the collective efforts of those entities that can influence ship designers, builders and operators to focus on the issue. That means the IMO, the International Association of Classification

Societies, (IACS), and national regulatory agencies responsible for structural design approval, such as the United States Coast Guard and Transport Canada - Marine Safety. Ship design is both a competitive and proprietary discipline, however these agencies are capable of providing not only the impetus but the guidance individual designers will need. This will not be a simple process, it will require a comprehensive analysis of existing designs to identify beneficial design features, and it will also require modeling of modified designs specifically related to sediment precipitation and flow during the latter stages of deballasting.

As sediment accumulation is a problem for all ships that trade in relatively shallow water and geographical areas where there is significant sediment run off, the process should not be restricted to ships designed with the Great Lakes trade in mind, but to all ships that carry significant ballast, in order to benefit both the industry as a whole and the Global environment.

While the work to date has concentrated on increasing the efficiency and safety of open ocean ballast exchange, one of the by-products is the potential for reducing residuals. More significantly from the Great Lakes perspective, there is the potential for reducing, or possibly eliminating the accumulation of sediment, and thus improving the ability of ships staff to maintain clean ballast tanks, particularly double bottom tanks. With the current conventional thinking on ballasting systems having a single point entry and exit for ballast water, or at the most a single point entry and two point exit where a separate stripping line exists, there are bound to be restricted flow patterns and eddies which are conducive to deposits of sediment accumulating. This becomes most apparent in peak (Photos 2.29 & 2.30) and double bottom ballast tanks where the structure is more complex, allowing accumulations to develop in locations distant from the bellmouth.

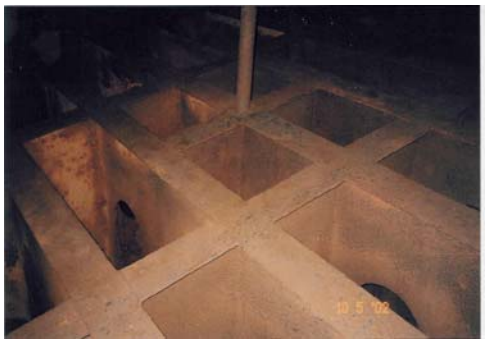


Photo 2.29 and 2.30: Deep floors and girders within a forepeak tank providing additional strength to the forefoot are particularly conducive to mud accumulations but more readily accessible for washing down.

In double bottom tanks, transverse floors, intercostals and longitudinal framing all provide obstructions to the flow to or from the bellmouth, particularly at the early stages of ballasting and the late stages of deballasting once the water level has dropped, first below lightening holes in the major members, then below the level of the top of the framing.

Photos 2.31 & 2.32: Longitudinal framing in a bulk carrier trading into the Great Lakes can vary in depth from 17-25 cm, and drainage to the bellmouth becomes particularly restricted once the water level in the tank drops below the top of these frames.

The pattern of sediment distribution was found to be essentially the same in every double bottom tank entered during the survey where the double bottom and hopper side tanks were common. Sediment precipitation in general was noted to be heavier in the forward part of the tank most distant from the bellmouth, but was most pronounced in the hopper side tank and along the shell at the turn of the bilge where the water column is greatest when the tank is full (Fig. 2.11). In this same general location deposits accumulate on the horizontal stringers on the side shell between any drainage holes that may exist (Photo 2.33).

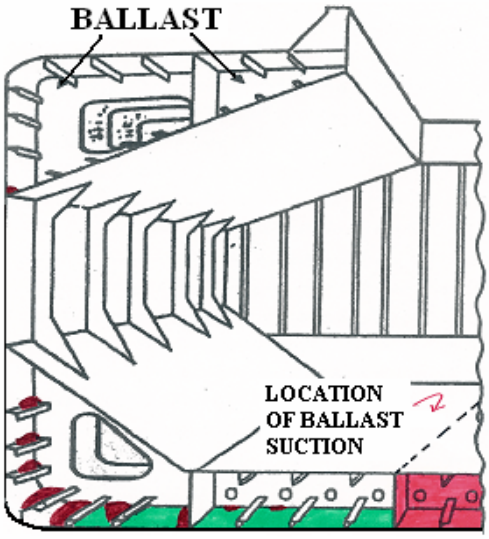


Fig. 2.11: While little sediment accumulates in the lower sections of the topside tanks, significant deposits can occur in the hopper side tank area starting along the outboard side of bilge longitudinals and the inner bilge girder. Unless drainage through the transverse floors has become blocked by scale or debris the area of bottom shell between girders directly ahead of the bow will remain relatively sediment free close to the bellmouth although deposits will occur on the forward side of transverse floors, increasing in size progressively toward the forward end of the tank

Photos 2.33 & 2.34: Sediment accumulating and compacting outboard of lower and upper framing in the bilge area, and between drainage holes in individual longitudinal frames.

On the bottom shell, accumulation occurs primarily on the outboard side of the longitudinal members (Photo 2.34) and increases progressively outboard to the bilge area. Once accumulation has started to any significant degree, particularly in the area along the turn of bilge, open-ocean flushing appears to only have limited effect in removing the deposits. Indications were that this was more a result of limitations in the limber holes in the longitudinal frames than in the act of flushing itself.

In the double bottom area the cells immediately adjacent to the bellmouth are usually found to be sediment free and any sediment accumulations will start on the forward faces of the transverse floors and the outboard faces of the bottom longitudinals, depending once again on the drainage characteristics of each individual tank.

2.7. Ballast Systems

Suggestions for design changes to ballasting systems to date have encouraged piping and valve modifications, primarily related to the introduction of water through multiple outlets in the tank to improve the prospects of complete exchange using the flow-through method.

One experiment that appears to have the most relevance to the residual sediment problem is being conducted by the Brazilian oil major PETROBRAS in conjunction with the Norwegian Classification Society, Det Norske Veritas, which has become known as the “Brazilian Dilution Method” of ballast exchange. This involves the installation of separate ballast lines for introduction and discharge of the ballast, with both systems being capable of operating concurrently. The significance of this design is that the ballast water is introduced through multiple points at the top of the tank, pumped out through the conventional ballast system bellmouth. In the initial conceptual design, and in an experiment that was conducted on one side tank of the tanker MV “Laurus”, the ballast input line and injections valves were on deck, presumably for economy and simplicity of design. In a permanent system, consideration may need to be given to an under deck installation to protect against heavy weather and/or mechanical damage, as well as freezing.

Introducing ballast water from the top of the tank is not in itself a new concept, it is common in the type of bulk carrier where the topside tanks have been designed both for the carriage of water ballast or grain, (photos 9 and10) and in fact in most cases can be used for washing out those tanks after grain carriage as the water discharges directly overboard through sluice valves. However taking that original concept a step further, and providing the ability not only to introduce water from the top of the tank in topside tanks, or combined side and double bottom tank, and from the top of the hopper space in a conventional double bottom, introduces the possibility of both washing down the horizontal members and the bilge area, the prime locations for sediment accumulations.

Figures 2.12 and 2.13 illustrate conceptually how such a system could be adapted to the two bulk carrier designs previously shown.

Rather than have the water introduced through a single point at one end of the tank, the water would be introduced either as a deluge or heavy spray through multiple orifices in a pipe running longitudinally through the tank, located specifically to wash down those surfaces and areas where sediment accumulates. This system could be utilized for normal ballasting, for occasional tank cleaning, and should circumstances require the introduction of a biocide in the NOBOB state to treat the surfaces of the tank most likely to harbor invaders.

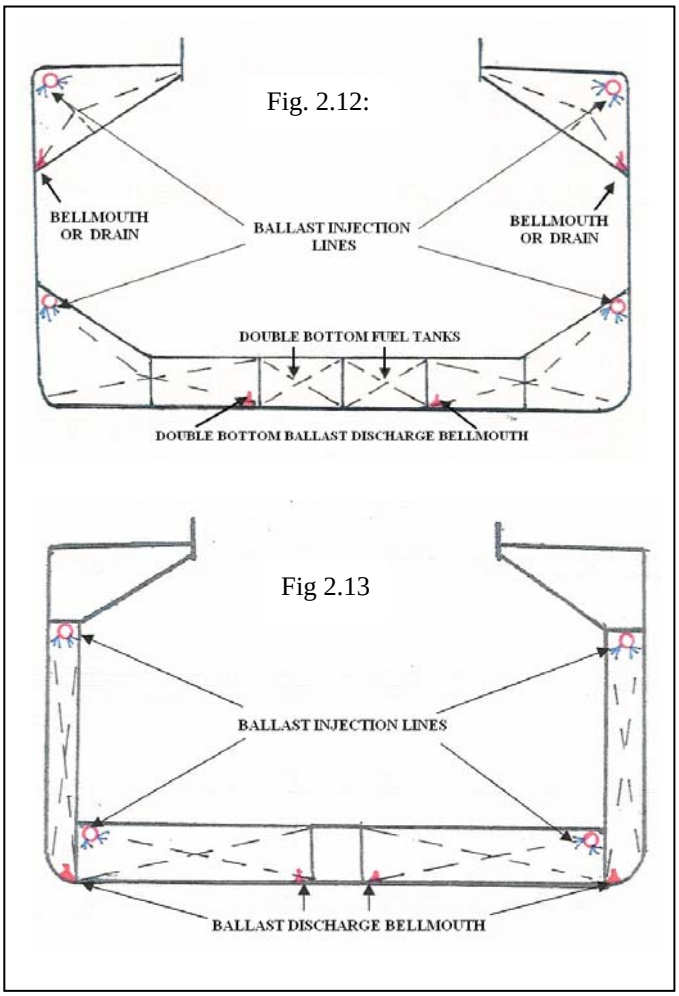


Fig. 2.12:

Fig 2.13

While such a concept could be retrofitted in existing ships, its benefits with respect to sediment removal would still be limited by each ship's ability to adequately drain and evacuate ballast from the individual tanks.

Probably the most innovative ballast management concept currently being studied with respect to ballast exchange, sediment accumulation, and the treatment of NOBOB ships is that of the "Ballast-Free Ship", being undertaken by the University of Michigan. While recognizing that ballast is an integral part of safe ship operation, the concept approaches the issue from the perspective of reduction of buoyancy rather than the addition of weight, and the hull design replaces the ballast tanks with ballast ducts through which the water flows as the ship is underway. Thus the ship transports no ballast, and any invaders that may inadvertently migrate will be subject to numerous changes of environment.

While the concept will have many technical hurdles to clear, with thoughtful design of drainage through the internal structure it should be possible to avoid sediment accumulation and transportation.

Chapter 3. Biological Assessment of Ballast Residuals in Tanks

The goal of this component of Task 1 was to fully characterize the biological content of the sediment and water residuals present in the empty tanks of NOBOB vessels. This biological characterization included microbes, pathogens, phytoplankton, zooplankton, and resting stages. We also characterized the physical and chemical composition of the residuals, with particular emphasis on salinity as it related to sources of water and management practices.

3.1. Summary of Residual Sampling

A summary of our sampling effort for Task 1 is provided in Table 3.1, with further details provided in Appendix 3. From December 2000 – December 2002 we entered and collected residual materials from 82 individual empty ballast tanks on 42 vessels. Five of these vessels were sampled on more than one occasion, so that we actually collected samples from 35 unique ships. Of the 42 ships sampled, 12 were accessed in U.S. ports and 30 in Canadian ports. This distribution was largely driven by the frequency of visits to a given port and the cargo loading schedules of the ship at these ports in terms of allowing adequate access to the empty ballast tanks.

The cooperation and responsiveness of the shipping industry was excellent throughout the study. We clearly found, however, that access to the double bottom tanks for sampling was often difficult due to time constraints in the ship's operating schedule or loading configurations that made it impossible to accommodate our sampling needs. This pattern was particularly true for the US ports. Despite the difficulties encountered, and a number of failed sampling opportunities, we exceeded our project goal of sampling 40 ships and far exceeded our sampling goals on the basis of having collected samples from 82 independent ballast tanks.

A breakdown of the types of ballast tanks accessed for sample collection is summarized in Table 3.2. The majority of our samples were collected from double bottom tanks (72%). These tanks were given the highest priority as they contained residuals from previous ballasting at foreign ports and were commonly ballasted and de-ballasted during trading operations within the Great Lakes. The second most frequently sampled tank type was the forepeak tank (22%). Access to the forepeak tank was often much less restricted than for double bottom tanks and this was often our fallback sampling option when access to the double bottom tanks was not available, or if we wanted a second tank on a given ship.

In most cases we were able to collect both water and sediment residuals from each tank for a total of 75 residual water samples, 73 wet and 4 dry residual sediment samples, and 65 plankton (net-filtered) samples (Table 3.3). Dry samples were designated as sediment that would crush into powder and had clearly been exposed to air long enough to have become fully desiccated. On two occasions we collected samples designated as slurry, which denoted a dense mixture of suspended sediment and surrounding water where the sediment could not be adequately segregated. This type of sample collection was discontinued early on in the study because it was determined to be more appropriate to analyze the water and sediment media separately.

Table 3.1. A summary of samples collected for Task 1, including temperature (deg. C) and salinity (ppt) of the water samples. Salinity is given for each sample, while the temperature is the mean value of all samples on a given ship because values typically agreed within one degree or less. (ND=not determined)

Date	Year	Jday	Port	Ship Code	Ship #	Tank #	Media Collected	Temp (oC)	Salinity (ppt)
7-Dec-00	2000	341	Hamilton	1001	1	1,2,3	water,sediment	ND	ND
8-May-01	2001	128	Hamilton	1002	2	4,5,6	water,sediment	8	24,44
22-May-01	2001	142	Cleveland	1003	3	7,8	water, sediment	12	22, 2
25-Jun-01	2001	176	Hamilton	1004	4	9,10	water,sediment	20	10,2
27-Jun-01	2001	178	Thorold	1005	5	11, 12	water,sediment	20	34, 32
30-Jun-01	2001	181	Windsor	1006	6	13, 14	water, sediment	22.6	34, 29
26-Jul-01	2001	207	Hamilton	1007	7	15	water, sediment	21	8, 9
28-Jul-01	2001	209	Hamilton	1008	8	17, 18	water, sediment	21.1	2, 5
4-Aug-01	2001	216	Hamilton	1009	9	19	sediment only	ND	ND
15-Aug-01	2001	227	Hamilton	1010	10	21	water, sediment	23.9	1
15-Aug-01	2001	227	Hamilton	1011	11	22	water, sediment	22.9	0
18-Sep-01	2001	261	Cleveland	1012	12	23, 24	water, sediment	20.7	0
1-Oct-01	2001	274	Hamilton	1007	13	25	water, sediment	17.1	5
5-Oct-01	2001	278	Cleveland	1013	14	26, 27	water, sediment	18.7	7
7-Oct-01	2001	280	Windsor	1007	15	28, 29	water, sediment	11.7	23, 3
22-Oct-01	2001	295	Cleveland	1014	16	30, 31	water, sediment	13.7	20, 22
25-Oct-01	2001	298	E. Chicago	1015	17	32, 33	water, sediment	10	70
8-Nov-01	2001	312	Burns Harbor	1016	18	34, 35	water, sediment	12	22, 22
19-Nov-01	2001	323	Burns Harbor	1017	19	36, 37	water, sediment	11.7	35, 35
21-Nov-01	2001	325	Hamilton	1018	20	38, 39	water, sediment	10.3	37
23-Nov-01	2001	327	Hamilton	1019	21	40, 41	water, sediment	9.8	7, 1
29-Nov-01	2001	333	Hamilton	1020	22	42, 43	water, sediment	8.4	2, 7
6-Jun-02	2002	157	Toronto	1021	23	44, 45, 46	water, sediment	9.6	35, 32, 30
6-Jun-02	2002	157	Toronto	1022	24	47	water, sediment	8.6	15
13-Jun-02	2002	164	Hamilton	1023	25	48, 49, 50	water, sediment	18.3	8, 2, 6
24-Jun-02	2002	175	Windsor	1024	26	51, 52	water, sediment	21, 21	16, 27
19-Jul-02	2002	200	Hamilton	1025	27	53, 54	water, sediment	21.2	19, 26
25-Jul-02	2002	206	Cleveland	1026	28	55, 56	water, sediment	22.2	18, 4
6-Aug-02	2002	218	Hamilton	1006	29	57, 58	water, sediment	19.6	37, 37
6-Aug-02	2002	219	Hamilton	1027	30	59, 60, 61	water, sediment	22.5	5, 8, 2
6-Aug-02	2002	221	Detroit	1028	31	62	sediment only	ND	ND
13-Aug-02	2002	225	Windsor	1029	32	63, 64	water, sediment	24.5	8, 3
15-Aug-02	2002	227	Hamilton	1030	33	65, 66	water, sediment	20.7, ND	22, 31
13-Sep-02	2002	256	Hamilton	1031	34	67, 68	water, sediment	22.6	57, 75
5-Oct-02	2002	278	Windsor	1027	35	69	water, sediment	20.2	36
11-Oct-02	2002	284	Windsor	1032	36	70,71	water, sediment	ND	32, 20
20-Oct-02	2002	293	Windsor	1033	37	72, 73	water, sediment	11.1	2, 1
23-Oct-02	2002	297	Burns Harbor	1007	38	74,75	water, sediment	13.7	1.9, 1.1
12-Nov-02	2002	316	Cleveland	1014	39	76, 77	water, sediment	12.3	26, 2
19-Nov-02	2002	323	Cleveland	1013	40	78,79	water, sediment	9.5	21, 20.6
26-Nov-02	2002	330	Hamilton	1034	41	80,81	water, sediment	7.5	28, 12
6-Dec-02	2002	340	Windsor	1035	42	82	water, sediment	-0.7	34

One of our stated sampling goals was to capture 3-6 photographs of each tank samples for inclusion in a final photographic database. In the course of our sampling efforts we realized that

we could not adequately represent or distinguish the variance in the distribution and amount of residuals in any meaningful way with just these few photographs. Therefore, we developed a new strategy to accumulate a series of photos that would: (1) define typical conditions observed in tanks, (2) describe structural issues within tanks pertaining to ballast residuals, and (3) capture typical sampling activities. These photos are available on our project web site: www.glerl.noaa.gov/nobob/.

Table 3.2. Summary of the number of ships and tank's sampled for Task I of the project.

	2000/2001	2002	TOTAL
No. of Ships	22	20	42
No. of Tanks	43	39	82
Forepeak	9	9	18
Double Bottom	30	29	59
Aft	1	0	1
Wing	3	0	3
Side	0	1	1

Table 3.3. Summary of Task 1 residual water and sediment samples collected for the entire project.

	2000/2001	2002	TOTAL
Residual water	37	38	75
Slurry sample	2	0	2
Wet sediment	37	36	73
Dry sediment	3	1	4
Zooplankton Net samples	32	33	65

Data Management

Data management and distribution is being coordinated by the project managers. First, we have developed a master database that summarizes the details of all sample collections for Task 1 and Task 2 experiments (see Appendix 3). This database provides details on ship, port, ballast-tank, sample-type, sample ID, temperature and salinity and is used as the master reference to link all associated data generated by NOBOB team members including ballasting history, residual assessments, chemical analyses and biological analyses. All field notes and sample distribution sheets are recorded electronically in EXCEL spreadsheets and original paper copies are maintained in notebooks as a backup. A statistical database is still under design to further analyze the chemical and biological results from the various project team members, as final manuscripts are being prepared. The database and final analyses will also include information on residual accumulation and ballasting history extracted from surveys conducted as part of this project.

3.2. Physical and Chemical Characterization

3.2.1. Introduction

A suite of physical and chemical measurements including temperature, salinity (or conductivity), dissolved nutrients, particulate organic carbon and nitrogen and chlorophyll were determined for the sediment and water residuals present in ballast tanks. These analyses were used to assess the overall environmental conditions within the tanks, provide supporting information to confirm survey results on ballasting history and aid the interpretation of biological content.

3.2.2 Methods

Temperature and salinity were determined on all water samples, except were noted in the sample summary Table 3.1. Temperature was measured directly in the ballast tank with a hand-held thermometer, or using a YSI model 85 meter. Salinity was either measured directly in the ballast tanks using the YSI-85 meter or with a hand-held refractometer back at the lab. Frequent cross-checks of these two instruments showed good comparison. Water samples for dissolved nutrients and chlorophyll concentrations were collected from the tanks in acid-washed, chemically clean polypropylene bottles and either processed in the parking lot within a few hours of collection or kept cold and dark until processed back at the laboratory. Water samples were analyzed for ammonium, phosphate, silica, and nitrate concentrations using automated, colorimetric procedures on an Auto Analyzer2, as detailed by the modification of Davis and Simmons (1979).

Sediment samples within the ballast tanks were analyzed for organic carbon and nitrogen, and a sub-set analyzed for grain size distribution. Sediment was collected using chemically clean, sterilized plastic spatulas and placed in chemically clean polypropylene buckets. Sediment was typically composited from scrapings taken throughout the ballast tank. Sub-samples were distributed to each of the labs for their respective analyses, which are described under separate sections below. Particulate organic carbon and nitrogen were measured using a Carlo Erba CHN elemental analyzer, after first freeze-drying the sample and then treating it with 2N HCl to remove inorganic carbon content. Total carbon is determined by combusting non-acidified samples.

3.2.3. Results

Water Residuals

Salinity of residual water samples ranged from 0 – 70 ppt, with 43 percent of the samples falling in a fresh- or brackish-water category (less than 10 ppt) (Fig. 3.1; Table 3.4). Four samples exhibited very high salinity values indicative of significant amounts of evaporation. These samples usually originated in upper wing tanks that were not ballasted as frequently as the double bottom tanks. The salinity of water samples often varied between tanks on the same ship, even when they were port and starboard tanks of the same cargo hold. Specifically, for 10

of 23 paired double bottom tanks the salinities differed by more than 5 ppt. This result validates our treatment of each individual tank as an independent sample.

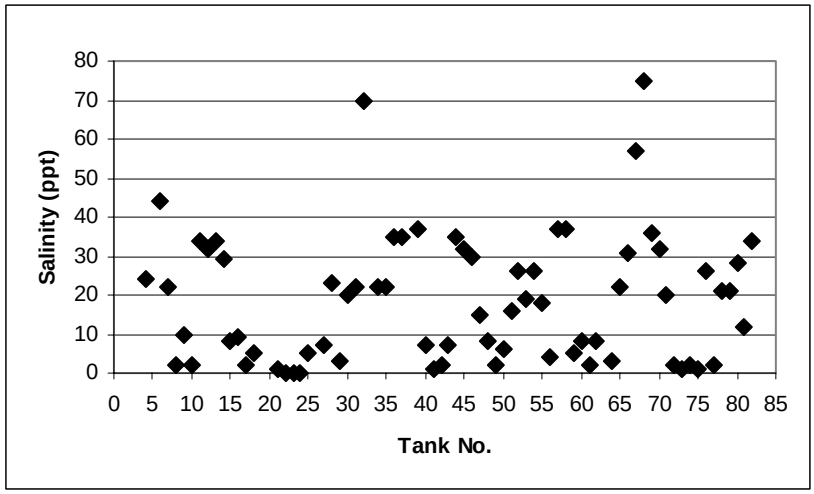


Fig. 3.1. Salinity concentrations of water residual samples collected for Task 1.

Table 3.4. Summary distribution of salinity values observed for residual water collected in our Task 1 samples from 82 empty ballast tanks on ships.

Salinity Range (ppt)	0-5	6-10	11-20	21-37	>40
No. Observations	22	10	7	30	5

As expected, temperatures were quite consistent between tanks on the same ship, usually varying by no more than 0.5 ^{o}C. Temperatures simply reflected the seasonal temperature cycle of the lakes and various harbors, although a direct comparison to ambient temperatures in the ports was not performed.

Dissolved Nutrients

We experienced several technical problems with the analyses of ammonia and phosphorus concentrations and tried several different approaches including the standard colorimetric procedures, ion-specific probes, and a fluorometric method. Despite these various approaches we were unable to get results that were consistently above detection limits or reliably above blank corrections from the salinity interference. Consequently we report here only results for nitrate and silica concentrations for the analyses of dissolved nutrients

Nitrate and silica concentrations varied significantly among samples, and often reach extremely high values compared to normal environmental samples (Fig 3.2 and 3.2). These results suggest that significant interaction occurs between the residual sediment and water or that there are significant sources of contamination within the tanks, such as coating materials.

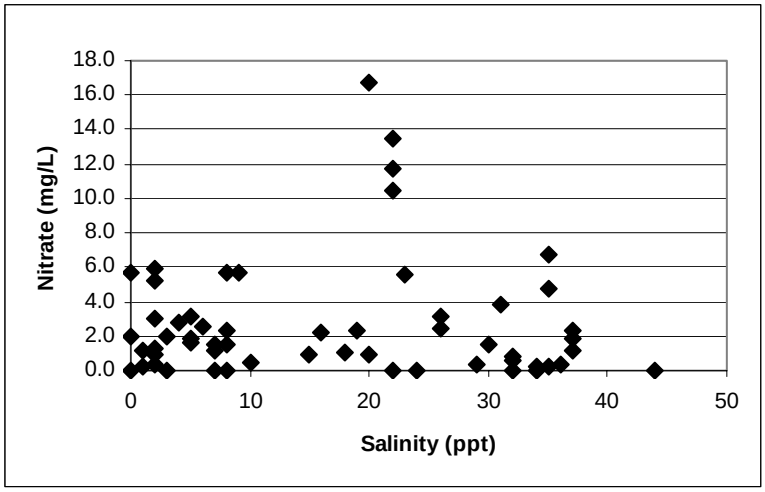


Fig. 3.2. Nitrate concentrations for water residual samples collected for Task 1, plotted against corresponding salinity values.

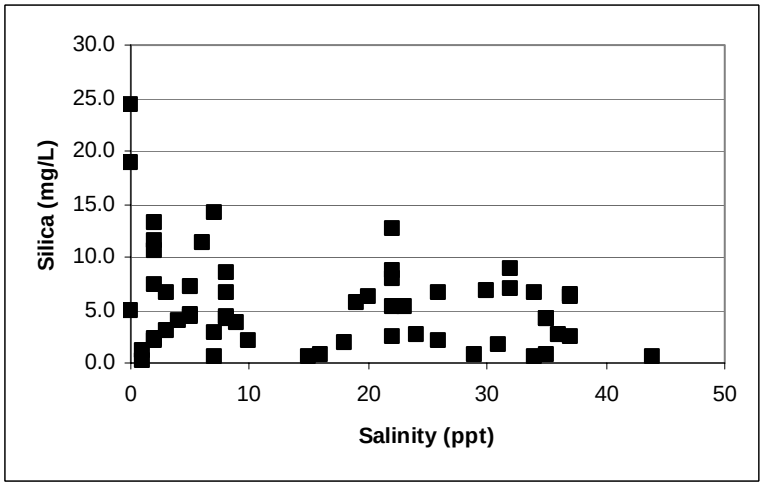


Fig. 3.3. Silica concentrations for water residual samples collected for Task 1, plotted against corresponding salinity values.

Neither of these nutrients showed a strong correlation with salinity. This lack of relationship again indicates that the residual water does not represent its initial source water because even though the residual water may be a mixture of initial source water, we would expect high salinity open-ocean water to have significantly lower nutrient concentrations compared to low salinity, riverine influenced water. Lastly, silica and nitrate concentrations were not strongly correlated to each other (Fig. 3.4), which implies that whatever diagenic or contaminant effects occur upon exposure in the ballast tank, they are not equally expressed among the different nutrient species.

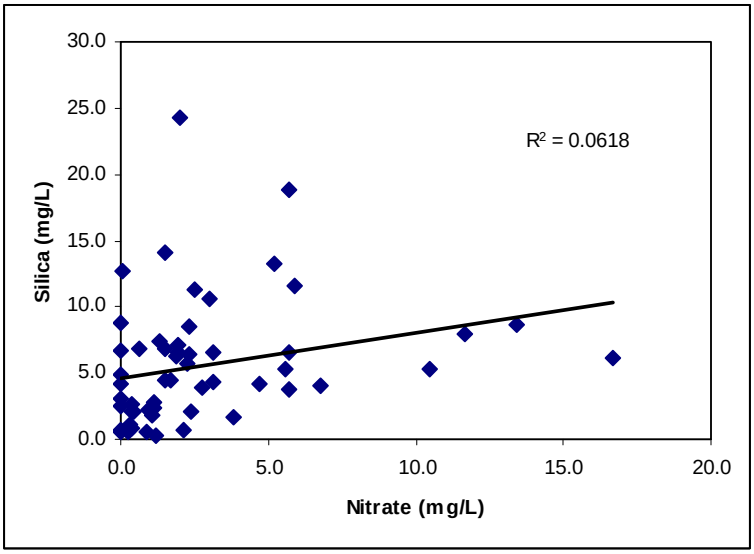


Fig. 3.4. Comparison of paired nitrate and silica concentrations for water residual samples collected for Task 1.

Sediment Residuals

Particulate organic carbon and nitrogen values in sediment residuals were highly variable, and ranged from between 2.5 – 39.7 percent Carbon (Fig. 3.5), and 0.11 – 0.48 percent Nitrogen (Fig. 3.6).

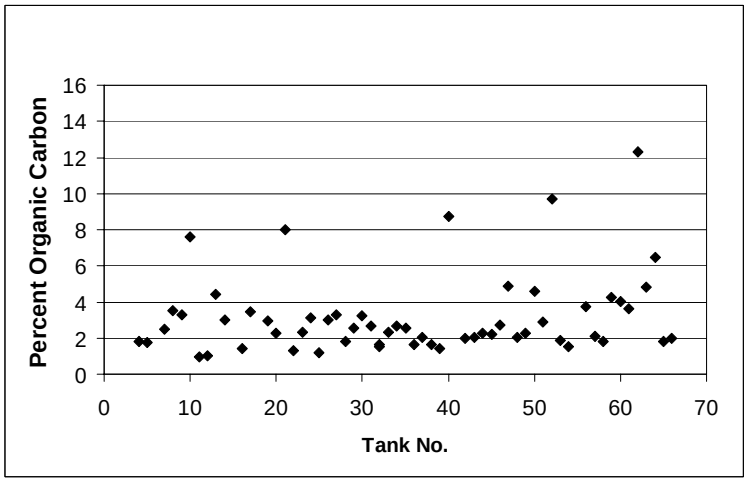


Fig 3.5. Particulate organic carbon concentrations for Task 1 sediment residuals samples.

Elevated values did not appear to be reflective of environmentally degraded source water, but rather were likely due to contamination by industrial lubricants, cleaners, or coatings applied to maintain the ship. The ratio of organic carbon to total carbon was also quite variable, ranging from 21 to 87% with a mean of 57%. The ratio of organic carbon to nitrogen ranged from 7 – 69, with a mean of 14.3. This large variance in the C:N ratio again indicates that diagenic or contamination processes are occurring at significant levels within the tanks.

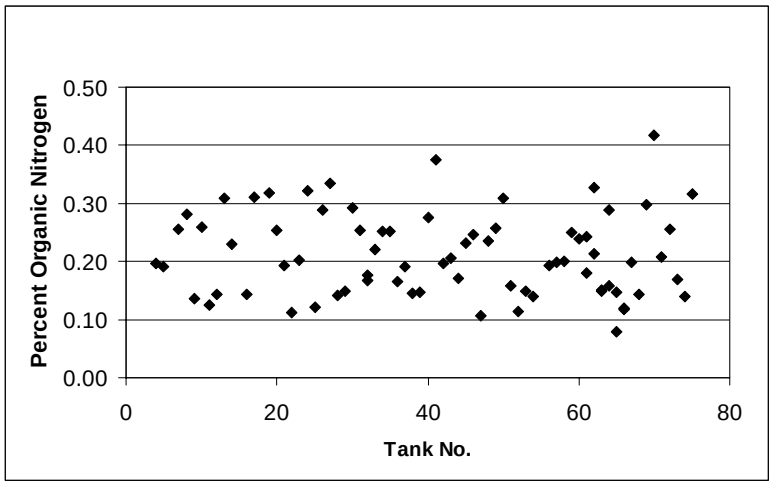


Fig 3.6. Particulate organic nitrogen concentrations for Task 1 sediment residuals samples.

3.2.4 Discussion

In general, nutrients concentrations for water and sediment ballast residuals did not appear to be a reliable indicator of ballasting history or the source water used for ballasting operations. Concentrations were too variable and did not correlate well with other parameters such as salinity that would allow for much interpretive value. Concentrations were often significantly above natural environmental levels, suggesting contamination within the tanks. These results should not be surprising given the nature of ship operations and industrial activities.

3.3. Microbial Analyses of Ballast Residuals

3.3.1. Introduction

The goal of ODU's portion of Task 1 was to characterize the microbial populations present in water and sediment ballast residuals. To that end, we counted virus-like-particles and total bacteria, measured chlorophyll-*a* and phaeopigments, and evaluated sole-carbon-source utilization by heterotrophic bacteria. We also screened residual water and sediment samples for the presence of selected indicator organisms and pathogens, including bacteria (enterococci, *E. coli*, *Vibrio cholerae* serotypes O1 and O139) and protozoans (*Giardia lamblia*, *Cryptosporidium parvum*, *Encephalitozoon intestinalis*, *Pfiesteria piscicida* and *P. shumwayae*), and dinoflagellates. These various measures and methods are listed in Table 3.5 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. Assays for enteric bacteria (2001 samples only) were performed by Dr. Alpha Diallo (Norfolk Department of Public Health). Dr. Thaddeus Graczyk (Johns Hopkins University) conducted analyses for *Cryptosporidium parvum* and *Giardia lamblia*, and for samples collected in 2002, also assayed for *Encephalitozoon intestinalis,* a microsporidian intestinal parasite. Tests for *Aureococcus anophagefferens* (2001 only) were conducted in collaboration with Drs. Linda Popels and Kathryn Coyne (University of Delaware). Analyses for *Pfiesteria piscicida* and *P. shumwayae* were performed by Dr. Parke Rublee (University of North Carolina Greensboro).

Table 3.5. Summary of microbial metrics determined in Task 1.

Microbial Metric	Method(s)
Non-specific or "bulk indicators"	
Total virus-like-particles	Direct counts (epifluorescence microscopy)
Total bacteria	Flow cytometry
Chlorophyll-*a*	Acetone extraction and fluorometric detection
Phaeopigments	Acetone extraction and fluorometric detection
Pathogens or indicator species	
Enteric bacteria (enterococci, *E. coli*)	Culture
Vibrio cholerae O1 and O139	Culture, biochemical testing, immuno-fluorescent antibodies, and PCR
Pfiesteria piscicida and *P. shumwayae*	PCR
Dinoflagellates	Culture
Aureococcus anophagefferens	PCR
Giardia lamblia, Cryptosporidium parvum, and *Encephalitozoon intestinalis*	Immunoassays and PCR
Functional groups	
Metabolic diversity of heterotrophic bacteria	Biolog GN2 plates (dark incubation)

3.3.2. Methods

Sample storage and shipping

Water and sediment samples for microbial analyses (including phytoplankton) were collected as described above, and then stored in the dark at 4°C before being shipped to ODU for processing and analysis. Transportation was expedited through use of overnight delivery services; however there were some samples that were more than 24 h in transit.

Microbiological analyses

Direct counts of virus-like particles. Virus-like particles (VLPs) were counted using the fluorochrome SYBR® Green I (Noble and Fuhrman 1998). Upon return to the laboratory, samples that could not be prepared immediately were fixed in formalin (2.7% final concentration). Fixed and unfixed samples were diluted 1:8 with 0.02 μm-filtered distilled, deionized water. Next, diluted samples were filtered onto 0.02 μm-pore size Anodisc filters (Whatman International Ltd.) and stained in the dark for 15 minutes at room temperature with a working solution of the nucleic acid stain SYBR® Green I (Molecular Probes, Inc.). Filters were placed on microscope slides with a 25 μl drop of antifade mounting solution and counted immediately or stored in the dark at −80°C until the VLPs were counted. Filters were randomly chosen (in groups of two), thawed in the dark at room temperature for about 5 minutes, and VLPs counted in 15-20 fields using an Olympus BX-50 System Microscope with a BX-FLA epifluorescent attachment. For each set of filters prepared, two control filters were prepared using only 0.02 μm-filtered distilled, deionized water, and their average VLP count was subtracted from values determined in field samples.

Flow-cytometry counts of bacteria. Water samples were fixed in a formaldehyde solution (final concentration 2.7%) and stored in the dark at 4°C until they were enumerated via flow cytometry. Analyses were done using a Becton Dickinson FACScan flow cytometer equipped with a 15 mW, 488 nm, air-cooled Argon ion laser. Simultaneous measurements of forward light scatter, 90-degree light scatter, and green fluorescence were made on all samples. PicoGreen (Molecular Probes, Inc.), a DNA-specific probe, was used to detect and enumerate bacteria (Veldhuis et al. 1997). Detectors (photomultiplier tubes) were in log mode and signal peak heights from excitation wavelengths were measured. The volume of samples was determined gravimetrically using an A-160 electronic balance (Denver Instruments Co.) whereby each sample was weighed prior to analysis and immediately after analysis. All samples were run at a low flow rate setting (approximately 20 μL min^{-1}).

Chlorophyll *a* and phaeopigment methods. Chl *a* samples were collected by filtering up to 500 mL of residual water onto 47 mm-diameter glass fiber filters (GF/F, Whatman International Ltd.) at a vacuum pressure of 100 mm Hg. Filters were wrapped in foil and stored at −80°C until the chl *a* and phaeopigments on the filters were extracted in acetone and measured fluorometrically (Parsons et al. 1992).

Detection of pathogenic viruses. It was our intent to use public-health methods developed for monitoring enteric viruses from different water types to test for the presence of enteroviruses and

human calicivirus (Huang et al. 1999; Metcalf and Jiang 1989). As discussed in an interim report, however, these analyses were not possible because of the unanticipated departure of Dr. Jason Jiang (formerly at the Eastern Virginia Medical School--EVMS), to whom this work was to be subcontracted. Subsequently, we initiated discussions with Dr. David Matson (also at EVMS) to see whether these analyses could be carried out in his lab. We performed a small experiment to determine whether Dr. Matson's techniques were applicable to samples of ballast water and sediments. Unfortunately, results from this "proof-of-concept" experiment were not encouraging. Because we did not have funds to support the development of these techniques (estimated as $30,000 to support a technician in Dr. Matson's laboratory), we did not perform these analyses.

Detection of enteric bacteria (2001 samples only). Up to 100 ml of water (or in some cases diluted sediment) was filtered through a 0.45 μm pore size membrane. The membrane was then triturated and transferred to an enrichment broth and incubated at a temperature appropriate for the growth of enterocci or *E. coli*. The pre-enrichment step enhances the recovery of stressed organisms in recreational water samples. Subsequently, selective enrichment was used to recover viable, culturable organisms, as described in APHA's Standard Methods for the Examination of Water and Wastewater and EPA method 1600.

Detection of Vibrio cholerae O1 and O139 (2002 samples only). Up to 100 ml of residual water (or sediment porewater extracted via centrifugation) was filtered (0.45 μm pore size), the filter placed on TCBS agar, and incubated overnight at 37°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. The biochemical method of Choopun et al. (2002) was used to identify V. cholerae and confirmed by PCR-based analysis of 16S-23S rRNA intergenic spacer regions (Chun et al., 1999). Isolates were tested with fluorescent monoclonal antibodies to determine whether or not they were serotypes O1 and O139 (Chowdhury et al., 1995). As positive controls for these analyses, we maintained 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 Oldach et al. (2000). Briefly, PCR primers were designed to unique regions of the small subunit ribosomal DNA of *P. piscicida* and *P. shumwayae*. Samples (50 to 200 ml of unpreserved water or several grams of sediment) were drawn onto glass fiber filters and immersed in a CTAB lysis buffer at room temperature, followed by a chloroform extraction and purification. 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.

Viability and enumeration of dinoflagellate cysts. Dinoflagellate cysts were isolated from a known volume of sediments using sodium metatungstate (Bolch 1997). After isolation, the suspension containing cysts was sub-sampled and transferred into 6-well plates. The plates contained f/10 culture medium (Guillard, 1973) made up to salinities of 0, 10, 20 and 30 with deionized water. Wells 5 and 6 contained sterile NaCl solution (of the same salinity as the original pore water) with no added nutrients. The plates were incubated at 18°C in the light

(12:12 L:D) and examined for dinoflagellate cysts and germinated swimming cells on days 1, 4, 8, 15, 30, and 60 days after incubation. In Study 1, one plate per sample was incubated, and some samples were enumerated (2 collected in 2001 and 5 collected in 2002). In Study 2, triplicate plates per fraction were incubated and cysts were not enumerated. Some identifications were made but have not been verified independently by a dinoflagellate taxonomist.

Detection of *Aureococcus anophagefferens* (2001 samples only). Samples were analyzed as detailed in Doblin et al. (2004). Briefly, between 100 and 300 ml of residual water was filtered serially to collect the size fraction of plankton that includes *Aureococcus* (1 - 5 μm). Nucleic acids were extracted from the filters and a PCR-based technique was used for detection.

Assays for pathogenic intestinal protozoans. Assays for *Cryptosporidium* and *Giardia* followed methods described in Graczyk et al. (1997), using a membrane filter dissolution method for recovery of the pathogens and immunofluorescence microscopy for direct enumeration of cells. Fluorescent identification of the oocytes from the filtration was based upon the comparison with fluorescent features of the enumerated oocytes in 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. Assays were also performed for a microsporidian, *Encephalitozoon intestinalis* (2002 samples only).

Sole-carbon source characterization of the heterotrophic bacteria community. Assays were conducted using GN2 Microplates (Biolog, Inc.) as described by Garland and Mills (1991). The microplate consists of 95 wells, each containing a different carbon substrate, and a control well. The list of carbon substrates includes carbohydrates, methyl esters, carboxylic acids, amides, amino acids, aromatics, amines, and polymers (Bochner, 1989). Each well also contains a tetrazolium dye that irreversibly turns purple should the substrate be utilized. Therefore, 95 time-series tests for substrate utilization (based on measurement of optical density) can easily be performed on a water sample, providing abundant data suitable for multivariate analyses, e.g., principal components analysis (see details of measurement and analytical methods in Choi and Dobbs, 1999).

3.3.3. Results

These results are divided into four sections. The first considers physico-chemical measures (temperature and salinity) over the course of the study and their relationship, if any, with so-called microbial "bulk metrics" (number of viruses and bacteria, concentration of phytoplankton pigments). In addition, we considered the bulk metrics in terms of one another and in terms of the amount of residuals and time since the tanks were last cleaned. The second section of the results summarizes our findings with respect to indicator organisms, pathogens, and harmful algae. The third section is a focused report on viability of dinoflagellates recovered from sediment residuals. The fourth and final section deals with sole-carbon source characterization of the heterotrophic bacteria community.

Physico-chemical measures, microbial bulk metrics, and ballast-tank conditions

During shipping seasons of 2001 and 2002, samples of ballast residuals (water and sediments) were collected from 82 tanks distributed among 42 ships. Sampling was conducted throughout the shipping season in 2001 and 2002, with good representation across different temperatures and ballast salinities (Figs. 3.7A and B). There was no relationship between salinity of ballast residuals and ambient air temperature (Fig. 3.8), indicating no seasonal relationship with residual salinities.

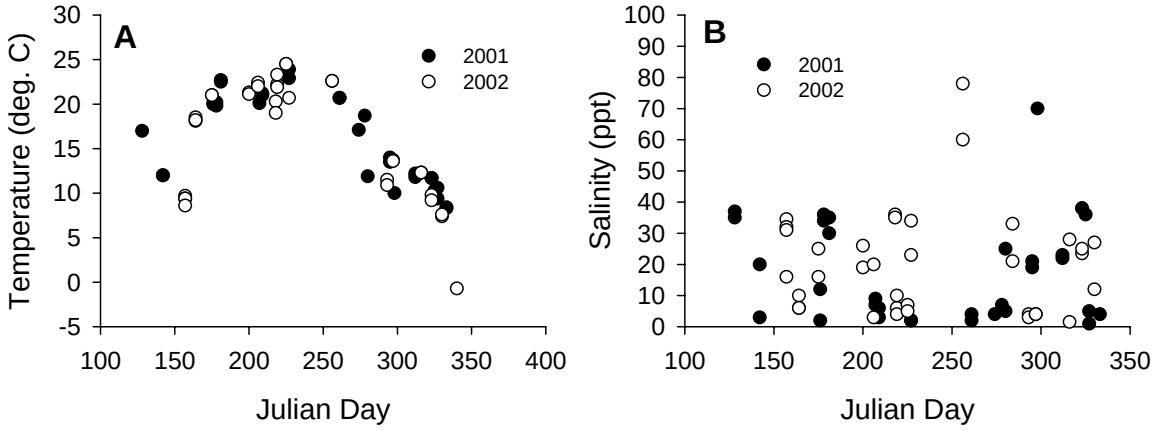


Fig. 3.7: (A) ambient (dockside) air temperature on days of sample collection and (B) salinity of residual samples collected in 2001 and 2002.

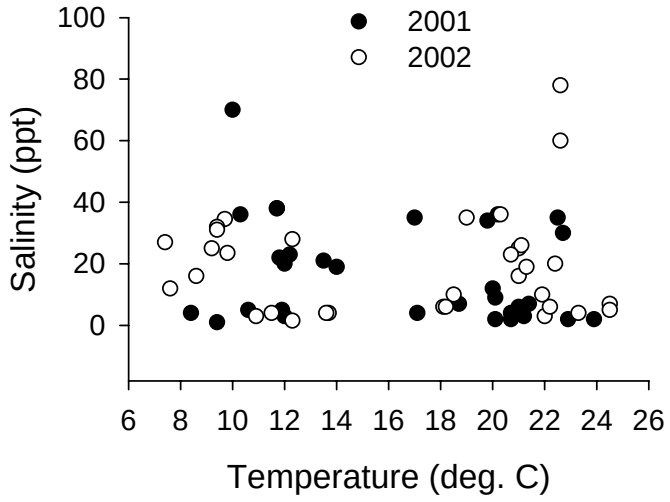


Fig. 3.8. Ambient air temperature on days of sample collection vs. salinity of residual ballast residuals collected in 2001 and 2002.

Abundance of virus-like particles (VLPs) and bacteria

In residual water, most VLP concentrations ranged between 10^7 and 10^9 ml^{-1} and all but one sample had between 10^5 and 10^9 bacteria ml^{-1} (Figs. 3.9, 3.10). In sediment porewater, VLP concentrations varied between 10^7 and 10^{11} ml^{-1} and bacteria concentrations ranged from 10^4 to 10^8 ml^{-1} (Figs. 3.9, 3.10).

While most of these VLP and bacterial concentrations fall within ranges to be expected in natural aquatic environments, there were some extraordinarily high values, e.g., porewater VLP concentrations in 2001 (Fig 3B, > 10^{10} ml^{-1}) and residual water bacteria concentrations in 2002 (Fig 3C, >10^8 ml^{-1}). There also were some very low values, e.g., VLP porewater concentrations in 2001 (Fig. 3B, < 5 X 10^7 ml^{-1}) and porewater bacteria concentrations in 2001 (Fig. 4B, <10^5 ml^{-1}). Overall, then, we conclude there was a very high degree of variation in the concentrations of VLPs and bacteria in these samples.

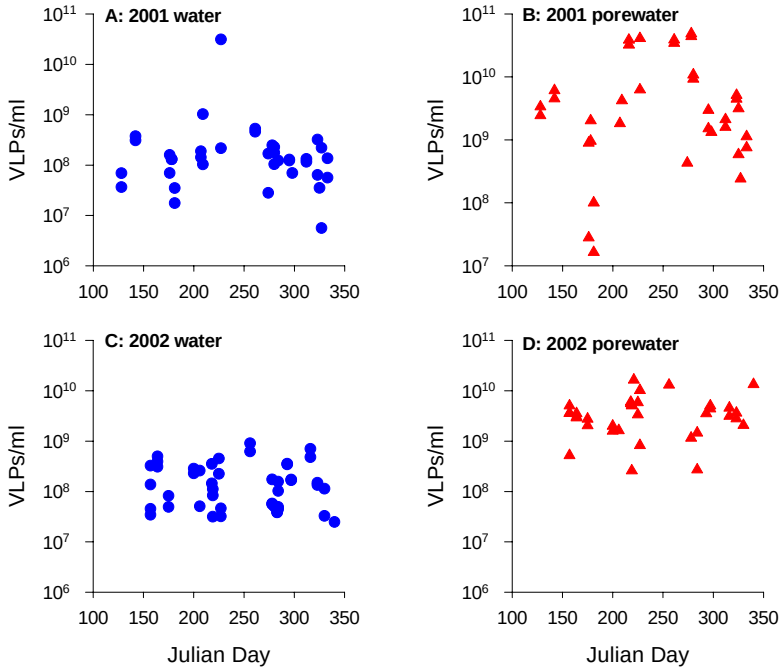


Fig. 3.9. Abundance of virus-like particles in residual water and porewater from residual sediment samples collected in 2001 and 2002.

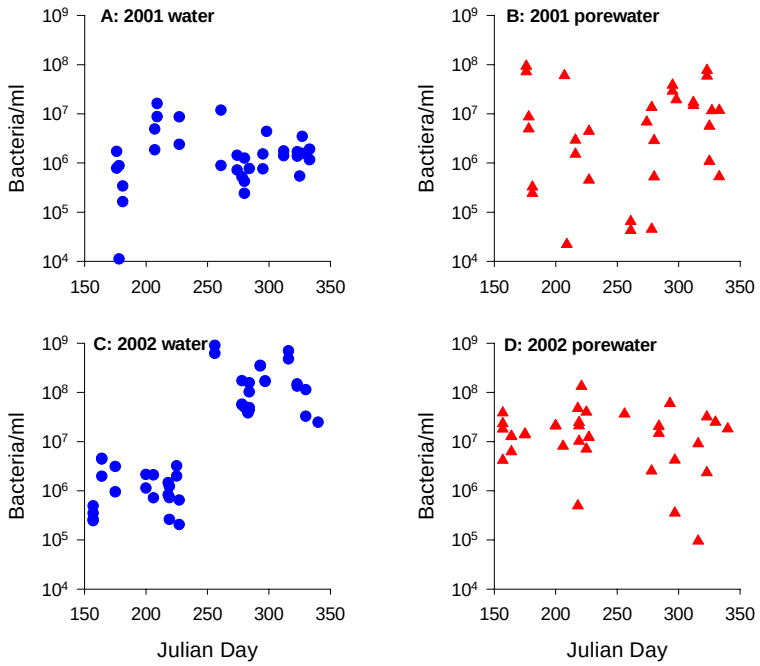


Fig. 3.10. Abundance of bacteria in residual water and porewater from residual sediment samples collected in 2001 and 2002.

There was no relationship between abundance of bacteria or virus-like particles and (dockside) ambient air temperature (Fig. 3.11), suggesting there was no overt seasonal effect on the concentration of total microorganisms.

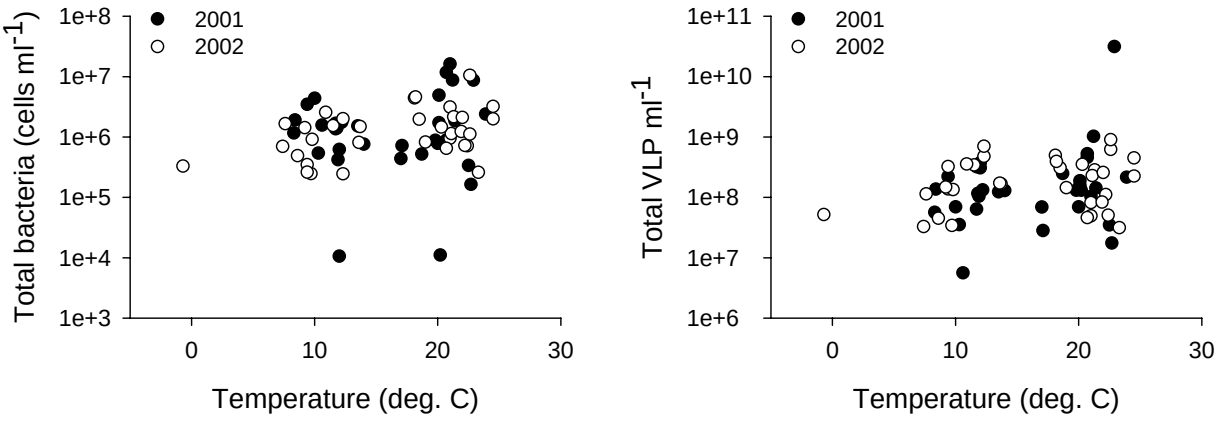


Fig. 3.11. Abundance of total bacteria and virus-like particles in residual water samples collected during 2001 and 2002.

In many aquatic systems, the density of naturally occurring viruses is regulated by the density of their hosts (typically bacteria). Thus, we looked for a positive correlation between VLP and bacteria concentrations in residuals, but a significant relationship emerged only in 2002 (Fig. 3.12).

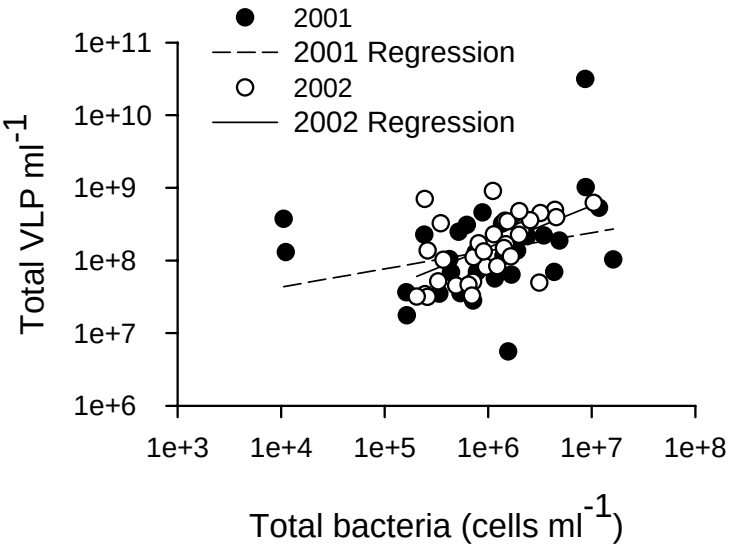


Fig. 3.12. Relationship between total bacteria and virus-like particles (VLPs) in residual water in 2001 and 2002. Although two regression lines are shown, the relationship is statistically significant only for the 2002 data.

Bacteria abundance in residual water had no significant correlation with the total amount of residual sediments on board ships, the number of months since tanks were cleaned or the relative proportion of residuals versus the total ballast capacity (analog for ship size) (Fig. 3.13A, B, and C, respectively).

Likewise, VLP abundance in residual water had no significant correlation with the total amount of residual sediments on board ships, the number of months since tanks were cleaned or the relative proportion of residuals versus the total ballast capacity (analog for ship size) (Fig. 3.14A, B, and C, respectively).

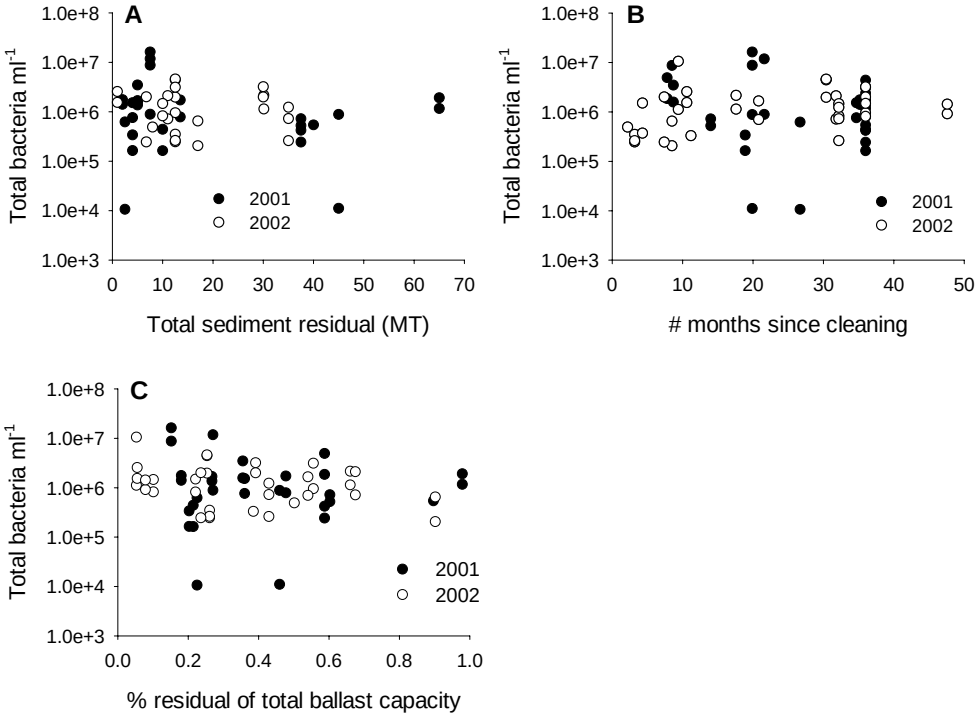


Fig. 3.13. Bacteria concentration in residual water with respect to (A) the total amount of sediment residual; (B) number of months since the ballast tank was cleaned; and (C) the proportion of residual material versus ballast capacity.

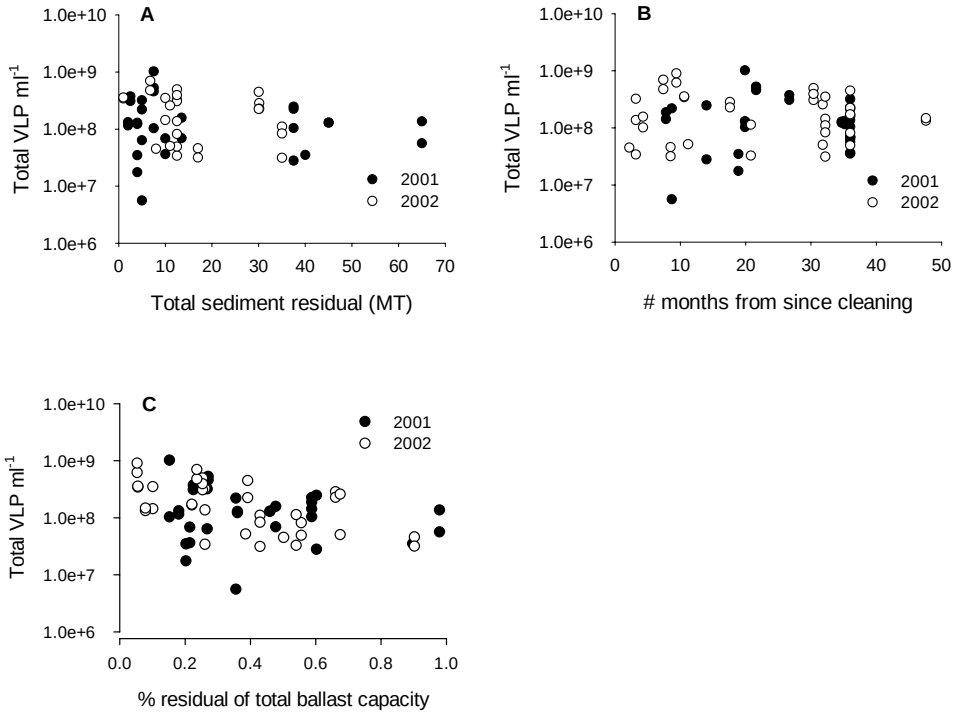


Fig. 3.14. VLP concentration in residual water with respect to (A) the total amount of sediment residual; (B) number of months since the ballast tank was cleaned; and (C) the proportion of residual material versus ballast capacity.

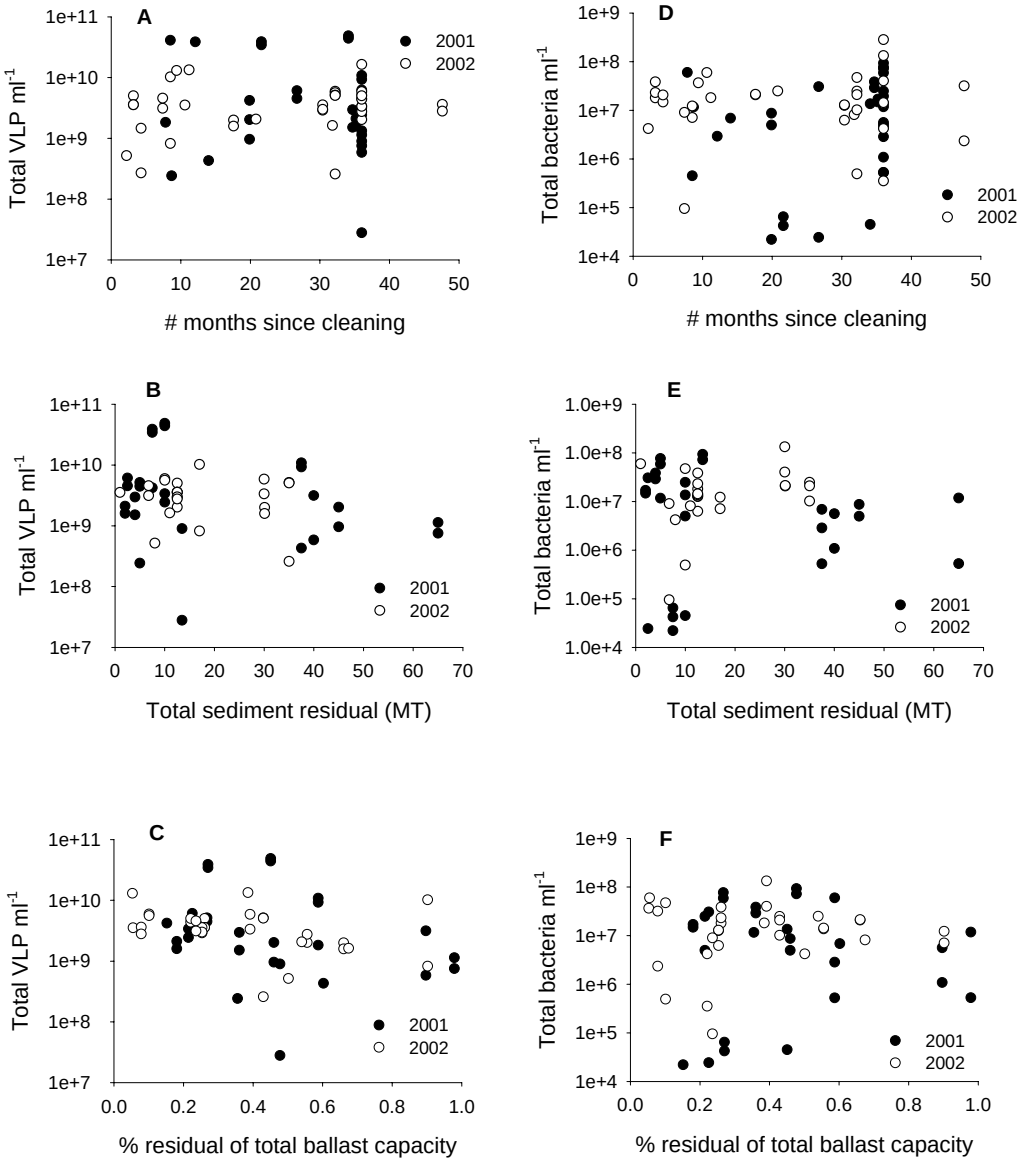


Fig. 3.15. VLP (A-C) and bacteria (D-F) concentrations in porewater extracted from residual sediments with respect to months since the ballast tank was cleaned, the total amount of sediment residual, and the proportion of residual material versus ballast capacity

As was the case with residual water, there were no obvious relationships between VLP or bacteria abundance with respect to months since the ballast tank was cleaned, the total amount of sediment residual, and the proportion of residual material versus ballast capacity (Fig. 3.15A-F).

Chlorophyll-a and phaeopigments (bulk indicators of phytoplankton biomass)

The abundance of photosynthetic organisms (as indicated by chlorophyll-*a* concentration) in residual water varied in substantially (Fig. 3.16), with chlorophyll-*a* averaging 0.89 ± 1.11 μg l^{-1} in 2001 (n = 34) and 1.47 ± 4.87 μg l^{-1} in 2002 (n = 37). With the exception of two tanks, chl-*a* to phaeopigment ratios were generally low (indicating a relatively high concentration of degraded pigments). Just as for VLPs and bacteria, the concentration of chl-*a* or its ratio to phaeopigments had no apparent relationship with the number of months since tanks were cleaned, the total sediment residual, or the % residual of the total ballast capacity. Thus, there appears to be no predictability with respect to biological residuals and these parameters.

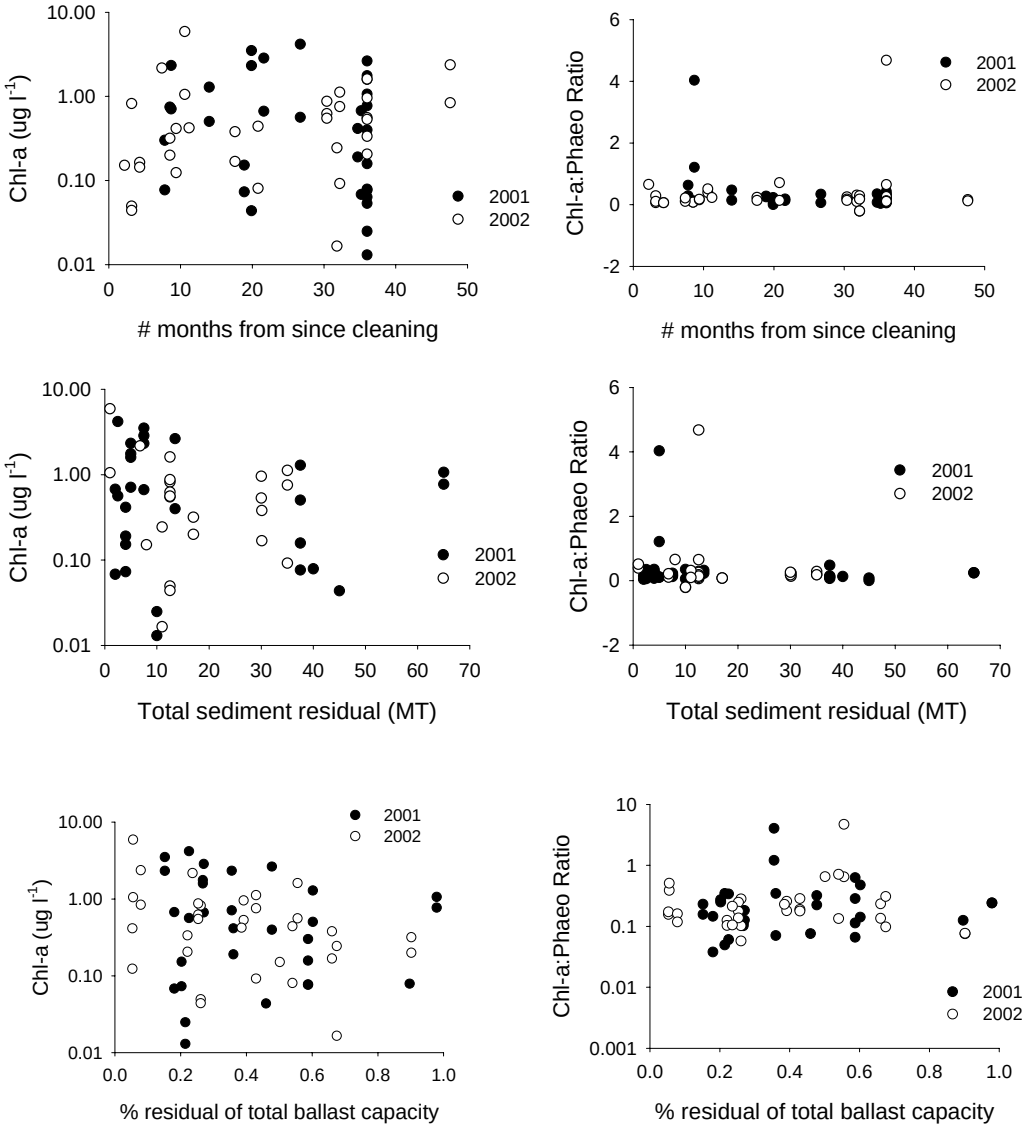


Fig. 3.16. Chlorophyll *a* and chlorophyll *a* to phaeophytin ratio in residual water with respect to months since the ballast tank was cleaned, the total amount of sediment residual, and the proportion of residual material versus ballast capacity.

Indicator organisms, pathogens, and harmful algae

Major findings are:

- We found microbial pathogens in ballast residuals, including *Vibrio cholerae*, *Cryptosporidium parvum*, *Giardia lamblia*, *Encephalitozoon intenstinalis*, *Pfiesteria piscicida*, *P. shumwayae*, and *Aureococcus anophagefferens* (see Fig. 3.17).
- We did not detect *E. coli* or enterococci in any of the samples tested.
- In all of the residual samples collected, 26 of 42 (62%) *ships* sampled tested "positive" for one or more pathogens.
- In all of the residual samples collected, 40 of 82 (49%) *tanks* sampled tested "positive" for one or more pathogens.
- There were few incidences of pathogen co-occurrence: 1 tank in 2001 and 2 tanks in 2002 had three pathogens. Four tanks in 2001 and 8 tanks in 2002 had two pathogens.
- There was no consistent temporal pattern in pathogen presence. In 2001, pathogens were detected throughout the sampling season (May to November), but more frequently in summer (June-July) than in fall (October-November). In 2002, pathogens were detected from June to November, but not in September or December (see Fig. 3.18).
- Data suggest ballasting operations in Antwerp (Belgium) are associated with ships carrying pathogens into the Great Lakes. Table 3.6 lists the location of ballasting operations of ships sampled in this study, prior to their most recent entry into the Great Lakes, ranked by the number of "positive" tanks. Tanks with water from Antwerp have the greatest pathogen frequency, with other European ports and the Port of Matanzas (Cuba) and Maracaibo (Venezuela) having moderate pathogen frequency.

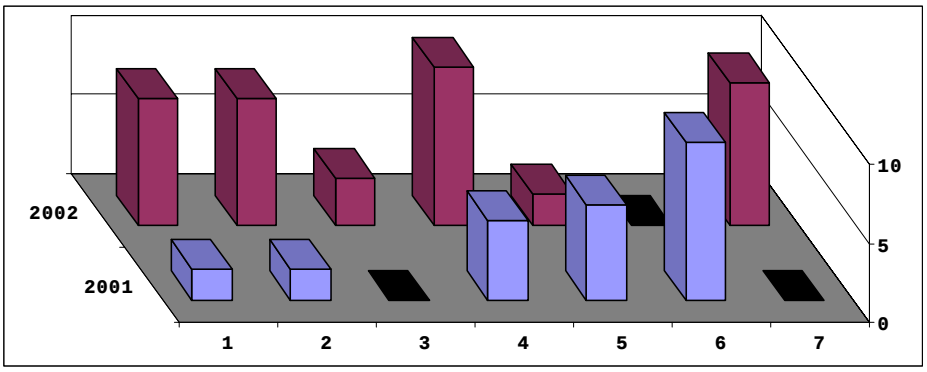


Fig. 3.17. Number of tanks testing "positive" for pathogens and harmful algae. 1 = *Giardia*; 2 = *Cryptosporidium*; 3 = *Encephalitozoon intenstinalis*; 4 = *Pfiesteria piscicida*; 5 = *P. shumwayae*; 6 = *Aureococcus*; 7 = *Vibrio cholerae*. The black rectangles (e.g., *Vibrio cholerae* in 2001) indicate there were no assays for that particular pathogen that year.

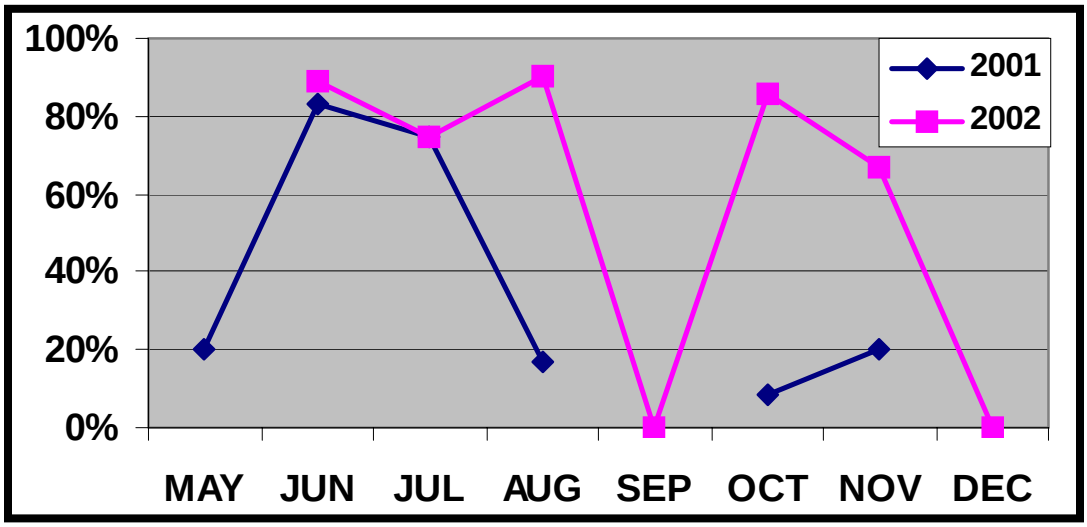


Fig. 3.18. Proportion of tanks testing positive for pathogens during the 2001 and 2002 sampling season.

Table 3.6. Ports where ballasting operations took place, ranked by number of "positive" tanks.

Previous Port		2nd Previous		3rd Previous	
Antwerp	8	Antwerp	6	Great Lakes	5
Matanzas	5	Ghent	4	Antwerp	4
Brunsbuttel	4	Rotterdam	3	Burns Harbor	3
Bremen	3	Bremen	3		
Rotterdam	3	Hamburg	3		
		Maracaibo	3		

Viability of dinoflagellates recovered from sediment residuals

A total of 17 sediment samples were incubated and monitored for dinoflagellate cyst germination (Table 3.7). In Study 1, 15 samples were tested by incubating one plate per fraction and cyst abundance was enumerated for 7 of the 15 samples tested. In Study 2, 9 samples were tested by incubating triplicate plates per fraction and cyst abundances were not enumerated.

Table 3.7. Residual sediments incubated for dinoflagellate cyst germination.

Study 1			***Study 2***	
Sample	Cyst abundance (g^{-1} sed.)	Cyst germination	Sample	Cyst germination
			1-01128-01ws	**30 (day 30)**
1-01128-02ws		**0,30 (day 12,33)**	1-01128-02ws	**30 (day 15)**
			1-01178-01ws	None
1-01178-02ws	367±126 (n=3)	None	1-01178-02ws	None
1-01274-01ws		None	1-01274-01ws	None
1-01312-01ws		**30 (day 3,9)**	1-01312-01ws	**30 (day 30)**
1-01312-02ws		**30 (day 2,15,30)**	1-01312-02ws	**30 (day 15)**
1-01323-01ws		None	1-01323-01ws	None
1-01323-02ws		None	1-01323-02ws	None
1-01333-02ws	80±76 (n=5)	**30 (day 30)**		
1-02157-01ws		None		
1-02164-03ws		None		
1-02175-02ws	850 (n=1)	**30 (day 30)**		
1-02200-01ws	417±104 (n=3)	None		
1-02206-01ws	383±82 (n=3)	None		
1-02218-01ws	90±42 (n=5)	None		
1-02221-01ws	130±45 (n=5)	None		

Total cyst abundance in residual sediments varied over an order of magnitude, from approximately 80 to 850 cysts per gram of sediment. Cyst abundance was low in each replicate count (maximum number observed was 17 in sample 1-02175-02ws). Thus total abundance estimates have a relatively large standard deviation (coefficient of variation ranged from 21-95%).

Of the 15 samples examined in Study 1, dinoflagellate germination occurred in 5 (33%). Germination frequency was similar in Study 2 (4 of 9 samples; 44%), with dinoflagellates emerging in the same samples as Study 1 (Table 3.7). Interestingly, Study 1 yielded marine dinoflagellates and a green flagellate, whereas Study 2 yielded marine dinoflagellates and an organism that is tentatively classified as belonging to the Class Cryptophyceae. (Cryptophytes belong to the division Chromophyta, which includes dinoflagellates, Dinophyceae). More definitive scanning electron microscope identifications are pending.

In no case did germination occur in all triplicate subsamples (Study 2). In fact, in only one sample (1-01128-02ws) did germination occur in more than one sub-sample. Summarizing both studies, there was no germination at any salinity other than 0 or 30, and there was no germination in unenriched wells. The time required for germination varied from 3 to 30 days, with most wells exhibiting germination by day 15 than any other time (Table 3.7).

There were similarities and differences in the types and number of organisms which grew in the sediment samples (Table 3.8). These organisms included, but were not limited to, centric diatoms, colonial diatoms, filamentous algae, and pennate diatoms. Organisms or objects observed but not noted were pollen grains, foraminiferans, and various animal parts or cell debris.

Table 3.8. Comparison of organisms observed in Study 1 and 2.

Sample	Study 1*	Study 2*
1-01128-01ws		SD, cd, chd, fa
1-01128-02ws	SD	SD, cd, chd, fa
1-01178-01ws		cd empty frustules
1-01178-02ws	fa	NO
1-01274-01ws	cd, pd	chd
1-01312-01ws	SD, cd	cd, chd, OC, pd, td
1-01312-02ws	SD	cd, chd, SD
1-01323-01ws		cd empty frustules
1-01323-02ws	cd, f, chd	cd empty frustules
1-01333-02ws	cd	

*Key: cd = centric diatom; chd = colonial diatom; f = foraminiferan; fa = filamentous algae; OC = oblong cryptophyte; pd = pennate diatom; SD = spherical dinoflagellate; td = triangular diatom; NO = no appreciable growth.

Differences and similarities between the two studies are summarized in Table 3.8. For example, in sample 1-01128-02ws, spherical dinoflagellate cells were observed in both studies; however in Study 2 the sample also yielded significant amounts of centric diatoms, colonial diatoms, and filamentous algae. Sample 1-01178-02ws showed drastic differences between the two studies. In Study 2, no organisms of significance grew; however, filamentous algae grew in Study 1. Sample 1-01274-01ws also had different organisms that grew during the incubation; Study 1 found centric and pennate diatoms in the sample, while Study 2 found only colonial diatoms. Sample 1-01312-01ws previously exhibited spherical dinoflagellates and centric diatoms, and in the current work centric diatoms, colonial diatoms, oblong cryptophytes, pennate diatoms, and triangular diatoms were observed. Sample 1-01312-02ws was slightly more similar and spherical diatoms were observed in both studies. In Study 2, however, centric and colonial diatoms were also observed. Finally, sample 1-01323-02ws showed centric diatoms, foraminiferans, and colonial diatoms in Study 1, and only centric diatom frustules in Study 2. These comparisons suggest that germination of many cell types and the level of germination per sample varies over time and among samples. These results also are consistent with a high level of spatial heterogeneity in the distribution of cysts in ballast-tank sediments.

The approximate average time for germination in Study 1 was 17.1 days (Table 3.7). While there were only two time points for germination in Study 2, the approximate average time was 18.5 days. Thus, the average times for germination were similar, even though the ranges observed were variable.

The salinity in which dinoflagellates germinated was also more variable in Study 1 compared to Study 2. Germination in the first study was seen not only at salinity 30, but at 0 as well. This

result potentially has important ecological concerns for the ports from which these samples were taken. The Great Lakes are a freshwater ecosystem, of course, and an invasion of a freshwater dinoflagellate (or at least, one tolerant of freshwater) has potential to negatively impact the indigenous freshwater plankton.

In summary, two independent studies of samples collected in 2001 showed consistent results, but differences in time to germination, salinity at which germination occurred, and growth of different organisms in individual plates together serve to highlight the variability within and between samples.

Sole-carbon source characterization of the heterotrophic bacterial community

Data sets were examined using principal-components analysis (PCA) in the manner described by Choi and Dobbs (1999). When all samples were considered, patterns were strongly affected by several outliers representing samples for which color development was extremely low (Fig. 3.19). In an attempt to better delineate trends, plates exhibiting no color development in five or more wells (of 95 wells containing sole-source carbon substrates) were eliminated from consideration. The resultant classification (Fig. 3.20) yielded no clear groupings of samples. The first two principal components (PCs) accounted for 71.3% and 3.7%, respectively, of total variation in GN2 plate data. The very high proportion of variance explained by PC1, however, was not correlated with either salinity of the residual water or dockside air temperature at time of sampling.

These results obtained with the entire two-year data set were essentially reiterated when each year (2000 and 2001) was analyzed separately (data not shown). Thus, there was no discernible or predictive relationship between salinity of the samples or ambient temperature and the waters' community-level metabolic response to the 95-carbon substrate array. Using a similar approach, we looked for trends between location of the previous ballasting port and PC scores, but none emerged.

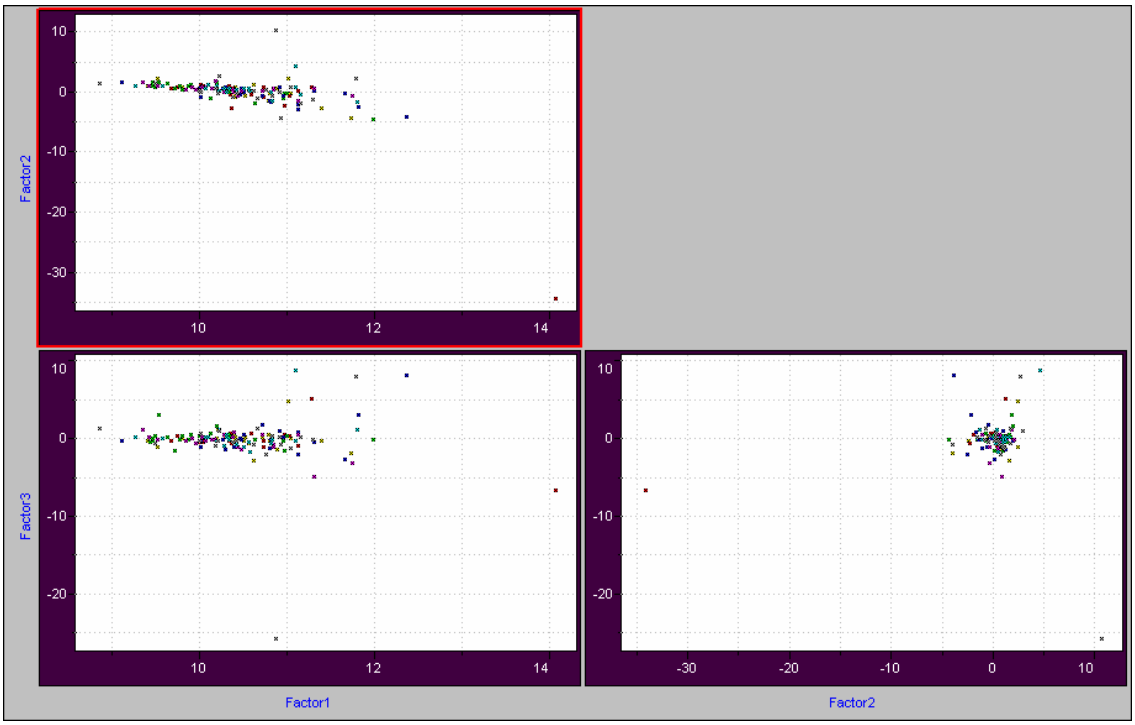


Fig. 3.19. Multivariate classification of aquatic heterotrophic bacteria communities, based on carbon-substrate utilization in Biolog GN2 plates. Two-dimensional plot of results from principal-components analysis of all samples collected in 2000 and 2001. Each point represents a sample.

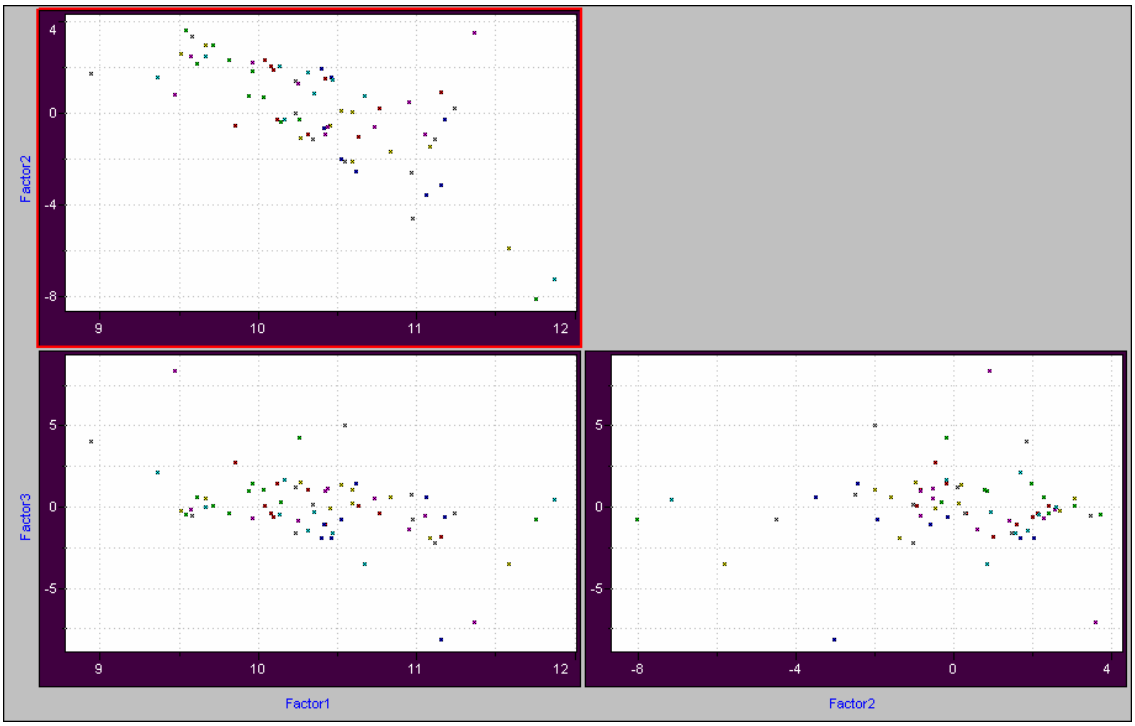


Fig. 3.20. As for Figure 3.19, except that several plates having 5 or more wells with extremely low color development were excluded from the data set.

3.3.4. Discussion

As part of a study intended ultimately to evaluate the risk of invasions associated with ocean-going vessels entering the Great Lakes, we characterized microbial components in ballast tanks of NOBOB ships. During 2001 and 2002, samples of ballast residuals (water and sediments) were collected from 82 tanks distributed among 42 ships. Our analyses of water and porewater included (among other measures) direct counts of virus-like particles (VLPs) and bacteria, enumeration of dinoflagellate cysts and tests of their viability, and assays for enteric bacteria, the bacterium *Vibrio cholerae*, and the protozoans *Cryptosporidium, Giardia*, and *Pfiesteria*.

Concentrations of VLPs and bacteria exhibited some extraordinarily high and low values, and no correlation with respect to salinity of the samples, ambient air temperature, time elapsed since last cleaning, mass of the ship's sediment residuals, or the percent of total ballast capacity comprised by residuals. Dinoflagellates and other unicellular algae found in sediments germinated in laboratory studies, even a year after their collection, emphasizing the importance of biological "resting stages" in consideration of ballast practices and management. Finally, in nearly half the tanks (40 of 82) and more than half the ships (26 of 42), we detected the presence of at least one species of harmful or pathogenic bacteria or protists—and sometimes more than one species. Of significant note, we did not detect enteric bacteria (*E. coli* and enterococci), but our sampling in this context was limited.

We considered these results with respect to the time of year samples were collected and the ships' previous ports-of-call. While no pattern emerged from the former analysis, the data suggest ballasting operations in Antwerp, Belgium--and other ports--are associated with ships carrying pathogens into the Great Lakes. This trend notwithstanding, it seems prudent to regard all NOBOB ships entering the Great Lakes as potential carriers of pathogens.

What is the relevance of these results with respect to management of ballast water and ballast residuals in the Great Lakes? If one considers the bona fide, documented introductions of organisms by ships' ballast waters, the emergent point is that invaders overwhelmingly are macroinvertebrates. With the exception of dinoflagellates (e.g., Hallegraeff 1998; Lilly et al. 2002), there is no conclusive evidence linking ballasting operations to successful invasions by aquatic microorganisms. Nonetheless, it would be simplistic and possibly very wrong to consider that aquatic microbial invasions do not occur or could not be mediated by ballast water. Unlike many of their invertebrate counterparts, microbial invaders cannot be seen without a compound microscope and their presence might only be noticed in spectacular cases, e.g., red tides or outbreaks of illness. Thus, there is a bias inherent in the detection of nonindigenous microorganisms. In considering such organisms, Wyatt and Carlton (2002) proposed a "smalls rule", i.e., the smaller the taxon, the less likely it is to be recognized as introduced and more likely to be considered indigenous. Therefore, tiny species are regarded as native, sometimes despite evidence to the contrary, when they should by default be considered to be cryptogenic, i.e., of unknown origin, until proven otherwise.

A managerial avenue more satisfying, perhaps, than pondering the "smalls rule", is to consider the predictions of risk-assessment models, especially ones developed specifically for the Great Lakes. MacIsaac et al. (2002) showed the propagule pressure of NOBOB ships entering the

Lakes was one to two orders of magnitude greater than ships that had exchanged ballast water at sea. Because NOBOB vessels predominate in incoming traffic to the Great Lakes, such ships appear to pose the greatest risk of new introductions.

Special Focus--Transport of harmful algae in NOBOB ships

It is well established that cyst-forming phytoplankton species are transported in ships' ballast tanks. However, there is increasing evidence that other phytoplankton species which do not encyst are also capable of surviving ballast transit.

The brown-tide organism, *Aureococcus anophagefferens* (hereafter *Aureococcus*) can grow under organic enrichment (Dzurica et al. 1989; Cosper et al. 1990; Berg et al. 1997; Gobler and Sañudo-Wilhelmy 2001) and at low light (Milligan and Cosper 1997) and survive at least 30 days in complete darkness (Popels and Hutchins 2002). In these respects, it is conceivably well suited for surviving transport in ballast tanks. **It was therefore selected as a focal point in our investigations on NOBOB vessels.** The remainder of this section of the report summarizes a detailed account already published in the peer-reviewed scientific literature (Doblin et al. 2004).

Brown tides have had significant detrimental impacts on the benthic communities in Narragansett Bay, Rhode Island, and the bays of Long Island, New York, and New Jersey (Bricelj and Lonsdale 1997), causing eelgrass dieback (due to decreased light penetration), and starvation and recruitment failure of commercially important scallop and mussel populations (Cosper et al. 1987; Tracey 1988). New data suggest there has been a dramatic extension of the known range of *Aureococcus* on the east coast of the US (Popels et al. 2003). Previously, it was known to exist in shallow estuaries from New York to Maryland, but not further south (Anderson et al. 1989). The newly defined range extends from Florida northward to New Hampshire (Popels et al. 2003), with blooms now being recorded in Virginia's coastal bays (Boneillo et al. 2003). Blooms have also recently been observed in Saldanha Bay, South Africa (Naidoo 1999; Pitcher and Calder 2000; Probyn et al. 2001). Thus, the distribution of brown tide seems to be rapidly increasing both within and outside the US, suggesting an anthropogenic dispersal vector, exploitation of a niche in previously uninhabited environments, or both.

Aureococcus is small in size (approximately 2-4 μm in diameter) and difficult to detect directly using regular microscopic observation, particularly at low background cell levels. To confirm the presence of the organism, we used an *Aureococcus*-specific primer for its detection in ballast residuals by PCR amplification of the 18S rRNA gene (Doblin et al., 2004).

Our investigations of NOBOB vessels demonstrated that commercial ships allow delivery of viable brown tide cells at endpoints of transits. Of 11 ships (total of 19 tanks) sampled from June-November 2001, 2 contained detectable levels of viable (actively transcribing) *Aureococcus* cells. This result is noteworthy given the ships' ballast histories, which indicate both tanks were ballasted twice with freshwater before being sampled, and had exchanged their ballast in the open ocean either on their last or second-last voyage, as much as a month prior to sampling. For a polyhaline (18-30 ppt) phytoplankton species to remain viable in the presence of water ≤5 ppt is certainly remarkable, and suggests that this should be taken into consideration as new ballast regulations are developed (e.g., IMO 2003).

For non-cyst forming phytoplankton such as *Aureococcus*, the presence of a large initial population entrained in the ballast tank is likely key to its successful transport and introduction, given the exponential decay of photosynthetic organisms during ballast tank confinement (Drake et al. 2002). Current ballast management procedures recommend that vessel captains avoid algal blooms when loading ballast (IMO 1997). However for *Aureococcus*, it appears that temperature may also be an important physiological constraint on its survival in ballast tanks. Brown tide was not detected in ballast water having temperatures below 17.6 °C, consistent with its temperature response in laboratory cultures (optimal temperature for growth ranges between 20 and 25 °C; Cosper et al. 1989). Other characteristics, such as the ability to survive in the dark and utilize organic substrates, may also facilitate ballast transport of *Aureococcus.* However, Popels and Hutchins (2002) found that *Aureococcus* survived best in the dark at *low* temperatures (e.g., 6°C and 12°C), presumably due to the decrease in metabolic activity, and found the addition of organic substrates had no effect on survival.

One of the most intriguing aspects of this organism's survival in ballast water is its apparent tolerance of low salinities--in contrast to its salinity preferences in the field (18 – 32 ppt; Anderson et al. 1993) and in culture (>22 ppt; Cosper et al. 1989). Brown tide was found in vessels with residual water salinities ranging from 2 to 34 ppt, and in both cases when *viable* cells were detected, tanks had taken on freshwater ballast during the last two ballast operations. In a previous study (Popels et al. 2003), *Aureococcus* was detected in locations that were periodically fresh (i.e. tidally influenced), but brown tide blooms have not occurred at these sites. Thus, it appears that *Aureococcus* is tolerant of low salinities in ballast tanks (perhaps due to buffering properties of the sediments), enabling it to survive a 30-day exposure to 2 ppt ballast-water. However, based on culture data (no growth at 0 ppt), it is unlikely that cells would grow on delivery to the freshwater Great Lakes.

While the possibility of an *Aureococcus* invasion of the Great Lakes is very, very low, the point we wish to make at the end of this report is that *Aureococcus* can be considered a model for what may well occur with other species of microorganisms. We know a good deal about the biology of *Aureococcus* and can detect it reliably. We feel confident, however, that other microorganisms, ones of which we are unaware or for which we did not assay, are found in the residual water and sediments of NOBOB vessels. Without knowing about their presence, much less their niche parameters (e.g, temperature and salinity tolerances), it is impossible to predict whether they might pose an invasive threat. With reference to the Precautionary Principle, however, we cannot dismiss such a possibility.

3.4. Phytoplankton

3.4.1. Introduction

The purpose of this part of the study was to examine phytoplankton transported by NOBOB vessels entering the Great Lakes, and to examine the potential for these phytoplankton to survive and possibly grow in the Great Lakes. In order to accomplish this objective we: 1) analyzed phytoplankton composition from residual sediment samples, 2) isolated and identified resting stages (cysts) of phytoplankton from ballast tank residuals, 3) conducted experiments with ballast tank sediment and water residuals to determine the survival and growth potential of these phytoplankton, and 4) determined whether the phytoplankton from experimental treatments were indigenous or nonindigenous species.

3.4.2. Methods

Residual ballast samples were collected from the empty tanks of NOBOB ships from December 2000 – December 2002 at various ports in the Great Lakes. Water samples were collected by hand pump from the bottom of the ballast tank. Sediment samples were collected aseptically by using spatulas. In most cases, two tanks were sampled per ship. For each ballast tank, environmental measurements (temperature and salinity of ballast water) and ship/tank identifiers (ship's age, time since ballast tanks were cleaned, ballast tank type- double bottom, side, forepeak, upper wing, and whether the tanks were flushed on the most recent trip) were determined. The water and sediment samples were stored at 4^0C during transport and storage until analyzed.

Once in the lab, ballast samples were prepared for phytoplankton analysis and germination experiments. Because it was extremely difficult to identify phytoplankton cells from raw sediment material due to the abundance of non-biotic material, we used an extraction procedure to isolate phytoplankton cysts and other algal material (Bolch 1997). For these sediment samples, a small (2 to 4 ml) sample was mixed with 30 ml of filtered water from the same tank, agitated using the super mixer for 2 minutes, washed with filtered water sample and then fractionated through 115 and 20 μm mesh sieves. The material retained on the 20 μm mesh was washed into a beaker and diluted to a known volume. Density gradient centrifugation was used to concentrate material (Bolch 1997). All phytoplankton identification and enumeration were conducted on a Leica DMR research microscope at 240-650X. Photomicrographs of cysts were taken with a Optronix 7500- camera system using Image-Pro software. Phytoplankton identification was based upon morphological criteria described in the literature.

The germination experiments were designed to determine the ability of phytoplankton in the ballast samples to survive and possibly grow (maybe considered a test of viability) in a variety of environments conditions. Because nonindigenous species may come from a variety of freshwater and marine environments, no single culture condition was deemed appropriate. Therefore, five different culture media were used in the germination experiments including: (1) a standard saltwater media- Guillard's Saltwater (Guillard 1975), **GS**, (2) a freshwater media comparable in macro and micro nutrients to the saltwater media- Guillard's Freshwater (Guillard

1975) **GL**, (3) the freshwater media that is most commonly used in our lab (Guillard and Lorenzen 1972)-**WC**, (4) 0.22 µm, filtered Lake Michigan water-**LW**, and (5) 0.22 µm, filtered Grand River water-**R**. Chlorophyll fluorescence was used as an indicator of phytoplankton abundance. For residual water samples, five mls of water was added to 45 ml of culture. For residual sediment samples, 2 ml of sediment was mixed with 30 ml of filtered tank-water to produce a slurry; then, 2 mls of this slurry were added to 45 ml of culture media. For controls, 45 ml of DDW was used in place of the culture media. All treatments were done in triplicate. For each treatment, phytoplankton abundance was monitored, and when a significant increase in abundance was noted, the experiment was terminated. At this time phytoplankton samples were taken for enumeration from all treatments. Phytoplankton samples were preserved with Lugol's solution and then prepared according to Dozier and Richardson (1975). Phytoplankton counts were only made on treatments where significant increases in fluorescence were recorded. Phytoplankton identification was based upon morphological criteria. When needed, wet mounts of experimental samples were also used to aid identification. Only healthy, vegetative cells containing cytoplasm were counted.

Statistical analysis of all data was performed with SYSTAT 8.0 and PRIMERv5.

3.4.3. Results

Composition

From 2001 and 2002, 57 residual sediment samples were analyzed for phytoplankton composition (Table 3.9). The composition of the phytoplankton community from each tank consisted primarily of diatoms (55%) and dinoflagellate cysts (24%) (Figure 3.21). Other algae made up 21% of the remaining cells. All of the dinoflagellate cysts found in our samples were marine in origin (Plates 1-6).

Table 3.9. Summary of samples analyzed for phytoplankton

2001	Type of Tanks	Number of Collection	Germination Experiment	Algal Extractions
Number of Ships		22	22	22
Number of Tanks		43	39	38
	Forepeak	9	8	7
	DBT	29	28	28
	Wing	3	2	2
	Aft	1	1	1
Number of Water		37	33	
Number of Wet Sediment		37	36	36
Number of Dry Sediment		3	2	2
Number of Slurry		2	0	0

Table 3.9 cont. Summary of samples analyzed for phytoplankton

2002	Type of Tanks	Number of Collection	Germination Experiment	Algal Extractions
Number of Ships		18	18	18
Number of Tanks		36	19	19
	Forepeak	8	3	3
	DBT	27	16	16
	Wing	0		
	Aft	0		
	Side Tank	1		
Number of Water		35		
Number of Wet Sediment		33	19	19
Number of Dry Sediment		1		

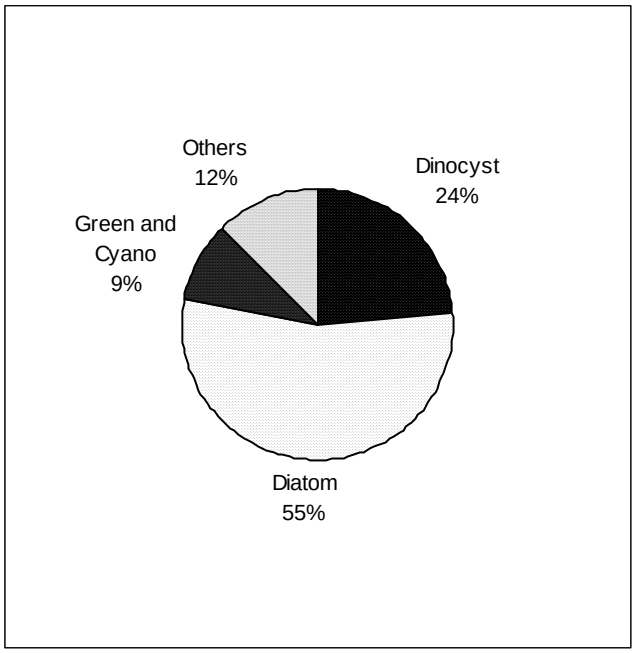


Fig. 3.21. Main component of extracted samples from the sediments of tanks

Only four of our samples did not have dinoflagellate cysts present. A total of 34 cyst species were identified (Table 3.10). The maximum number of dinoflagellate cyst species present in any one sample was 13 (Figure 3.22). Approximately 45% of the species were found in only one sample, which included mostly *Protoperidinium* and *Gonyaulax* species. Four species were found in over 20% of the samples. These include *Alexandrium minutum,* which was found in 15 samples, and *P. oblongum*, *A. hiranoi* and *Polykrikos schwartzii* which were all found in 10 samples. Species that were more intermediate in their occurrence include: *G. polyedra* and *A. lusitanicum* (8 occurrences each); *S. trochoidea*, *P. excentricum*, and *G. catenatum* (6 occurrences); *A. affinis, G. verior, P. sublinerme, P. leonis*, and *P. conicum* (5 occurrences); and *A.tamarense* (4 occurrences). The species present exhibited few clear associations among the samples, when relative abundances were analyzed. For the 2001 samples, there were only 6 instances (out of 19 identified clusters) where species associations were more similar than dissimilar.

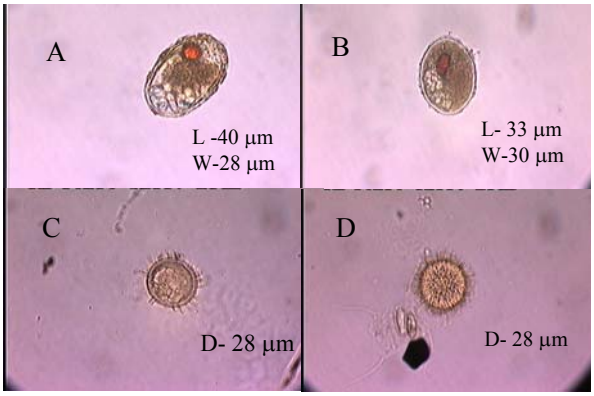


Plate I. (A) *Alexandrium catenella*
(B) *A. minutum / lusitanicum*
(C-D) *Diplopelta parva*

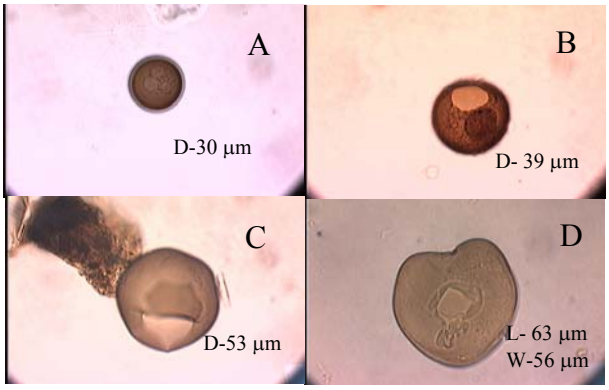


Plate II. (A) *Protoperidinium americanum*
(B) *P. punctulatum*
(C) *P. thorianum*
(D) *P. subinerme*

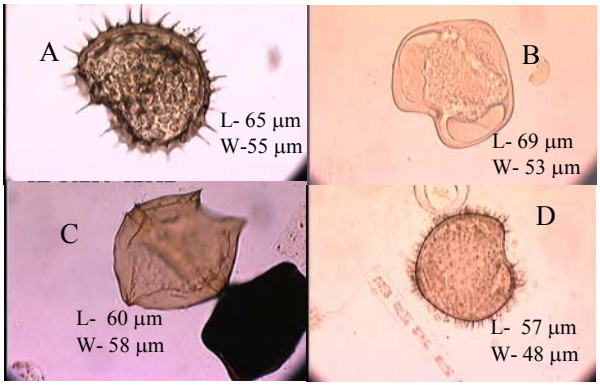


Plate III. (A) *Protoperidinium conicum*
(B) *P. oblongum*
(C) *P. leonis*
(D) *P. claudicans*

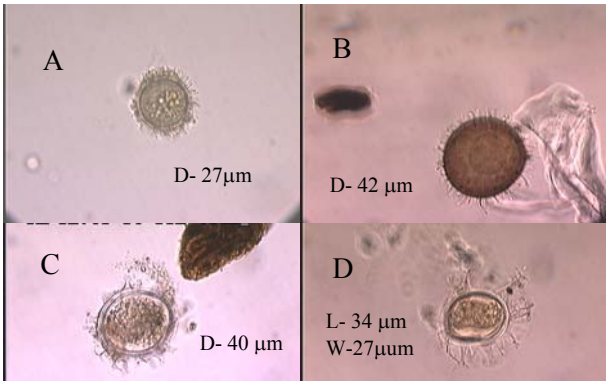


Plate IV. (A) *Gonyaulax grindleyi*
(B) *G. polyedra*
(C) *G. spinifera*
(D) *G. scrippsae*

Plate V. (A-B) *Gymnodinium catenatum*
(C) Polykrikos schwartzii
(D) *Protoperidinium nudum*

Plate VI. (A-C) *Scrippsiella trochoidea*
(D) *S. crystallina*

Table 3.10. List of dinoflagellate species (cyst forms) found in our samples.

Species Name	**Species Name**
Alexandrium affinis	*Protoperidinium claudicans*
Alexandrium catenella	*Protoperidinium conicoides*
Alexandrium hiranoi	*Protoperidinium conicum*
Alexandrium lusitanicum	*Protoperidinium denticulatum*
Alexandrium minutum	*Protoperidinium divaricatum*
Alexandrium tamarense	*Protoperidinium excentricum*
Brigantedinium sp.	*Protoperidinium leonis*
Diplopelta parva	*Protoperidinium nudum*
Gonyaulax grindleyi	*Protoperidinium oblongum*
Gonyaulax polyedra	*Protoperidinium punctulatum*
Gonyaulax verior	*Protoperidinium subinerme*
Gonyaulax scrippsae	*Protoperidinium thorianum*
Gonyaulax spinifera	*Polykrikos schwartzii*
Gymnodinium catenatum	*Scirppsiella crystallina*
Protoperidinium americanum	*Scrippsiella lachrymose*
Protoperidinium avellana	*Scrippsiella trochoidea*
Protoperidinium minutum	

The environment and ship/tank variables associated with each sample were analyzed by PCA and compared to number of dinoflagellate cyst species present in the same sample. The number of dinoflagellate species appeared to be negatively correlated with PC1 and PC2. The dominant variables contributing to PC1 were ship's age (0.48) and salinity (0.43). The dominant variable contributing to PC2 was whether or not the tanks were flushed (-0.58). The number of dinoflagellate species was negatively correlated with whether or not a tank was flushed ($r = -0.43$, $p<0.01$) and ship age ($r = -0.34$, $p = 0.03$).

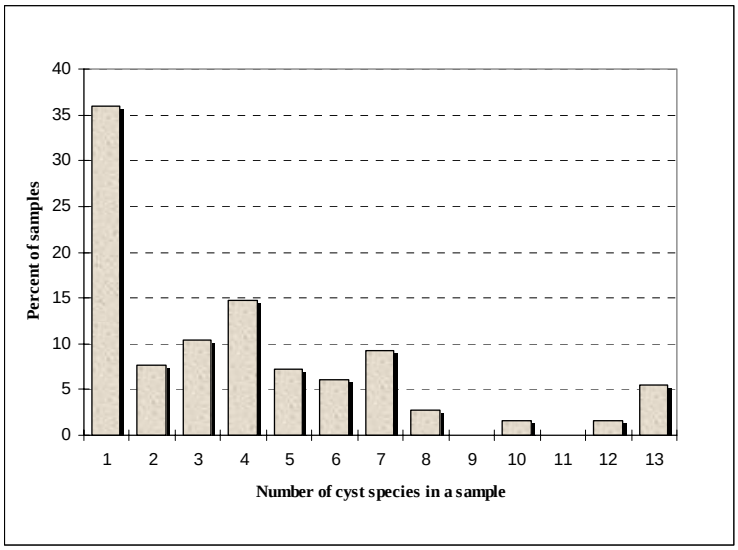


Fig. 3.22. Number of dinoflagellate cyst species found in samples

Several harmful dinoflagellate cysts were identified according to the literature (Hamer et al. 2000; Hamer et al. 2001). These are toxic, bloom-forming, marine species. Among them are cysts belonging to potentially toxic PSP-causing species of the genus *Alexandrium.* Based upon morphological descriptions in the literature, five *Alexandrium* species were identified, which are *A. affinis; A. hiranoi; A. lusitanicum; A. minutum; A. tamarense/catenella*. *A. minutum* was most common, occurring in 33% of samples. The number of *Alexandrium* species was correlated with temperature in the ballast tank (r = 0.52). Other harmful, bloom-forming species found in our samples included *Gonylaux polyedra*, *Gymnodinium catenatum* and *Scrippsiella trochoidea*. The vegetative cells of *Scrippsiella trochoidea* were found in one sample.

A few preliminary experiments were conducted where isolated cysts were added to WC media to determine if germination was possible in a freshwater media. No germination was noted for any of the marine dinoflagellates.

Experimental

Experimental results of germination and growth from both sediment and water residuals are presented in this next section. In our experiments, maximum fluorescence (biomass) was typically reached after 2-4 weeks. Every ballast sample produced significant phytoplankton growth (evidenced as increased fluorescence) in at least one culture media treatment (Table 3.11). In 2001, both common laboratory freshwater media, WC and GL, produced germination and growth in at least 80% of the samples (Table 3.11). Filtered Grand River water (R) and Lake Michigan water produced growth in at least 75% and 47% of the samples, respectively. The lowest response was found for standard saltwater media, GS, which produced positive growth in only 41 % of the experiments. In 2002, common freshwater media, GL and WC, produced growth in 100% and 63% of the samples, whereas GS media produced growth in 53% of the samples. Filtered Grand River water produced growth in 95% of the samples, but filtered Lake Michigan water produced the lowest response at 21%.

Table 3.11. Percent of ballast residual samples where significant growth was noted for different culture media.

	Percent With Significant Growth	
Culture Media	**2001**	**2002**
WC	82	63
GL	84	100
GS	41	53
LW	47	21
R	75	95

Not only was there tremendous variability in the growth response of each treatment, but the dominant species in each treatment also varied with media (water or sediment) even within the

same sample. This variability was not easily predicted, as it depended on the species composition of the sample and the preferences of each species to the growth media.

Diatoms were the dominant species that grew in all of our experiments, with lesser amounts of green algae, small flagellates and dinoflagellates. A total of 154 phytoplankton species were found in our experimental treatments, among them were 41 taxa (30 identified species) of nonindigenous diatoms (Table 3.12). All of these nonindigenous diatoms found in the experimental treatments were marine in origin (described from a marine environment). Nine of these nonindigenous species have been reportedly found in the Great Lakes (*Actinocyclus normanii, Actinocyclus normanii fo. subsalsa, Coscinodiscus radiatus, Cyclotella distinguenda, Navicula pelliculosa, Pleurosira laevis, Skeletonema costatum, Skeletonema subsalsum, Surirella ovata v. crumena*; Stoermer et al. 1999).

Although nonindigenous species were present in nearly 70% of the treatments showing positive growth, they were a relatively rare component of the phytoplankton assemblage that developed. Indigenous freshwater species dominated most treatments. Examining the results from freshwater treatments only (all treatments except GS) is most relevant to the Great Lakes. In most freshwater treatments where increased fluorescence was noted, nonindigenous species constituted <5% of total phytoplankton abundance (Fig. 3.23); in <5 % of the treatments where increased fluorescence was noted, nonindigenous species constituted >50% of total phytoplankton abundance. Nonindigenous species constituted >20% of the total phytoplankton abundance in 14% of the treatments.

Table 3.12. Phytoplankton species found in experimental treatments (NIS denoted in red).

Genus	Species	Genus	Species	Genus	Species
Actinoptychus	undulatus	Cyclotella	Comta	Nitzschia	angustata
Actinocyclus	normanii fo.subsalsa	Cyclotella	meneghiniana	Nitzschia	apiculata
Actinocyclus	normanii	Cymbella	cymbiformis	Nitzschia	communis
Amphora	subangularis	Cymbella	cuspidata	Nitzschia	dissipata
Coscinodiscus	eccentricus	Dactylococcopsis	rhaphidioides	Nitzschia	fonticola
Coscinodiscus	radiatus (?)	Dictyosphaerium	pulchellum	Nitzschia	frustulum
Cyclotella	distinguenda	Diploneis	Ovalis	Nitzschia	gracilis
Cymatosira	belgica	Epithemia	Sorex	Nitzschia	kuetzingiana
Delphineis	surirella	Fragilaria	brevistriata	Nitzschia	microcephala
Dictyocha	fibula	Fragilaria	construens var venter	Nitzschia	palea
Dimeregramma	minor	Fragilaria	crotonensis	Nitzschia	recta
Diploneis	weissflogii	Fragilaria	pinnata	Nitzschia	sigma
Licmophora	communis	Golenkinia	radiata	Nitzschia	subcapitellata
Navicula	palliculosa	Gomphonema	angustatum	Nitzschia	tryblionella
Nitzschia	granulata	Gomphonema	intricatum	Nitzschia	tryblionella var debilis
Nitzschia	panduriformis	Gomphonema	olivaceum	Nitzschia	tryblionella var victoriae
Nitzschia	compressa	Gomphonema	parvulum	Pediastrum	boryanum
Odontella	aurita	Gyrosigma	acuminatum	Pleurosigma	angulatum
Paralia	sulcata	Gyrosigma	distortum	Rhodomonas	minuta var nannoplanctica
Pleurosira	laevis	Gyrosigma	kuetzingii	Rhoicosphenia	curvata
Raphoneis	amphiceros	Lagerheimia	subsalsa	Rhopalodia	gibba
Skeletonema	costatum	Lagerheimia	balatonica	Scenedesmus	abundans
Skeletonema	subsalsum	Lagerheimia	subsalsa	Scenedesmus	acuminatus
Surirella	ovata var crumena	Melosira	varians	Scenedesmus	arcuatus
Terpsinoe	americana(?)	Meridion	circulare	Scenedesmus	armatus
Thalassiosira	angulata	Monoraphidium	arcuatum	Scenedesmus	bijuga
Thalassiosira	eccentrica	Monoraphidium	contortum	Scenedesmus	brevispina
Thalassiosira	oestrupii	Monoraphidium	fontinale	Scenedesmus	dimorphus
Thalassiosira	leptopus	Monoraphidium	mirabile	Scenedesmus	intermedius
Thalassiosira	punctigera	Navicula	anglica	Scenedesmus	opoliensis
Achnanthes	hauckiana	Navicula	cryptocephala var veneta	Scenedesmus	protuberans
Achnanthes	lanceolata	Navicula	cryptotenella	Scenedesmus	quadricauda
Achnanthes	linearis	Navicula	gracilis	Schroederia	setigera
Achnanthes	minutissima	Navicula	fluens	Sphaerocystis	schroeteri
Actinastrum	hantzschii	Navicula	gregaria	Stephanodiscus	astraea
Amphora	ovalis	Navicula	lanceolata	Stephanodiscus	astraea var minutula
Amphora	coffeaeformis	Navicula	minima	Stephanodiscus	dubius
Amphora	perpusilla	Navicula	pelliculosa	Stephanodiscus	hantzschii
Asterionella	formosa	Navicula	pupula	Surirella	ovata
Aulacoseira	alpegina	Navicula	pupula var rectangularis	Surirella	ovata var pinnata
Aulacoseira	distans	Navicula	pygmaea	Surirella	ovalis
Aulacoseira	granulata	Navicula	radiosa	Synedra	acus
Aulacoseira	islandica	Navicula	radiosa var tenella	Synedra	affinis
Aulacoseira	italica	Navicula	reinhardtii	Synedra	fasciculata var truncata
Campylomonas	marssoni	Navicula	salinarum	Synedra	puchella
Chlorolobion	braunii	Navicula	scutelloides	Synedra	rumpens
Cocconeis	disculus	Navicula	simplex	Synedra	ulna
Cocconeis	placentula	Navicula	tripunctata	Tabellaria	fenestrata
Crucigenia	quadrata	Nitzschia	accomodata	Tetraedron	caudatum
Cryptomonas	erosa	Nitzschia	acicularis	Tetraedron	muticum
Cyclotella	atomus-like	Nitzschia	amphibia	Tetrastrum	glabrum
Cyclotella	bodanica				

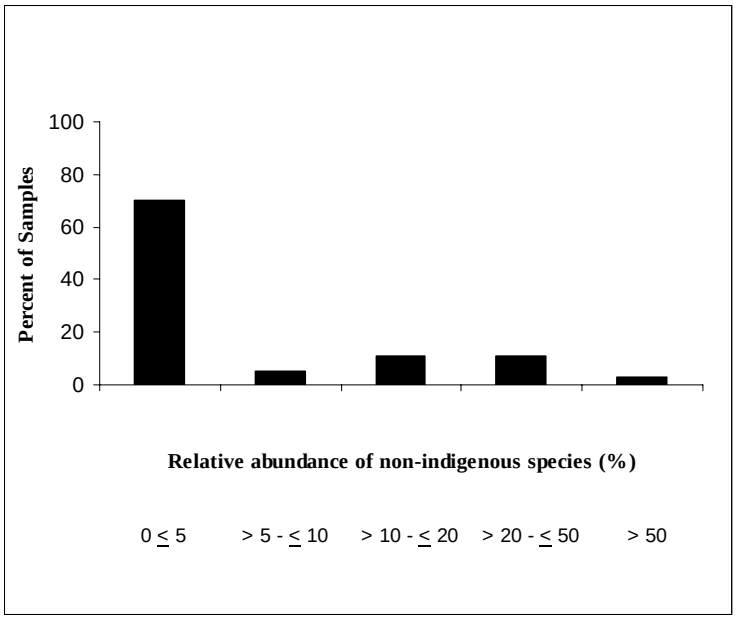


Fig. 3.23. Percent of samples with different relative abundances of nonindigenous species. Freshwater treatments only.

The number of nonindigenous species present in a particular freshwater culture treatment where significant growth occurred varied from 0-8 species, with a mean of two (Fig. 3.24). Almost 30% of our experimental treatments did not have any nonindigenous species present, and only 18% of the experiments had more than 4 nonindigenous species present.

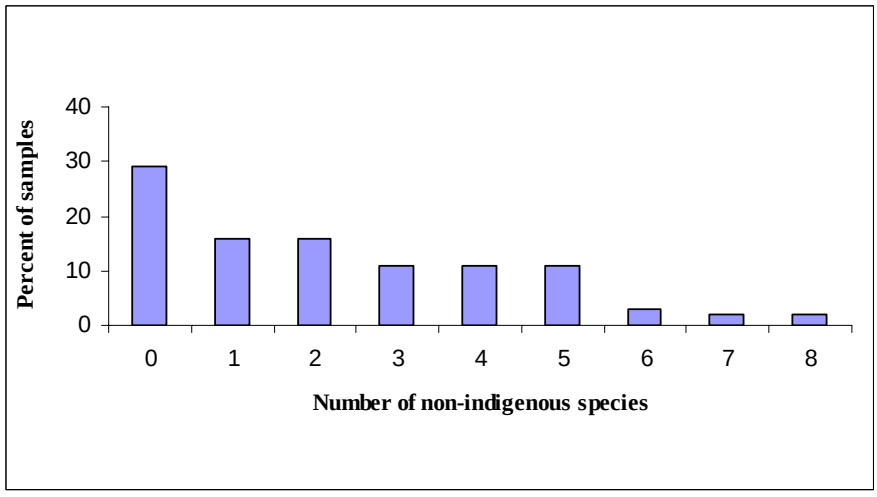


Fig. 3.24. Percent of samples containing specific number of nonindigenous species. Freshwater treatments only.

Marine dinoflagellates did not germinate and grow in any treatments, even the GS (saltwater) treatment. This may be surprising given that marine dinoflagellates grow in saltwater environments and marine dinoflagellates were found in the sediment/water inocula. This demonstrates the difficulty of extrapolating from limited laboratory conditions to the natural environment.

The viability potential of nonindigenous species in our experiments is determined by a number of factors such as abundance of the species in the inocula, and the suitability of the species to survive in a specific experimental treatment. Only a few taxa were found in a number of samples. Among the 41 nonindigenous taxa only ten appeared in more than 10% of the samples (Table 3.13). These taxa included: *Odontella aurita, Thalassoisira* sp., *Thalassiosira ecentrica, Actinophycus undulates, Skeletonema costatum, Paralia sulcata, Raphoneis amphiceros, Actinocyclus normanii, Actinocyclus normanii* fo *subsalsa,* and *Coscinodiscus* sp.

Table 3.13. Percent frequency of occurrence of nonindigenous taxa in experimental treatments.

Species	Percent frequency in samples
Cymatosira sp.	0
Amphora subangularis	2
Chaetoceros sp.	2
Cyclotella distinguenda	2
Cymatosira belgica	2
Delphineis sp.	2
Grammatophora sp.	2
Licmophora communis	2
Navicula palliculosa	2
Nitzschia compressa	2
Pleurosira laevis	2
Surirella ovata var crumena	2
Terpsinoe americana	2
Thalassiosira angulata	2
Thalassoisira punctigera	2
Actinocyclus sp.	4
Campelodiscus sp.	4
Delphineis surirella	4
Dimeregramma minor	4
Skeletonema sp.	4
Unknown marine	4
Actinoptycus sp.	5
Coscinodiscus radiatus	5
Diploneis weissflogii	5
Nitzschia granulata	5
Nitzschia panduriformis	5
Thalassoisira leptopus	5
Coscinodiscus eccentricus	7
Dictyocha fibula	7
Skeletonema subsalsum	7
Thalassiosira oestrupii	7
Odontella aurita	11
Thalassiosira sp.	13
Thalassiosira eccentrica	16
Actinoptycus undulatus	18
Actinocyclus normanii fo subsalsa	19
Skeletonema costatum	20
Paralia sulcata	21
Raphoneis amphiceros	23
Actinocyclus normanii	25
Coscinodiscus sp.	29

3.4.4. Discussion

A significant number of nonindigenous phytoplankton, both in vegetative and resting stages, were found in the ballast residuals of NOBOB ships. Moreover, some nonindigenous species survived and grew when appropriate environmental conditions were encountered; some of those conditions mimicked those found in the Great Lakes. Therefore, there can be little doubt that residual sediment and water from ballast tanks of NOBOB ships are a potential vector for the introduction of nonindigenous phytoplankton species into the Great Lakes.

If nothing is done to prevent the introduction of nonindigenous species into the Great Lakes via the ballast residuals of NOBOB ships, we can predict the following phytoplankton would be likely invaders based on their abundance in ballast water/sediment and their ability to survive in our freshwater treatments. These taxa include: *Odontella aurita*, *Thalassiosira ecentrica*, *Actinophycus undulates, Paralia sulcata*, *Raphoneis amphiceros*, *Thalassiosira oestrupii,* and a *Coscinodiscus sp*.

3.5. Active Invertebrates in Residual Ballast

3.5.1. Methods

Taxonomic composition of residual ballast

Residual sediment was collected and analyzed on a subset of 64 tanks taken from 36 ships. Sediment was collected from at least five areas of each tank, usually along longitudinal shell frames away from drainage flows, using sterile scoops and spatulas. Where possible, approximately 5 kg of sediment was collected into a bucket from each tank. After exiting the ship, the sediment was thoroughly stirred and two 500 g subsamples were weighed and preserved in 95% ethanol; the remaining sediment was kept unpreserved at 4°C for analysis of resting stages (see below). On one occasion only one 500 g subsample could be collected from a tank, and for one tank only one subsample could be examined due to excessive oil contamination in the sample. Temperature of residual ballast water was recorded at the time of collection (see below) using a Fisher Scientific thermometer, and used as a proxy measure of sediment temperature. On return to the laboratory, sediment pore water salinity was measured of the supernatant extracted by centrifugation of a 200 g sediment subsample (3300 G for 15 min) using an optical refractometer. In the laboratory, sediment samples were washed through a 45-µm mesh sieve to remove fine sediment. Associated animals were subsequently removed from the remaining sediment using the colloidal silica Ludox HS40.

Residual ballast water samples and associated taxa were collected and analyzed on a subset of 64 tanks taken from 34 ships. Where possible, 50 L of residual water was pumped into carboys using a hand bilge, passed through a 30-µm mesh net, and the retained material preserved in ethanol. Salinity of residual water was measured using an optical refractometer at the time of collection. Organisms from water and sediment samples were enumerated and identified to the lowest level practical using dissecting and compound microscopes. Nematodes from sediment samples were identified from a subset of 16 tanks from 10 ships. For analyses, sediment and water samples were expressed as numbers of organisms·kg^{-1} (wet weight) and organisms·L^{-1}, respectively.

Relationships between the total abundance of organisms in residual water or sediment and temperature, pore water salinity, total residual sediment, time since last ballasting and total ballast capacity were analyzed using stepwise linear regression using Statistica 6 (StatSoft, Inc., 2003). For all analyses, abundances and environmental variables for both sediment and water samples were averaged for each ship, since tanks within ships shared common ballast histories. In addition, we investigated the relationship of total abundance of invertebrates to region of ballast origin.

Some of the organisms present in ballast residuals likely do not have the potential to invade due to physiological constraints (e.g., salinity tolerance). Thus, total organism abundance may overestimate invasion risk posed by live animals in residual ballast. We therefore explored for trends in the region of ballast origin using the combined total abundance of fresh- and brackish-water rotifers, cladocerans and copepods per ship. Since copepods living both in freshwater (e.g., *Nitocra hibernica*, *Cyclops strenuus*) and brackish water (e.g., *Onychocamptus mohammed*,

Schizopera borutzkyi) have invaded the Great Lakes, this subset of organisms was chosen due to their well defined taxonomy and salinity preferences. We did not include species with preferences for higher salinity (i.e., those preferring highly brackish to marine) in the risk assessment.

Propagule supplies

Total propagule supplies of active sediment and water animals entering the Great Lakes per year were estimated based on average densities of organisms entering in ships, the average volume of water or sediment components, and the total number of NOBOB ships discharging water in the Great Lakes per year. Data on NOBOB ship activity between 1994 and 2000 was obtained from Colautti et al. (2004). Estimation of propagule supplies for freshwater and brackish animals were calculated similarly, as an indication of actual risk.

To determine whether sediments and water pose an invasion risk, we compiled a list of the NIS of copepods and cladocerans found in ballast residuals that are capable of surviving freshwater and brackish water conditions. Taxa were ordered based on frequency and abundance potentially discharged, and a comparison made of the relative frequencies and abundances of those that have and have not invaded.

3.5.2. Results

Taxonomic composition of residual sediments

Collected organisms belonged to a broad array of taxonomic groups (Appendix 4), although meiofaunal groups dominated numerically. Three of the 36 ships had no taxa present in their sediment samples. Nematodes dominated the overall relative abundances (91%), followed by harpacticoid (5%) and cyclopoid copepods (3%). Nematodes occurred in 91% of ships entering the Great Lakes, harpacticoids 46%, and cyclopoids 49%. Based on our samples, these taxa contribute almost 99% of all organisms entering the Great Lakes associated with ballast sediment. Of the remaining taxa, polychaetes were the most abundant and were relatively common (<1% abundance; 23% of ships). Other taxa had low abundances and were recorded from few or single tanks (i.e., <20% of ships).

Nematodes were the most species rich group, with 48 taxa recorded from the subset of ten ships. This total includes numerous taxa not reported from the Great Lakes, or North America. A total of 35 copepod species were identified from the 33 ships containing live invertebrates. Twenty harpacticoid species were identified, three of which are native to the Great Lakes: *Bryocamptus zschokkei*, *Canthocamptus staphylinoides* and *Nitocra spinipes*. Three others are NIS already established in the Great Lakes: *Nitocra hibernica*, *Onychocamptus mohammed* and *Schizopera borutzkyi*. Additionally, two are freshwater taxa not known to have populations in the Great Lakes: *Bryocamptus pygmaeus* and *Canthocamptus staphylinus*. Four others are brackish water fauna (*Halectinosoma curticorne*, *Harpacticus uniremis*, *Microarthridian littorale* and *Schizopera baltica*), while the remaining eight species are typical of more saline conditions. Twelve cyclopoid species were identified, eleven of which are freshwater species. Six of these

species are known from the Great Lakes, including *Cyclops strenuus*, a probable invader, which was recorded from two ships. Four species, *Mesocyclops leuckarti*, *Paracyclops fimbriatus*, *Thermocyclops crassus* and *T. oithonoides* are freshwater species that do not have established populations in the Great Lakes. One species of marine calanoid copepod, and two species of marine poecilostomatoid copepod, were also recorded. Two epibenthic cladoceran species (*Alona quadrangularis*, *Ilyocryptus sordidus*) were recorded, both of which are cosmopolitan taxa presumably native to the Great Lakes. Another group with a reported invasion history in the Great Lakes, the oligochaetes, comprised only four species and 0.2% of animals recorded and were present in 14.3% of ships.

Taxonomic composition of residual waters

Taxonomic composition of water fauna differed greatly from that of sediments (Appendix A). There was overlap in occurrence of some taxa, especially where species occur naturally in both habitats (e.g., cyclopoid copepods), while some others were epibenthic species likely sampled incidentally. One ship contained no taxa in the residual water samples, although several additional ships were not sampled due to an absence of easily collectable residual water; five additional ships had no taxa in one of two tanks sampled. Copepods comprised the most abundant group in residual waters (97.3% of abundance per ship; 66.0% nauplii, 20.4% cyclopoids, 10.8% harpacticoids, with calanoids and poecilostomatoids comprising the remainder). Rotifers were the next most abundant taxon at 1.2% of total abundance. Remaining taxa collectively comprised <1.5% of total abundance.

Copepods were the most species rich group, with five calanoid, twelve cyclopoid and ten harpacticoid taxa recognized. This total includes thirteen species already recorded from the Great Lakes, including three established NIS (*Eurytemora affinis, Schizopera borutzkyi, Cyclops strenuus*). Ten of the remaining fourteen species are marine taxa, which presumably would not survive if introduced to the Great Lakes, leaving four freshwater or brackish-water species (cyclopoid copepods: *Acanthocyclops venustus*, *Cyclops abyssorum*, *Eucyclops serrulatus*, *Halicyclops* sp.) that could potentially tolerate conditions in the lakes. At least eight cladoceran species were recorded, of which three are not established in the Great Lakes; one of these, *Daphnia magna*, is a North American species, while the other two, *D. cristata* and *D. atkinsoni*, are European natives. Both of the latter species, however, were recorded as single individuals only. *Bosmina maritima* was also recorded, which is a known invader. Seven rotifer species were identified, all of which are native to the Great Lakes. At least three *Gammarus* species (Amphipoda) were identified, all of which are from European estuarine brackish waters. Small bivalves were recorded on several ships, including *Driessena* veligers. However, these were typically present in low abundance and frequency overall.

Determinants of propagule supply

A statistically significant relationship was found between pore water salinity and total abundance of animals within sediment residuals, with lower abundances at higher salinities, although the explained variance was low (stepwise multiple regression $r^2 = 0.267$; $p < 0.005$). None of the other variables assessed were important in determining animal abundances ($p > 0.05$). Similar results were found for residual water data, with a significant negative relationship between

salinity and total invertebrate abundance (stepwise multiple regression $r^2 = 0.198$; $p = 0.012$). Twenty percent of the NOBOB ships examined entered the Great Lakes with freshwater (0-2‰), 23% with brackish (3-10‰) and 57% contained saltwater (>11‰) residuals (n=35). Salinity was broadly related to region of ballast origin (Table 3.14). Ships that last ballasted at ports in the Great Lakes, North Sea, Baltic or Mediterranean and Black Sea regions all had generally low salinity residual ballast water and sediment (usually < 10‰). No clear relationship existed between total numbers of animals and region of ballast origin, except that areas with medium to high salinities (> 20‰) had lower median abundances than those with salinities < 20‰. However, examination of fresh and brackish water animals showed a clear affinity for ships that had most recently loaded ballast at low salinity ports, with those on the North Sea, Great Lakes and Baltic Sea having the highest abundances as compared to other regions. This may indicate that fresh and brackish water taxa are relatively transient, and dependent on the last ballast source. Nematodes, on the other hand, may be more or less resident.

Propagule supplies transported to the Great Lakes

The average annual number of NOBOB ships entering the Great Lakes between 1994 and 2000 was 484, of which 249 subsequently loaded and then discharged mixed Great Lakes ballast water into the Great Lakes (Colautti et al. 2004). Ships averaged 15 metric tonnes (= 15000 kg) of residual sediment and 46.8 metric tonnes (= 46800 L) of residual water. Thus, at average animal densities of 1322 $ind \cdot kg^{-1}$ in ballast sediment, NOBOB ships carried approximately 49.5×10^8 individuals into the Great Lakes from sediment per year (Table 3.15). Similarly, the average number of propagules carried annually in residual water is 12.7×10^7. Thus, on average a total of 50.7×10^8 sediment and water-borne animals may have the opportunity for introduction to the Great Lakes each year by NOBOB vessels. However, only 22.6×10^7 propagules are freshwater or brackish rotifers, cladocerans and copepods that may pose a risk of invasion. Some of these taxa may already exist in the Great Lakes, or have originated from previous ballasting in the Great Lakes. Thus, propagule supply of nonindigenous freshwater and brackish copepods and cladocerans carried in sediments (see Table 3.16), excluding those already invaded, results in 25.9×10^6 individuals per year. From residual water, the propagule supply of these organisms (excluding those already invaded) is 20.5×10^4 per year. Thus, most of the nonindigenous propagule supply from the water fraction comprises species that have already invaded, while the sediment fraction contains many that have not yet invaded. However, as discussed below (*Comparison of shipping sub-vectors*), taxa living in, or associated with, ballast sediment probably have little opportunity for discharge from tanks. As a result, taxa associated with residual water appear to pose a greater risk than that of sediments.

Twenty-one nonindigenous copepod and cladoceran species recorded from residual sediments and water, including those already invaded, were examined based on propagule supplies to the Great Lakes (Table 3.16). In general, species that enter the Great Lakes more frequently, and are presumably released more frequently, were more likely to have established populations (e.g., *Schizopera borutzkyi*, *Eurytemora affinis*) than those that were released less frequently or in smaller numbers (e.g., *Daphnia cristata*, *Daphnia atkinsoni*).

Table 3.14. Range and median values of salinity, total animals, and freshwater to brackish rotifer, cladoceran and copepod taxa recorded from sediment and water residuals in ballast tanks of ships entering the Great Lakes. Region of ballast origin is ordered by median salinity. Salinity ranges for residual sediments were recorded from the pore water salinity of the sediment.

	n	salinity range	salinity median	total animals range	total animals median	Fresh-water-brackish animals range	Fresh-water-brackish animals median
Residual Sediment		‰	‰	ind·kg^{-1}	ind·kg^{-1}	ind·kg^{-1}	ind·kg^{-1}
Great Lakes	1	4.0	4.00	98	98.00	11	11.00
North Sea	15	0.0-28.0	5.00	24.33-5510	465.00	0-905	19.50
Baltic	3	2.0-12.0	8.00	14-2538	178.50	0-157.6	2.00
Mediterranean and Black Sea	3	2.0-29.0	15.50	0-19911	9955.50	0.0	0.00
North-west Pacific	4	19.0-34.5	24.33	0-1127.5	90.83	0-1.33	0.25
Other	2	19.0-31.5	25.25	16-94.33	55.16	0.0-2.50	1.25
West-Central Atlantic	8	4.8-54.0	35.25	0-280.66	45.00	0.0	0.00
Residual Water		‰	‰	ind·L^{-1}	ind·L^{-1}	ind·L^{-1}	ind·L^{-1}
Baltic	2	4.0-4.5	4.25	0.17-3.94	2.06	0.08-0.43	0.26
Great Lakes	2	2.0-8.0	5.00	1.26-15.16	8.21	1.24-12	6.62
North Sea	13	0-26.5	7.00	0.09-143.5	1.08	0-40.4	0.10
Mediterranean and Black Sea	2	1-15	8.00	0-0.24	0.12	0	0.00
West-Central Atlantic	8	4-66	27.75	0.04-2.25	0.44	0-1.85	0.11
Other	2	22.5-35	28.75	0.01-0.06	0.04	0	0.00
North-west Pacific	4	0-37	32.15	0.09-11.46	0.11	0-0.15	0.00

Table 3.15. Propagule loads potentially released into the Great Lakes from residual sediment and water of NOBOB ships. Annual density carried based on 249 NOBOB entries that load and subsequently discharge ballast water in the Great Lakes system per year.

	Volume of residuals	Animal density	Annual density carried
Residual sediment	(tonnes)	(ind·kg)	(ind·year)
Total animals	15.0	1322.48	49.5×10^8
Freshwater and brackish animals	15.0	53.01	19.8×10^7
NIS animals, including invaded	15.0	7.78	29.1×10^6
NIS animals, excluding invaded	15.0	6.92	25.9×10^6
Residual water	(tonnes)	(ind·L)	(ind·year)
Total animals	46.8	10.92	12.7×10^7
Freshwater and brackish animals water	46.8	2.37	27.6×10^6
NIS animals, including invaded	46.8	0.13	15.7×10^5
NIS animals, excluding invaded	46.8	0.02	20.5×10^4

Table 3.16. Nonindigenous copepod and cladoceran species recorded from residual ballast sediment and water capable of surviving in brackish or freshwater habitats. Abundance is the number of organisms transported in ships based on average densities in NOBOB ships, average volumes of sediments and water in the ballast tanks of the ships, and the number of NOBOB ships discharging ballast into the Great Lakes per year. Taxa are ordered based on a) frequency and b) abundance. Asterisks (*) denote NIS already established within the Great Lakes. Habitat: fw = freshwater taxa; br = brackish water taxa.

		Sediment		Water		
	NIS	No. ships	Abundance (x 10^5)	No. ships	Abundance (x 10^4)	Habitat
Schizopera borutzkyi	*	4	13.89	1	0.63	br
Eurytemora affinis	*			4	7.42	fw/br
Canthocamptus staphylinus		3	9.08			fw
Onychocamptus mohammed	*	3	5.30			br
Cyclops strenuous	*	2	10.65	2	126.26	fw
Nitocra hibernica	*	2	2.59			fw
Paracyclops fimbriatus		1	217.96			fw
Schizopera baltica		1	3.20			br
Bryocamptus pygmaeus		1	3.20			fw
Mesocyclops leuckarti		1	2.13			fw
Eucyclops serrulatus				1	12.73	fw
Harpacticus uniremis		1	1.07			br
Thermocyclops crassus		1	1.07			fw
Thermocyclops oithonoides		1	1.07			fw
Acanthocyclops venustus				1	5.31	fw
Bosmina maritima	*			1	1.77	fw
Halicyclops sp.				1	1.77	br
Cyclops abyssorum				1	0.71	fw
Daphnia atkinsoni				1	0.36	fw
Daphnia magna				1	0.36	fw
Daphnia cristata				1	0.36	fw

3.6. Dormant Invertebrates in Residual Ballast

3.6.1. Methods

Residual sediments were collected from 69 tanks, on 39 NOBOB ships, for analysis of the dormant invertebrate community. Four ships were sampled twice during the sampling period, with each independent trip into the Great Lakes considered a unique ship sample since new ballast had been held in tanks between sampling periods.

Resting stage density counts

After thorough mixing, four 40 g sediment subsamples (wet weight) were taken from each ballast tank sample and preserved in 95% ethanol. Resting stages were enumerated under a dissecting microscope after separation from coarse sediment using the colloidal silica Ludox® HS 40. Average density calculated from the four subsamples was subsequently converted to density of resting stages per tonne of sediment.

Hatching experiments

Unprocessed sediments were stored in plastic containers in the dark at 4 °C for at least four weeks to allow a refractory period before hatching experiments commenced. After this time, sediments were stirred manually, and 40 g subsamples were removed in four 10 g allotments. Synthetic pond water of 0‰ salinity or serial dilutions (8, 16, or 32‰) of filtered, natural seawater were used as hatching media. All experiments were conducted using a light:dark cycle of 16:8 hours. We conducted two types of studies: maximum diversity and whole sediment experiments.

Maximum diversity experiments were designed to promote maximum hatching abundance of the dormant taxa in the sediment community to assess species richness and abundance across ships. Resting stages were separated from sediments collected from five tanks (four ships), selected for high density of resting stages, using a sugar flotation method. Four replicates were incubated in each of four treatments: 0‰ and 8‰ media at each of 10 °C and 20 °C. Dishes were checked for emergence every 24 hours for the first ten days, and every 48 hours for the subsequent ten days. All hatched individuals were immediately removed for enumeration and identification. Sediments from an additional two tanks were incubated only in 0‰ medium at 20 °C for ten days. Variation in total abundance and species richness hatched between salinity and temperature treatments was analyzed using two-way multivariate analysis of variance (MANOVA; Systat 8.0, SPSS Inc., 1998). The two 10-day experiments were excluded from analyses for consistency. If a significant multivariate effect was observed, univariate analysis of variance (ANOVA) was performed to discern the effect of salinity and temperature on each dependent variable. As these experiments were extremely labor-intensive, an unreplicated 40 g sediment sample was prepared in the same way, incubated in 0‰ medium at 20°C for 20 days, for all remaining sediments (50 tanks from 28 ships). In this manner, we could collect information on richness and abundance of common species from a broad array of ships with reduced overall effort.

Whole sediment experiments were designed to give a more realistic estimate of hatching rates and species richness *in situ*. The protocol used was modified from that of May (1986) and Duggan et al. (2002). Four 40 g subsamples were removed from each of nineteen tank sediments (sixteen ships) and placed into 500 mL glass vessels. One hundred and fifty mL of 0‰ medium was added to each vessel before incubation at 20 °C. Vessels were swirled by hand to mix the sediment with the medium. Vessels were examined for emergence of invertebrates every 48 hours for 20 days by carefully decanting the medium through a 45 μm mesh screen. All material retained on the mesh was washed into a counting tray for enumeration and identification.

Three additional salinity treatments (8, 16 and 32‰) were added for a subset of the whole sediment experiments (ten tanks from eight ships) to determine if brackish or saltwater taxa were also present in the sediment egg bank. Variation in total abundance and species richness hatched between salinity treatments was analyzed using one-way MANOVA. Sediments analyzed for 20 days in both the replicated diversity and whole sediment experiments in 0‰ medium at 20 °C were analyzed by one-way MANOVA to determine if total abundance and species richness hatched differed as a result of experimental method (n = 5). Again, all significant MANOVA results were subsequently investigated using ANOVA and Bonferoni *post hoc* tests were used to determine differences between the four salinity levels on each dependent variable.
For all experiments conducted in the laboratory, hatched individuals were removed to separate vials and raised to maturity, when possible, to aid in identification. Taxa were identified using standard taxonomic keys. No individuals were recovered from control vials at any time. All waste generated during the experiments was autoclaved prior to disposal to minimize the possibility of environmental contamination.

Analysis of ballast history

We examined ballast history information to determine whether risk identified from hatching trials was related to each ship's activities. Stepwise multiple regression was used to determine if any of the ballast history variables (i.e., residual water salinity, total ballast capacity, volume of residual sediment and month of last ballast uptake) were important predictor variables of resting stage density or total abundance of hatched invertebrates. Subsequently, analysis of covariance (ANCOVA) was used to investigate the relationships between both egg density, and total abundance hatched, with previous regions of ballast uptake.

Estimation of propagule pressure

We calculated the number of viable dormant propagules, π_φ, carried by any ship as:

$$\pi_\varphi = \delta\varphi\tau \quad (1)$$

where δ is the density of resting stages per tonne of sediment for that vessel, φ is the proportion of resting stages that are viable and τ is the amount of sediment in tonnes aboard the vessel. We calculated propagule pressure for the 34 ships analyzed by the maximum diversity experiments above, using parameter values generated in 0‰ medium at 20 °C. To deduce the number of

viable NIS propagules carried, π_v, we added a term, ν, to indicate the proportion of viable propagules that are considered nonindigenous to the receiving area:

(2) $$\pi_v = \delta\varphi\tau\nu$$

where ν is the product of the number of nonindigenous individuals divided by the total number of individuals.

3.6.2. Results

The density of invertebrate resting stages in ship sediments had a lognormal distribution, ranging from 4.0 x 10^4 to 9.1 x 10^7 resting stages·tonne^{-1} (median and mean values of 7.2 x 10^5 tonne^{-1} and 3.6 x 10^6 tonne^{-1}, respectively). Taxonomic identity, based upon resting stage morphology, was made for 12 groups in the sediments (see Fig. 1). Diapausing eggs of rotifers, particularly *Brachionus* species, dominated (77.9%) resting stage abundance. This pattern was influenced by one ship with an extremely high density of *Brachionus* diapausing eggs (65.3% of resting stage abundance for all ships), although the same general pattern held true even if that ship was excluded.

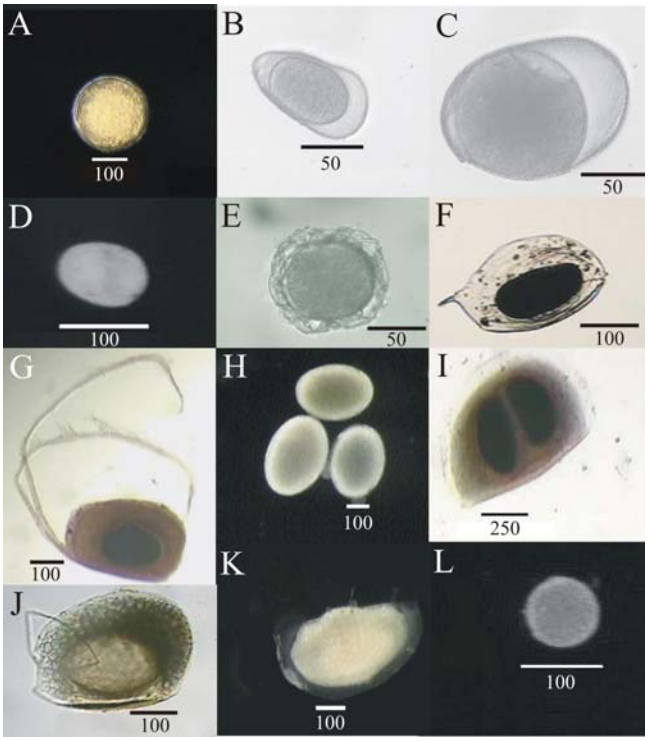


Fig. 3.24. Resting egg morphotypes recovered from ballast sediments. Rotifera: (A) *Asplanchna girodi,* (B) *Brachionus budapestinensis,* (C) *B. calyciflorus,* (D) *Filinia* spp., (E) *Ploesoma* spp.; Cladocera: (F) *Bosmina* spp., (G) Chydoridae, (H) various Cladocera, (I) *Daphnia* spp., (J and K) *Moina* spp.; Copepoda: (L) calanoid copepod. Scale bars (μm) are included on each image.

Sufficient quantities of sediment for laboratory experiments were lacking in three ships, limiting assessments to density of resting stages. For the remaining 36 ships, we hatched 76 distinct taxa. Twenty-one NIS were identified, consisting of 14 rotifers and seven cladocerans (Table 3.17). One of the NIS identified, *Bosmina maritima*, has already become established in the Great Lakes. Both the total abundance and frequency of occurrence of NIS was low in comparison to species considered native to the Great Lakes (Appendix 5).

In the maximum diversity experiments, 59 taxa were hatched from the replicated trials, although this number may be conservative owing to the presence of unidentifiable juvenile invertebrates. Species richness ranged from 0 to 20 taxa per sediment, with a median value of 3. Thirteen additional unique taxa, of 45 in total, were identified from the 50 unreplicated diversity trials. All taxa were hatched from true diapausing stages; no quiescent copepodids were recovered by this method. The rotifer *Synchaeta bacillifera* and the cladoceran *Evadne nordmanni*, were the only organisms that hatched exclusively in brackish water. Rotifers were the most species rich group, comprising 75% of all species hatched. Cladocerans comprised the second richest taxon, representing 23% of hatched species. Copepod nauplii were hatched from 14 sediments, but could not be identified to the species level and were considered as one taxon in consequence. The 0‰, 20 °C treatment group had both the highest abundance and greatest species richness hatched, followed by the 0‰, 10 °C treatment group (Fig. 3.25). Both total abundance and species richness were significantly affected by experimental temperature and salinity (MANOVA, $p < 0.01$). Univariate analyses indicated that higher salinity and lower temperature each suppressed total abundance and species richness of hatched taxa independently, and there was no interaction effect for salinity*temperature on either variable (ANOVA, $p < 0.05$).

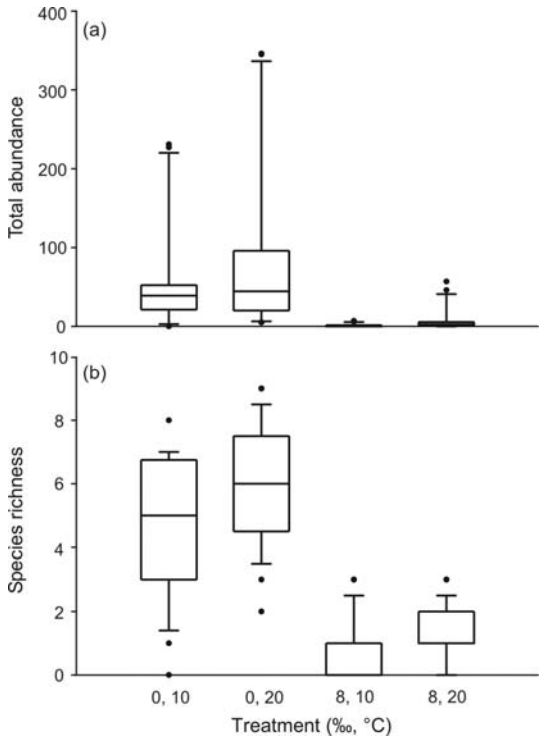


Fig. 3.25. Box plots of (a) total abundance and (b) species richness for organisms hatched from residual sediments of five ballast tanks during replicated maximum diversity experiments. Note scale difference for each y-axis.

In the whole sediment experiments, 21 taxa hatched, although six sediments had no animals emerge under any treatment regime. Three taxa, *Acanthocyclops robustus*, *Nitocra lacustris* and an unidentified juvenile cyclopoid, were found as quiescent copepodids. All remaining taxa were hatched from diapausing eggs. Species richness ranged from 0 to 13 taxa per sediment, with a median value of 2. The rotifers *Synchaeta baltica* and an unidentified *Synchaeta* sp., and copepod nauplii, were the only taxa that hatched exclusively in saltwater. Rotifers and copepods were the most species rich groups, comprising 76% and 14% of all species hatched from whole sediments, respectively. Copepod nauplii hatched from 6 sediments, and were again considered as a single taxon. One cladoceran, *Daphnia magna*, hatched from ephippial eggs. The experimental salinity treatment had a significant influence on total abundance and species richness hatched (MANOVA, $p < 0.001$). Univariate analyses further revealed that increased salinity suppressed both total abundance and species richness (ANOVA, $p < 0.001$). However, pairwise contrasts revealed that total abundance was significantly greater at 0‰ than for all other treatments (Bonferoni *post hoc* test, $p < 0.001$), while species richness did not differ between the 0 and 8‰ treatments (Bonferoni *post hoc* test, $p > 0.05$; see Fig. 3). Burial in sediment significantly decreased both total abundance and species richness of hatched taxa, with 0-43% of individuals hatched from isolated resting stages emerging from buried resting stages (MANOVA, $p < 0.001$; Fig. 3.27). Again, this effect was significant for both total abundance and species richness independently (ANOVA, $p < 0.001$).

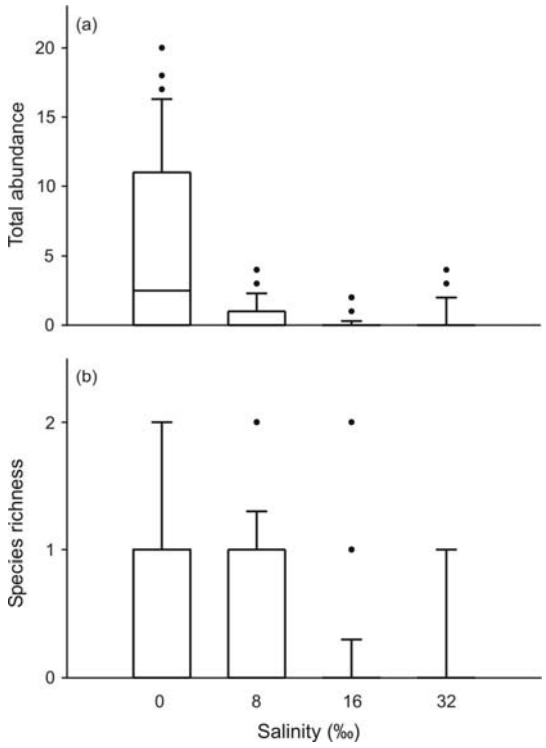


Fig. 3.26. Box plots of (a) total abundance and (b) species richness for organisms hatched from residual sediments of eight ballast tanks during whole sediment experiments. All treatments were incubated at 20 °C. Note scale difference for each y-axis.

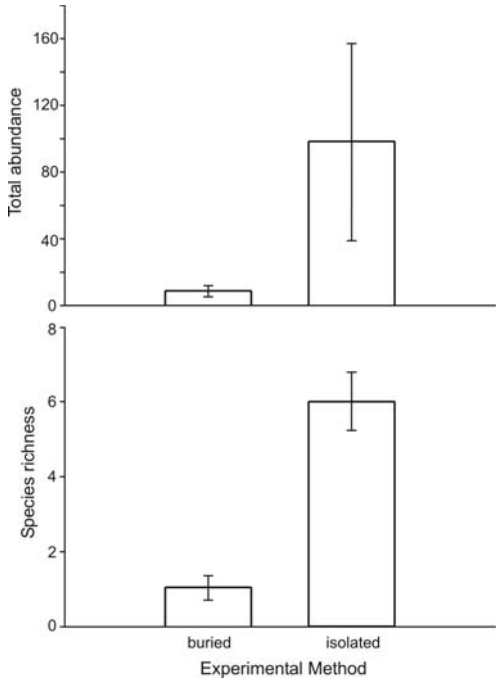


Fig. 3.27. Mean ± standard error (a) total abundance and (b) species richness of organisms hatched from residual sediments of five ballast tanks in whole sediment (buried) and maximum diversity (isolated) experiments. All replicates were incubated in 0‰ growth media at 20 °C. Note scale difference for each y-axis.

The two dominant regions for most recent site of ballast uptake were the North Sea (n = 14) and west-central Atlantic Ocean (n = 8). The Great Lakes basin was the most frequent penultimate source of ballast (n = 14). Since total abundance of hatched individuals was significantly related to the density of resting stages (linear regression, $r^2 = 0.49$, $p < 0.001$), relationships to ballast history variables are nearly identical and we only present results for analysis of resting stage density. Resting stage density was weakly related to the salinity of residual ballast water (stepwise multiple regression, $r^2 = 0.195$, $p = 0.013$). All other ballast history variables were found to be unimportant in relation to resting stage density ($p > 0.05$).

Incorporation of experimental values for resting stage density, viability and sediment tonnage into our propagule pressure model (Eqn. 1) revealed that NOBOB ships in this study carry up to 1.2×10^8 viable resting stages·ship^{-1}, with a mean density of 1.0×10^7 (Fig. 5). However, resting stages from sediments of 13% of ships sampled could not be induced to hatch in the laboratory under any conditions, and were apparently non-viable in freshwater. Thirty-two percent of the ships sampled carried resting stages of NIS, at densities up to 4.5×10^6 resting stages·ship^{-1} (Eqn. 2).

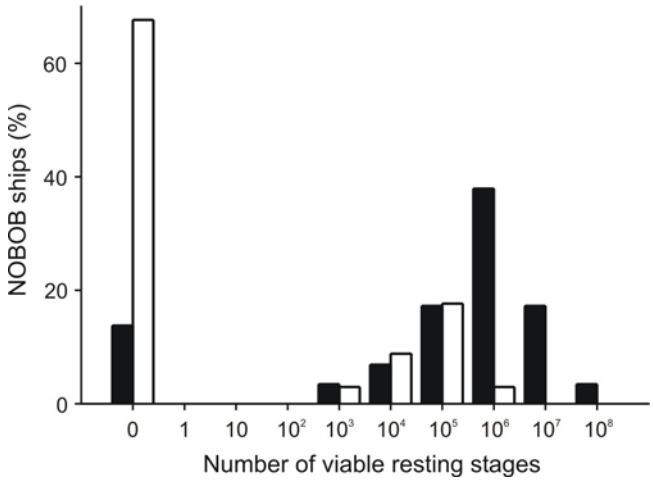


Fig. 3.28. Density of viable resting stages transported in sediments of 34 ships designated as 'no ballast on board'. Solid bars designate egg density distribution for all taxa; open bars represent only those species considered nonindigenous to the Great Lakes.

Table 3.17. Species hatched from diapausing eggs in residual ballast sediment that are considered nonindigenous to the Great Lakes. Species are listed in order of decreasing risk, according to propagule pressure and suitability of habitat. Occurrence identifies the number of ships that the species was collected from (out of a possible 35). Abundance lists the cumulative mean number of individuals that emerged from 40 g sediment for all ships on which each species was found. Species hatched in 0‰ medium during laboratory experiments were considered a match for the habitat in the Great Lakes.

Species Name	Occurrence	Abundance	Habitat Match
Daphnia magna†	4	6	Y
Filinia passa†	4	3.5	Y
Brachionus leydigi†	4	3	Y
Filinia cornuta†	3	3	Y
Asplanchna girodi†	2	1	Y
Cephalodella sterea†	1	4.75	Y
Bosmina maritima§	1	2	Y
Diaphanosoma orghidani	1	1.25	Y
Brachionus forficula	1	1	Y
Brachionus nilsoni†	1	1	Y
Conochilus coenobasis†	1	0.5	Y
Diaphanosoma mongolianum	1	0.5	Y
Cephalodella ?stenroosi	1	0.3	Y
Alona rustica	1	0.25	Y
Brachionus bennini†	1	0.25	Y
Brachionus diversicornis	1	0.25	Y
Diaphanosoma sarsi	1	0.25	Y
Hexarthra intermedia†	1	0.25	Y
Moina affinis	1	N/A	Y
Synchaeta baltica	1	2.75	N
Synchaeta bacillifera	1	2.25	N
Evadne nordmanni†	1	0.5	N
Pleopis polyphemoides	1	N/A	N

Note: †denotes species with broad geographic distribution. §denotes species already established in the Great Lakes.

3.6.3. Discussion

Comparison of shipping sub-vectors

The mean density of active animals transported in residual sediments in this study, 49.5 x 10^8 $year^{-1}$, is higher than that of dormant stages from the same sediments, 24.5 x 10^8 $year^{-1}$. However, many of the active and dormant sediment taxa are buried or have adaptations to ensure they remain in association with sediments, even during flow or turbulent conditions, and will have little chance for discharge from ballast tanks. As such, only a small proportion of these (approximately 8% or less) will enter the lakes with discharged ballast at their final Great Lakes port-of-call. Risk is likely to vary by taxon, however. For example, nematodes will occur within the sediment and are less likely to be discharged, while more epibenthic taxa (e.g., harpacticoids) may be discharged more readily. This may reflect why most of the common epibenthic nonindigenous organisms found in this study have already invaded the system. In contrast, active invertebrates in residual water are available for discharge at a mean density of 12.7 x 10^7 $year^{-1}$. Despite the density of active and dormant taxa in sediments being greater than the number of invertebrates in residual water, planktonic animals probably have greater opportunities for discharge with ballast water (see MacIsaac et al. 2002). Evidence for this comes from the difference between the numbers of freshwater and brackish NIS which have and have not invaded the system to date; a large proportion of the nonindigenous propagule supply in the water fraction comprises of taxa that have already invaded (87%), while only 11% of the nonindigenous propagule supply in sediments have invaded to date. Thus, despite the sediments containing higher densities of nonindigenous propagules overall, only the most frequently occurring epibenthic species may be able to invade the Great Lakes system. Further, *in situ* hatching studies suggest that less than 1% of invertebrate diapausing eggs will hatch and be available for introduction (see Task 2 section). Therefore, residual ballast water may pose the greatest risk for introduction of invertebrates.

Chapter 4. Great Lakes NOBOB Ballast Tank Mesocosm Experiments

The goal of Task 2 was to assess the potential for biota and resting stages resident in ballast tank residual water and sediment to invade the Great Lakes under actual ship operating conditions. Specifically, we evaluate the survival dynamics of organisms during Great Lakes transport and to what extent hatching of resting stages in filled tanks may occur.

4.1. Microbial and Phytoplankton

4.1.1. Introduction

From a microbial perspective, the goal of Task 2 was to determine whether phytoplankton, bacteria, and viruses (including pathogenic or harmful forms) survived transit through the Great Lakes (GL) under normal ballasting operations. Specifically, we wanted to determine whether there was: (1) an increase in density of microbes after tanks were filled (due to resuspension of bottom sediment), (2) a change in the viability of phytoplankton during transit through the GL, (3) a "die-off" of pathogens under normal shipping operations within the GL, and (4) changes in the density and composition of the microbial populations during repeated ballast loads and discharges.

4.1.2. Methods

Sample storage and shipping

Water and sediment samples for microbial and phytoplankton analyses were collected in the same way as Task 1 samples, then stored in the dark at 4 °C before being transported or shipped to GLERL or ODU for processing and analysis.

Virus, Bacteria and Phytoplankton analyses—population metrics

Total populations of viruses and bacteria were enumerated in the same way as Task 1. Viruses were enumerated by filtering water and sediment pore water samples onto 25-mm diameter 0.02 µm filters (viruses; Anodisc), then staining with the nucleic acid stain SYBR® Green (Molecular Probes, Inc.). After incubating filters in the stain, they were rinsed, mounted, and examined under epifluorescence microscopy at 1000X magnification. Bacteria were enumerated using a flow cytometer. Water samples were fixed in a formaldehyde solution (final concentration 2.7%) and stored in the dark at 4°C until they were enumerated via flow cytometry. Analyses were done using a Becton Dickinson FACScan flow cytometer equipped with a 15 mW, 488 nm, air-cooled Argon ion laser. Simultaneous measurements of forward light scatter, 90-degree light scatter, and green fluorescence were made on all samples. PicoGreen (Molecular Probes, Inc.), a DNA-specific probe, was used to detect and enumerate bacteria (Veldhuis et al. 1997). Detectors (photomultiplier tubes) were in log mode and signal peak heights from excitation wavelengths were measured. The volume of samples was determined gravimetrically using an A-160 electronic balance (Denver Instruments Co.) whereby each sample was weighed prior to analysis and immediately after analysis. All samples were run at a low flow rate setting (approximately 20 µL min^{-1}).Chlorophyll-*a* was used as an indicator of phytoplankton biomass.

Chlorophyll *a* (chl *a*) samples were collected by filtering 300-1000ml of ballast water onto 47mm-diameter glass fiber filters (GF/F Whatman) at a vacuum pressure of 100mm Hg. Filters were wrapped in foil and stored at –85°C until the chl *a* on the filters was extracted in acetone and measured fluorometrically (Parsons et al. 1984). Phaeopigment concentration was quantified by acidifying the chl-*a* samples with 5% hydrochloric acid and again determining the sample's fluorescence.

Bacterial community composition

Although not originally set out in the proposal, Denaturing Gradient Gel Electrophoresis (DGGE) analyses was used to provide an indication of community composition (Fig. 4.1) for Experiment 1 and 2. DGGE separates polymerase chain reaction (PCR) amplified DNA (or complementary DNA from extracted RNA) fragments based on minor differences in gene sequence (Muyzer 1993). PCR products are fractionated by gel electrophoresis through a gradient of increasing chemical denaturant, to provide DNA "fingerprints" of eukaryotic (phytoplankton) and prokaryotic (bacteria) communities. These DNA fragments may then be isolated from the gel, sequenced, and compared to known sequences (e.g. Ribosomal Database Project) in order to identify the species or ribotype of interest. DGGE provides a composite profile of the community that can be compared to other community profiles and while it is not quantitative, allows for selection, sequencing and identification of individual bands. Further, comparison of RNA and DNA profiles can provide an estimate of viability, because the RNA pool within a nucleic acid sample is contributed primarily by organisms that are metabolically active and transcribing RNA rather than organisms whose DNA is simply present.

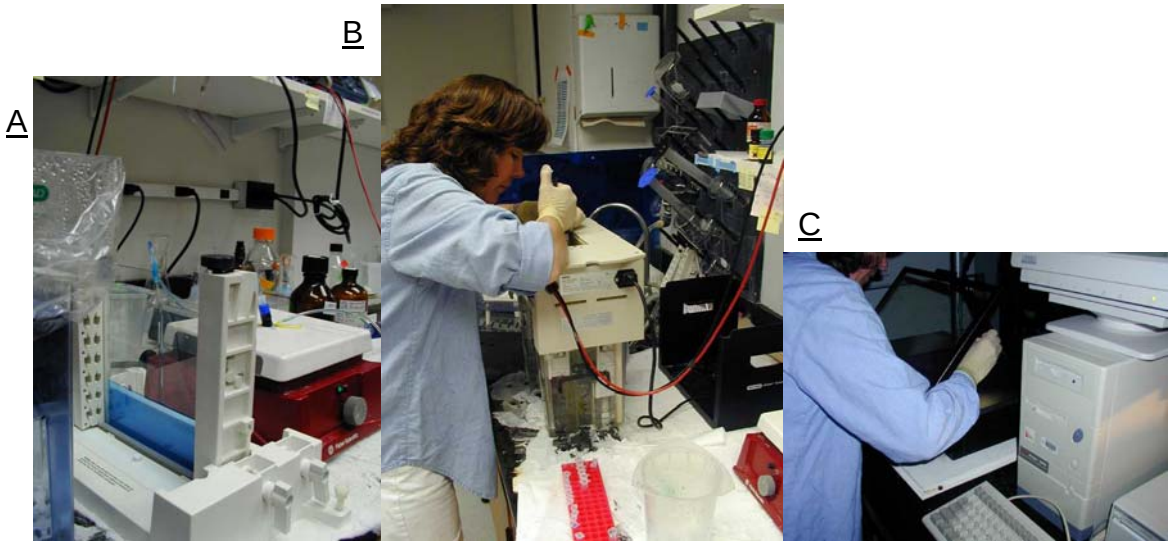


Figure 4.1. Denaturing Gradient Gel Electrophoresis procedure. A: pouring gel, B: loading gel, and C: visualizing gel under a UV transmissometer while picking out bands to sequence.

Pathogen analyses—detection and viability

Similar to Task 1 analyses, we investigated a suite of pathogens and indicator species including:

- enteric bacteria (indicator species, e.g., *E. coli*, enterococci);
- *Vibrio cholerae* (bacterial cause of human cholera)
- *Cryptosporidium parvum*, *Giardia duodenalis*, *Encephalitozoon intestinalis* (protozoan parasites of human intestines)
- *Pfiesteria piscicida, P. shumwayae* (dinoflagellates associated with fish kills)
- *Aureococcus anophagefferens* (not pathogenic, but dense blooms of "brown tide" are associated with mortality of eelgrass and shellfish)

Pathogen screening techniques involved PCR (polymerase chain reaction) and immunoassays. In addition, we assessed viability of selected pathogenic organisms using a combination of biochemical measures and culture techniques. For more information on methods, please see Task 1 section.

Phytoplankton viability analyses

Grow-out incubations were used to assess viability of phytoplankton. Germination experiments were completed for 2001, 2002, and 2003 experiments. As soon as possible after sampling at each time point, 5ml of harbor or ballast water was aliquoted into culture vessels containing 45ml of culture media. If residual sediments were being examined, 2ml of sediment was added to 30ml of filtered ballast water (collected at the same time as residuals), mixed, and then 2ml of this mixture was added to 30ml of filtered ballast water. Treatments included enriched Lake Michigan (GL) water and unenriched Lake Michigan water (L). Samples were then incubated for 2-4 weeks, with growth being monitored by *in-vivo* fluorescence using a Turner Designs 10-AU fluorometer. At the end of incubations, samples were preserved in Lugols solution and species were enumerated using settling chambers and an inverted microscope (Utermöhl 1958).

Species diversity was calculated using the formula:
Diversity = 1 – B
where B = sum $\{N_i (N_i -1)/N (N-1)\}$ and i is each different species in the sample.

4.1.3. Results

Summary of Experiments

In all, five Task 2 experiments were completed for the project between the time period of October 2001 – September 2003 (Table 4.1). This number exceeded our initial sampling goals for the project of three Task 2 experiments. In particular it should be noted that we used the project deadline extension to complete 3 additional experiments in 2003. Each of the experiments covered a voyage lasting between 10 and 11 days, and involved either three or four ports of call. Initial and final sampling ports are given in Table 4.1.

Table 4.1. Summary of Task 2 experiments undertaken on ships transiting the Great Lakes

Experiment #	Date	Day	Sample code	Port
1	10/01/2001	0	2-01274-T0	Hamilton
	10/07/2001	6	2-01280-T1	Windsor
	10/11/2001	10	2-01284-T2	Chicago
2	10/05/2002	0	2-02278-T0	Windsor
	10/07/2002	2	2-02280-T1	Detroit
	10/10/2002	5	2-02283-T2	Burns Harbor
	10/11/2002	6	2-02284-T3	Milwaukee
3	07/2/2003	0	2-03183-T0	Hamilton
	07/6/2003	4	2-03187-T1	Windsor
	07/10/2003	8	2-03191-T2	Burns Harbor
	07/12/2003	10	2-03193-T3	Thunder Bay
4	07/14/2003	0	2-03195-T0	Hamilton
	07/17/2003	3	2-03198-T1	Detroit
	07/24/2003	10	2-03205-T2	Milwaukee
5	09/15/2003	0	2-03258-T0	Cleveland
	09/19/2003	4	2-03262-T1	Windsor
	09/21/2003	6	2-03264-T2	Burns Harbor
	09/26/2003	11	2-03269-T3	Duluth

Virus, Bacteria, and Phytoplankton analyses—population metrics

In Experiment 1, total virus-like particle (VLP) abundance and chl-*a* (chlorophyll *a*, a primary photosynthetic pigment) concentration in residual ballast water upon entry into the Great Lakes was relatively low (Table 4.2). However, with the addition of Hamilton Harbor water (as indicated by a salinity decrease), VLP abundance increased by a factor of 20, bacteria abundance increased by a factor of 2, and chl-*a* concentration increased by an order of magnitude (Table 4.2). Over the next 10 days, VLP and bacteria abundance and chl-*a* concentration declined to their initial levels (Fig. 4.2, Table 4.2). The ratio of chl *a* to phaeopigments (degradation products of chl *a*) was low initially, increased on addition of Hamilton Harbor water, then declined less precipitously than chl *a* concentrations (due to a decrease in the concentration of phaeopigments when chl-*a* concentration stayed relatively constant).

Table 4.2. Changes in salinity, total virus-like-particle (VLP) and bacteria abundance, as well as chlorophyll *a* concentration and the ratio of functional to degraded chlorophyll (Chl-*a*:Phaeo) during Experiment 1.

Date	Port	NOBOB ID	Salinity (ODU)	Total VLP ($x10^9$/mL)	Total Bact. ($x10^6$/mL)	Chl-a (µg/L)	Chl-a: Phaeo
10/01/2001	Hamilton	1-01274-01	4	0.08	0.72	0.50 (0.04)*	0.48
10/01/2001	Hamilton	2-01274-T0	1	1.51	1.43	5.22	2.72
10/07/2001	Windsor	2-01280-T1	4	0.18	1.25	0.60	1.34
10/11/2001	Chicago	2-01284-T2	0	0.12	0.77	0.48 (0.03)*	1.57

* standard deviation

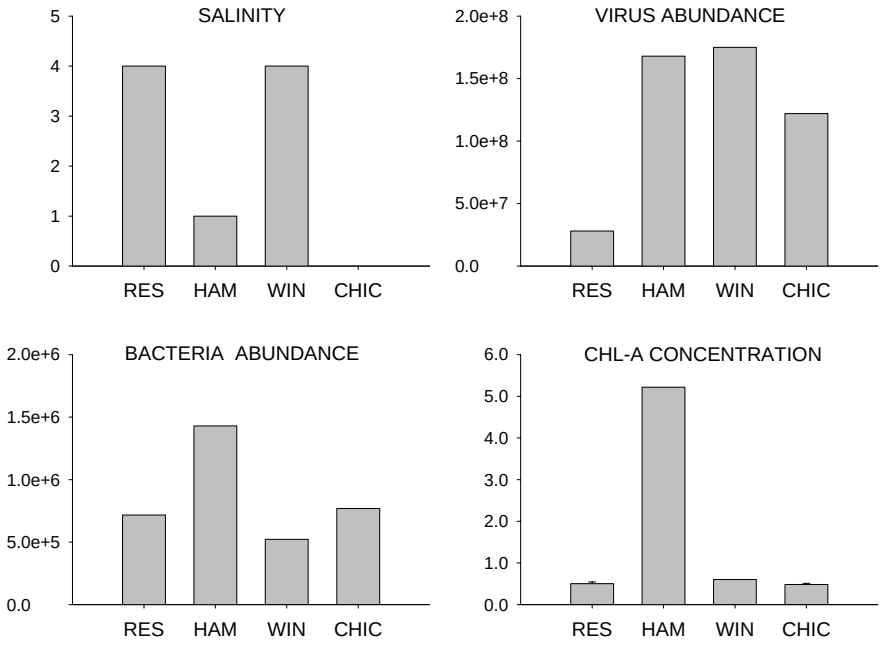


Fig. 4.2: Task 2 Experiment 1: Salinity, virus abundance, bacteria abundance and chlorophyll-*a* concentration in ballast tanks during a GL vessel transit from Hamilton (initial port), to Windsor (3 days later), to Chicago (6 days later). Units as in Table 4.2.

For Experiment 2, the initial salinity of residual water was 36 ppt and was reduced to approximately 2 ppt upon filling the tank with harbor water in Windsor. All population metrics, except for VLPs, increased with the addition of harbor water to the empty tank (Table 4.3). Note that VLP abundance was significantly lower (approximately two orders of magnitude) than for Experiment 1. VLP and bacteria abundance as well as chl-*a* concentration then declined during transit through the Great Lakes, but remained higher than their original levels at the last port sampled (Table 4.3, Fig. 4.3). There was little difference in VLP concentration between surface and bottom samples, but bacteria abundance and chl-*a* concentrations were different (either higher or lower) in the surface compared to bottom samples at T0 (Windsor), T1 (Detroit), and T3 (Milwaukee).

Table 4.3: Changes in total virus-like-particle (VLP) and bacteria abundance, as well as chlorophyll-*a* concentration and the ratio of functional to degraded chlorophyll (Chl-*a*:Phaeo) during Experiment 2.

Date	Port	NOBOB ID	Sample type	Total VLP ($x10^7$/mL)	Total Bact. ($x10^6$/mL)	Chl-a (µg/L)	Chl-a: Phaeo
10/05 /2002	Windsor	1-02278-T0	residual	17.3	0.63	0.64	0.09
10/05 /2002	Windsor	2-02278-T0	surface bottom	5.57 5.74	3.27 1.99	1.68 2.10	1.25 0.93
10/07 /2002	Detroit	2-01280-T1	surface bottom	5.15 5.06	2.80 2.31	0.42 (0.02)* 0.62 (0.03)	0.92 0.83
10/10 /2002	Burns Harbor	2-02283-T2	surface bottom	3.88 3.84	1.66 1.75	0.24 (0.01) 0.27 (0.00)	0.89 0.76
10/11 /2002	Milwaukee	2-02284-T3	surface bottom	4.90 4.40	1.94 1.49	0.64 (0.08) 0.48 (0.05)	2.94 0.77

* standard deviation

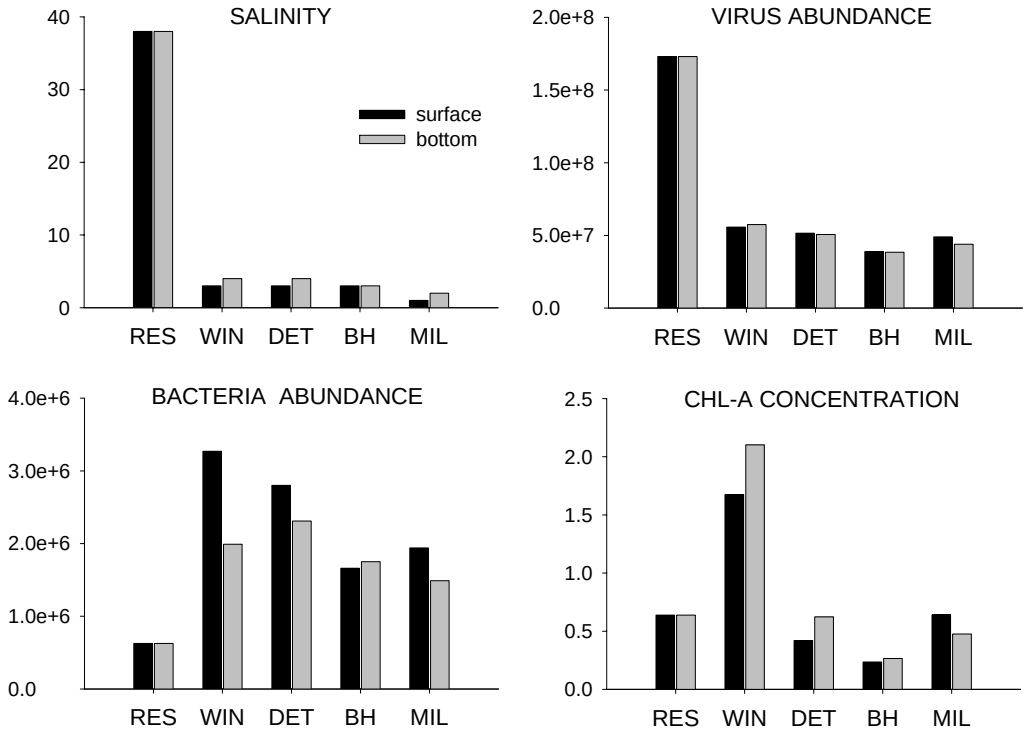


Fig. 4.3: Task 2 Experiment 2: Salinity, virus abundance, bacteria abundance and chlorophyll-*a* concentration in ballast tanks during a GL vessel transit from Windsor (initial port), to Detroit (2 days later), to Burns Harbor (5 days later) and Milwaukee (6 days later). Samples were collected from the upper and lower portions of the tanks. Units as in Table 4.2.

In all of the other Task 2 experiments (see Tables 4.4 – 4.6), VLP and bacteria abundance declined by about a factor of 2 during ballast transit. Provided more water wasn't added to tanks, chlorophyll-*a* concentration also declined significantly—e.g. by 97% during Experiment 3 (Table 4.4), and 98% during Experiment 5 (Table 4.6).

Table 4.4: Changes in salinity, total virus-like-particle (VLP) and bacteria abundance, as well as chlorophyll-*a* concentration and the ratio of functional to degraded chlorophyll (Chl-*a*:Phaeo) during Experiment 3.

Date	Port	NOBOB ID	Salinity (ODU)	Total VLP ($x10^8$/mL)	Total Bact. ($x10^6$/mL)	Chl-a (µg/L)	Chl-a: Phaeo
07/02 /2003	Hamilton	2-03183-H	0.5	5.47	2.33 (0.06)*	3.69	7.24
07/02 /2003	Hamilton	2-03183-T0	0.5	4.54	2.32 (0.70)	4.07	0.92
07/06 /2003	Windsor	2-03187-T1	1	3.47	0.66 (0.00)	0.58	0.29
07/10 /2003	Burns Harbor	2-03191-T2	2	1.65	0.99 (0.33)	0.08	0.09
07/12 /2003	Thunder Bay	2-03193-T3	2	1.51	0.65 (0.02)	0.14	0.31

* standard deviation

Table 4.5: Changes in salinity, total virus-like-particle (VLP) and bacteria abundance, as well as chlorophyll*a* concentration and the ratio of functional to degraded chlorophyll (Chl-*a*:Phaeo) during Experiment 4.

Date	Port	NOBOB ID	Salinity (ODU)	Total VLP ($x10^8$/mL)	Total Bact. ($x10^6$/mL)	Chl-a (µg/L)	Chl-a: Phaeo
07/14 /2003	Hamilton	2-03195-H	0.5	5.08	1.43 (0.05)*	2.43	1.45
07/14 /2003	Hamilton	2-03195-T0	0.5	4.24	1.76 (0.06)	4.41	1.16
07/17 /2003	Detroit	2-03198-T1	1	1.53	0.74 (0.02)	1.63	0.58
07/24 /2003	Milwaukee	2-03205-T2	1	1.65	0.81 (0.02)	5.98	0.89
07/24 /2003	Milwaukee	2-03205-H	1	1.50	1.88 (0.09)	2.69	1.42

* standard deviation

Table 4.6: Changes in salinity, total virus-like-particle (VLP) and bacteria abundance, as well as chlorophyll-*a* concentration and the ratio of functional to degraded chlorophyll (Chl-*a*:Phaeo) during Experiment 5.

Date	Port	NOBOB ID	Salinity (ODU)	Total VLP ($x10^9$/mL)	Total Bact. ($x10^6$/mL)	Chl-a (µg/L)	Chl-a: Phaeo
09/15 /2003	Cleveland	2-03258-H	1	No Data	2.49 (0.59)*	9.43	1.81
09/15 /2003	Cleveland	2-03258-T0	1	No Data	1.98	5.96	2.35
09/19 /2003	Windsor	2-03262-T1	1	No Data	1.40 (0.03)	0.52	0.43
09/21 /2003	Burns Harbor	2-03264-T2	1	No Data	1.22 (0.15)	0.37	0.30
09/26 /2003	Duluth	2-03269-T3	1	No Data	0.75 (0.05)	0.12	0.15
09/26 /2003	Duluth	2-03269-H	1	No Data	2.80 (0.29)	5.24	3.27

* standard deviation

Bacterial community composition

Additional analyses for bacteria diversity were carried out during Experiment 1 (subcontracted to Microbial Insights; www.microbe.com) and 2 (collaborator, Dr. Kathryn Coyne, University of Delaware). The bacteria community was characterized by profiling a conserved region of the 16S rDNA gene using denaturing gradient gel electrophoresis (DGGE). The most intense band observed at the top of the gel in Fig. 4.4 is alpha proteobacteria (red box). The three bands that appear in the lower portion of the gel during the second (Port of Windsor) and third event (Port of Chicago) (third and fourth lanes, respectively) are gram-positive bacteria. There is great similarity in the profiles at each port, especially in the alpha-proteobacteria group. However, bacteria diversity seems to increase with transit through the GL (i.e. more bands present in second and third events compared to first), but there is one band (highlighted in yellow), which first increases in intensity then decreases almost to non-detection.

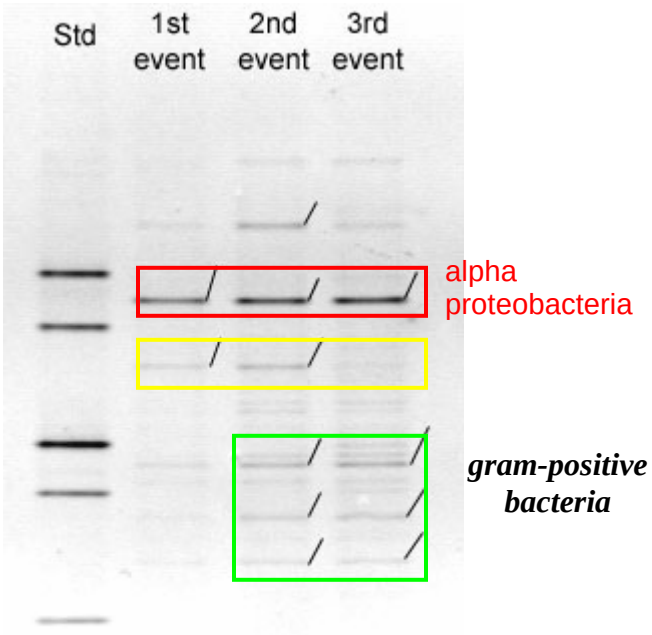


Figure 4.4: Image of DGGE gel showing banding patterns derived from amplification of bacterial 16S rDNA fragments from Task 2 Experiment 1. The first lane contains a DNA standard (positive control). The 1st event represents unfiltered Hamilton harbor water after it had been pumped into a ballast tank containing relatively small (residual) volumes of sediment and water. The water then sat in the tank for 6 days and 10 days (2nd and 3rd events, respectively) before being discharged in Chicago (outbound port).

As in Experiment 1, the DGGE analysis in Experiment 2 revealed a change in the composition of the microbial community during transit through the GL. Several unidentified groups (one is shown in red in Fig. 4.5A), decline in abundance throughout the six-day experiment. Other groups are either present in the residual sample at the beginning of the experiment but are not detectable once the tank is filled (shown in blue in Fig. 4.5A), or are present in the water after filling but not when the tank is empty (i.e. in the residual; shown in green in Fig. 4.5A). Comparative analysis of DNA versus RNA indicates some microbial groups that are present are not actively transcribing RNA (i.e. are nonviable; shown in green in Fig 4.5B). However, there are other groups that seem to be very active (i.e. viable; see red box in Fig 4.5B).

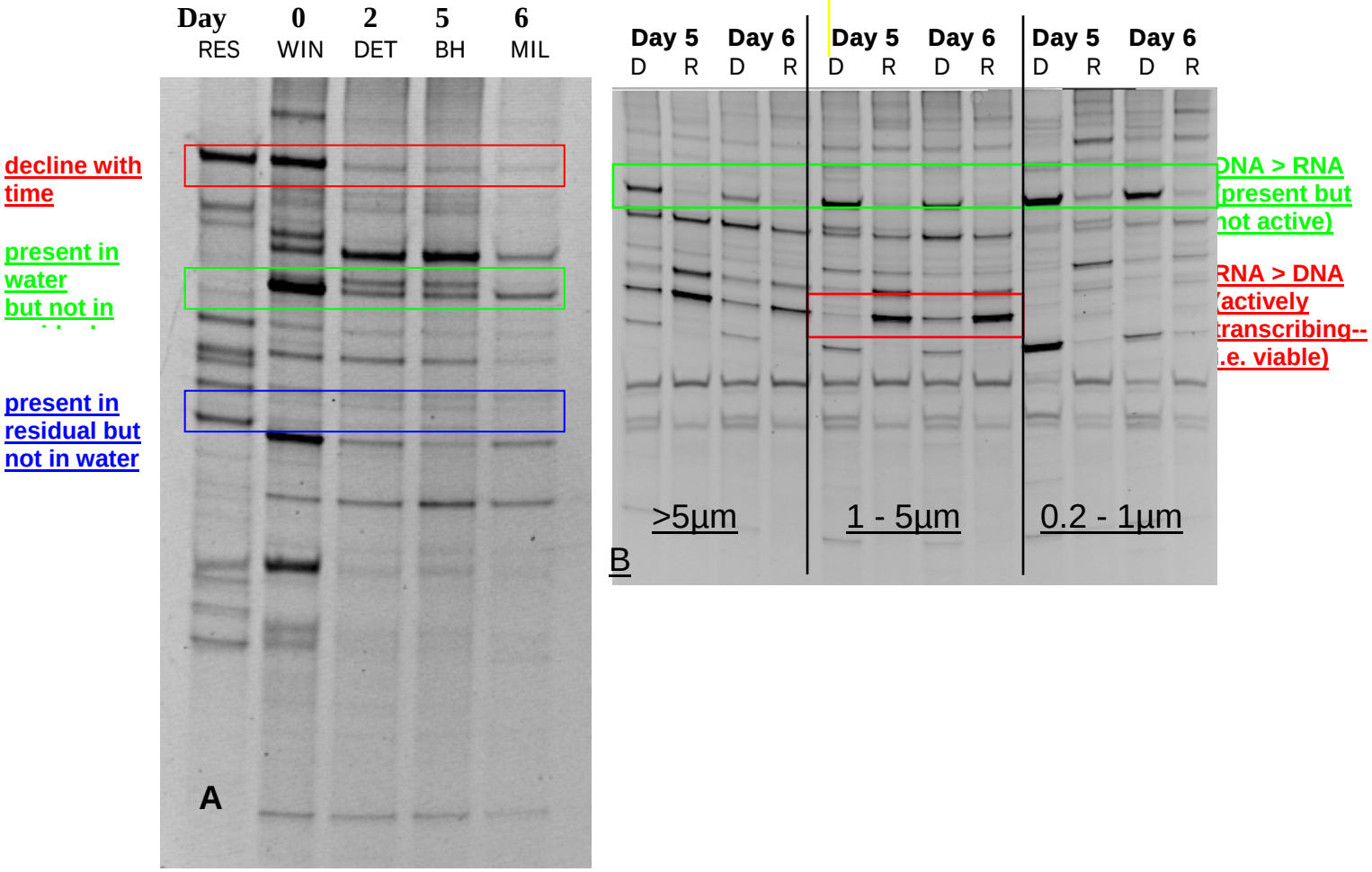


Figure 4.5: Images of DGGE gel showing banding patterns derived from amplification of bacterial 16S rDNA fragments from Task 2 Experiment 2. (A) DNA, 1 -5 µm fraction; the first lane represents residual water, the second lane water from the same tank after filling in the Port of Windsor, third lane, water after it had been sitting in the tank for 2 days (collected in the Port of Detroit), 4th lane, water after it had been sitting in the tank for 5 days (collected in the Port of Burns Harbor), and 5th lane, water after it had been sitting in the tank for 6 days (collected in the Port of Milwaukee). (B) DNA and RNA from water which had been sitting in the tank for 5 and 6 days (collected at the Port of Burns Harbor and Milwaukee, respectively).

Pathogen analyses

Different pathogens were detected during 2001 and 2002 experiments. In Experiment 1, the dinoflagellate *Pfiesteria piscicida* was detected in the initial residual sample, directly after filling and 10 days later in Chicago, the final port sampled. During the same experiment, *Aureococcus*, another harmful estuarine phytoplankter, was found in the tank after filling and at all subsequent ports—a significant finding, given the low salinity of the ballast water (for more information, see Doblin et al., 2004).

In Experiment 2, the pathogenic protozoan *Encephalitozoon intestinalis* was detected after water had been present in the tank 2 days (Port of Detroit). *Cryptosporidium*, another pathogenic protozoan, was detected after 5 and 6 days of ballast tank confinement (Ports of Burns Harbor and Milwaukee, respectively). From this it is clear that pathogens are detected intermittently

during most ballast transits through the Great Lakes, but there is large variability between experiments.

Phytoplankton analyses

In all experiments, phytoplankton growth was significantly greater in enriched Lake Michigan water (GL) compared to unenriched Lake Michigan water (L) (Fig. 4.6). During Experiment 1, growth rates of phytoplankton were higher in ballast water collected on day 10 compared to the original harbor water used to fill the tank on day 0. Interestingly, there were no marine species observed in the harbor and initial (i.e. day 0) samples, but after incubation, marine species comprised about 1% of total species present. Further, there were other species that appeared in tanks at the end of experiments that weren't detected in initial samples—e.g. *Actinocyclus* (Figs. 4.7 and 4.8).

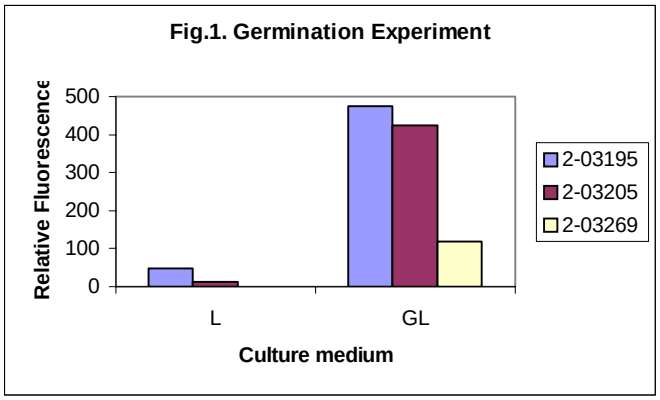


Fig. 4.6. Growth of phytoplankton in unenriched (L) and enriched (GL) Lake Michigan water during 3 of 5 Task 2 experiments (other data not shown).

Fig. 4.7. Image of *Actinocyclus* sp. cyst present in ballast tanks.

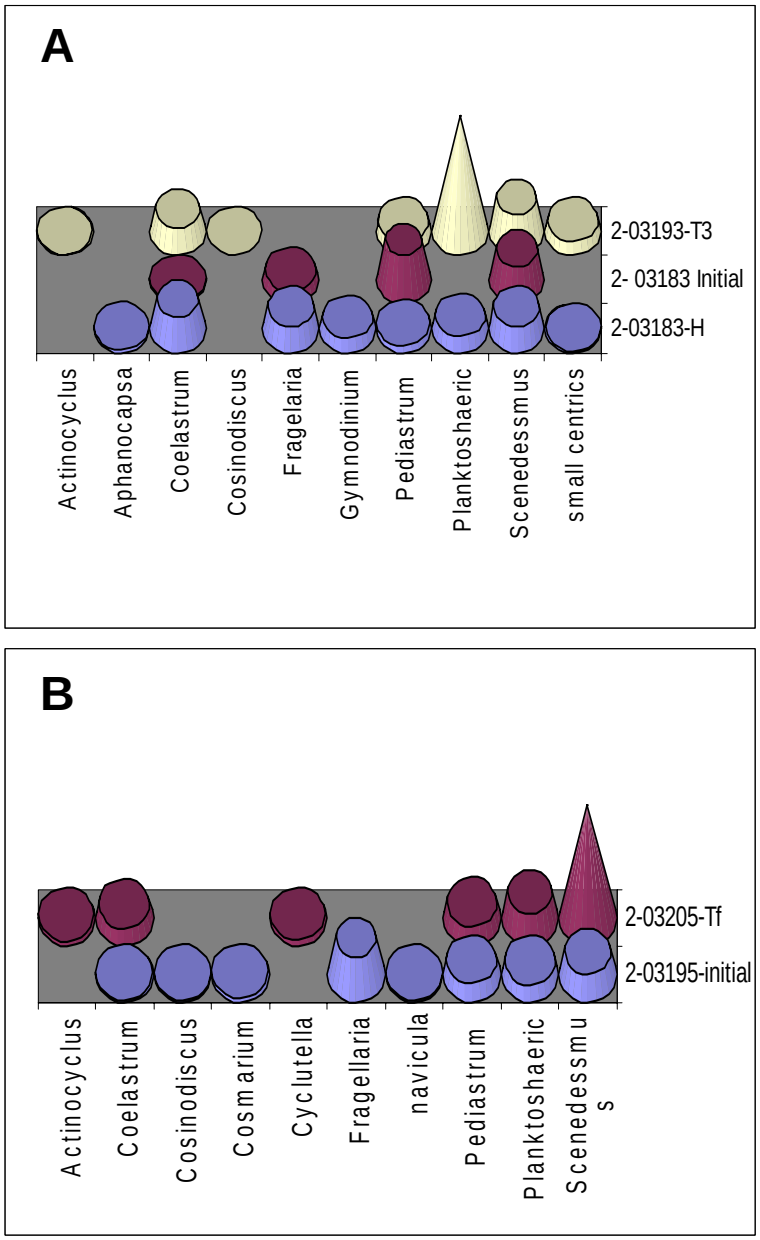


Fig. 4.8: Species composition of phytoplankton in ballast water collected during (A) Experiment 3; and (B) Experiment 4.

For all experiments performed in 2003, phytoplankton species diversity declined during vessel transit (Fig. 4.9 A, B and C). Further, there was a shift in species dominance (see Fig. 4.10 A, B and C) such that on average, the pennate diatoms, *Fragillaria* sp. and *Melosira* sp. decreased in abundance and the cyanobacterium *Microcystis* increased in abundance. In contrast, the abundance of the centric diatom *Coscinodiscus* sp. remained relatively constant (Fig. 4.11).

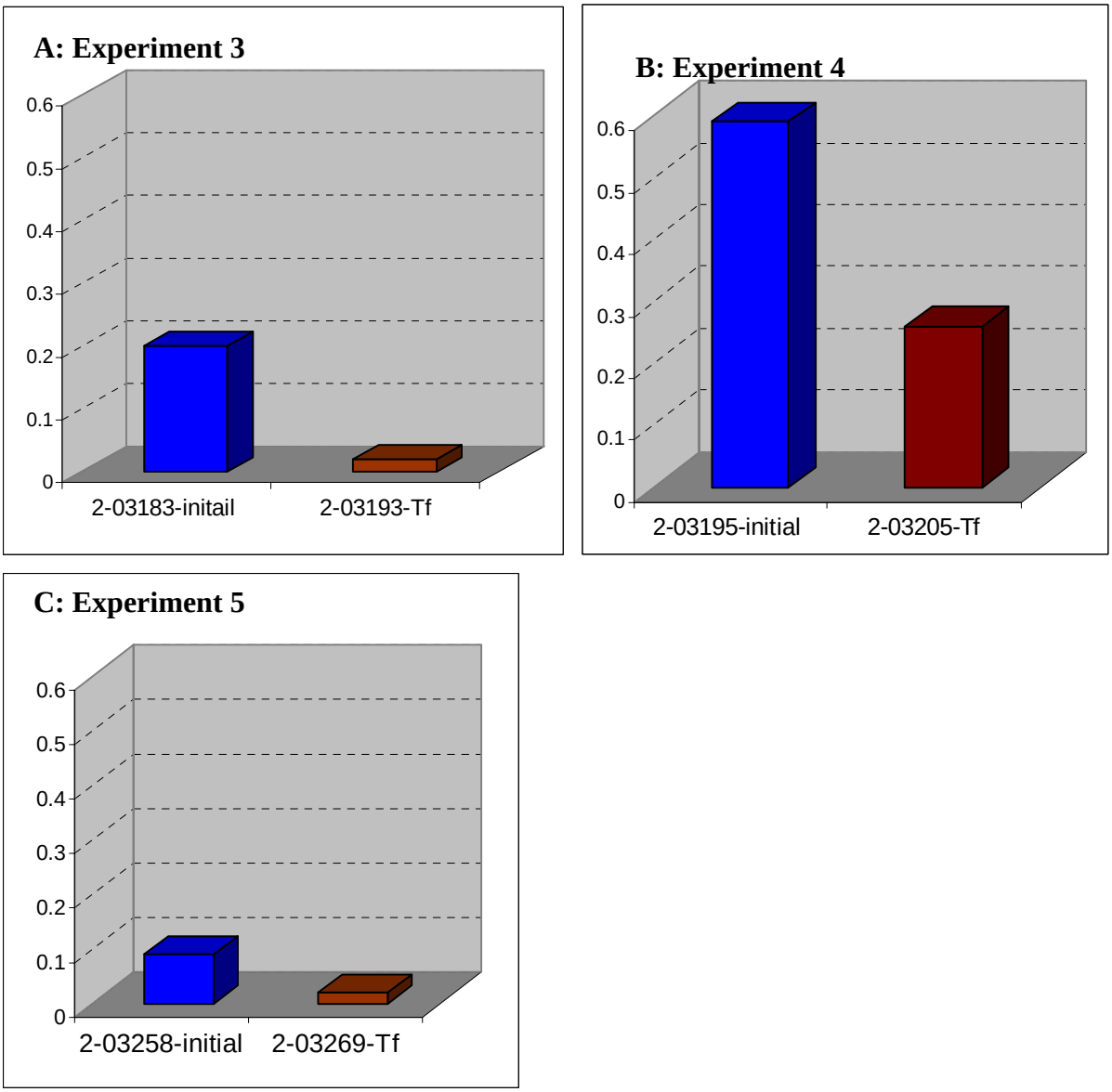


Fig. 4.9: Phytoplankton species diversity index of initial and final Task 2 samples collected during Experiments 3, 4, and 5 (2003).

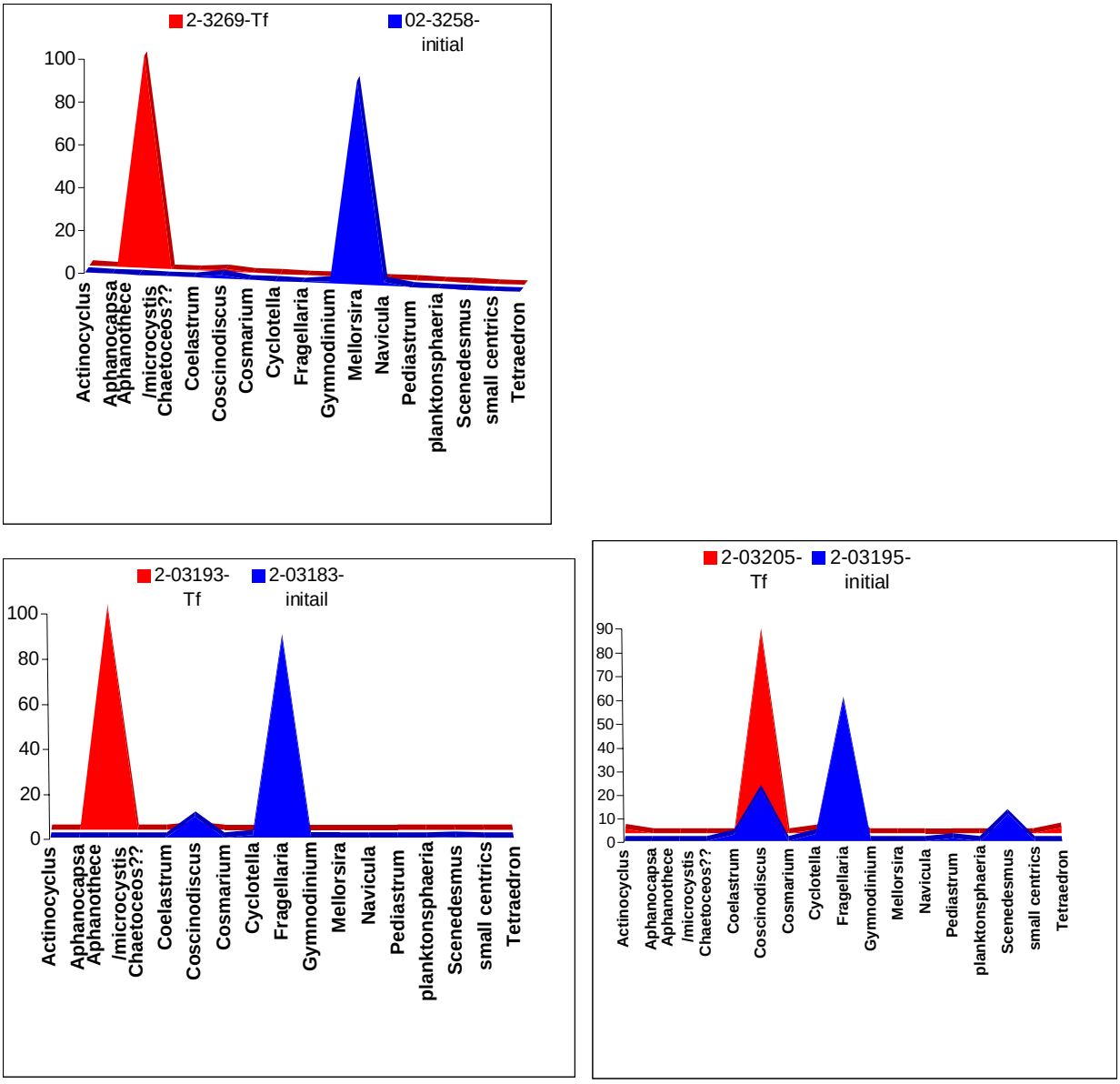


Figure 4.10: Species composition changes between initial and final samples during Experiment 3, 4, and 5 (2003).

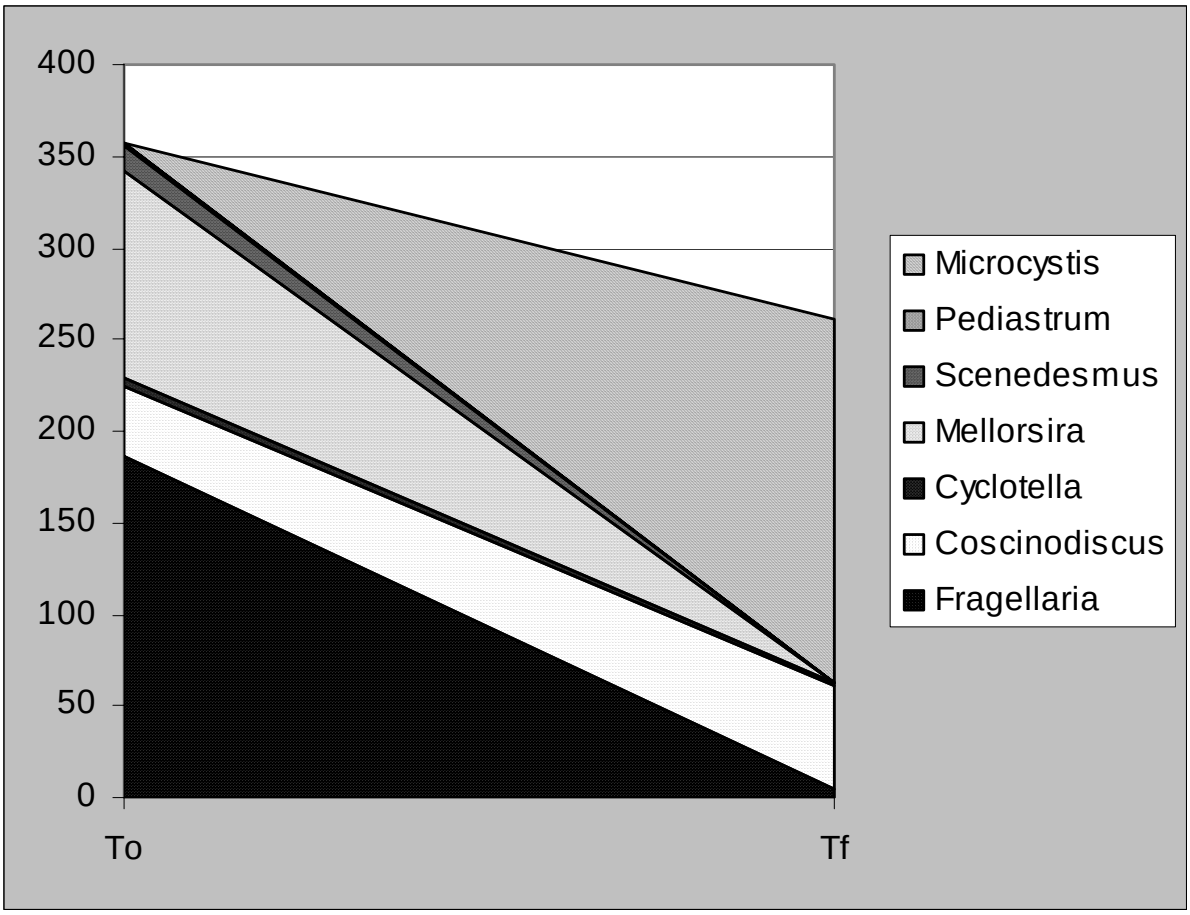


Fig. 4.11: Broad changes in phytoplankton abundance during vessel transit in the Great Lakes. Data summarized from Experiments 3, 4 and 5, 2003.

4.1.4. Discussion

The results of Task 2 studies clearly show a significant decline in abundance (50 –98%) of microbial populations (viruses, bacteria and phytoplankton) during ballast transit through the GL. By comparing NOBOB tanks versus those that were recently filled, we also observed that there was a short-term increase in microbial density at the top of tanks after ballasting. In the case of bacteria and viruses, this was likely due to resuspension of bottom sediments (that generally have higher inventories than overlying residual water—see Task 1 section of this report). The increase in chlorophyll-*a* (and commensurate increase in the ratio of functional versus degraded photosynthetic pigments) with tank filling was more likely the result of fresh phytoplankton being taken up rather than cells being resuspended. This is because there is an exponential decrease in chl-*a* concentration with ballast tank confinement, irrespective of whether the ship is operating within the Great Lakes or on the open ocean (see Fig. 4.12).

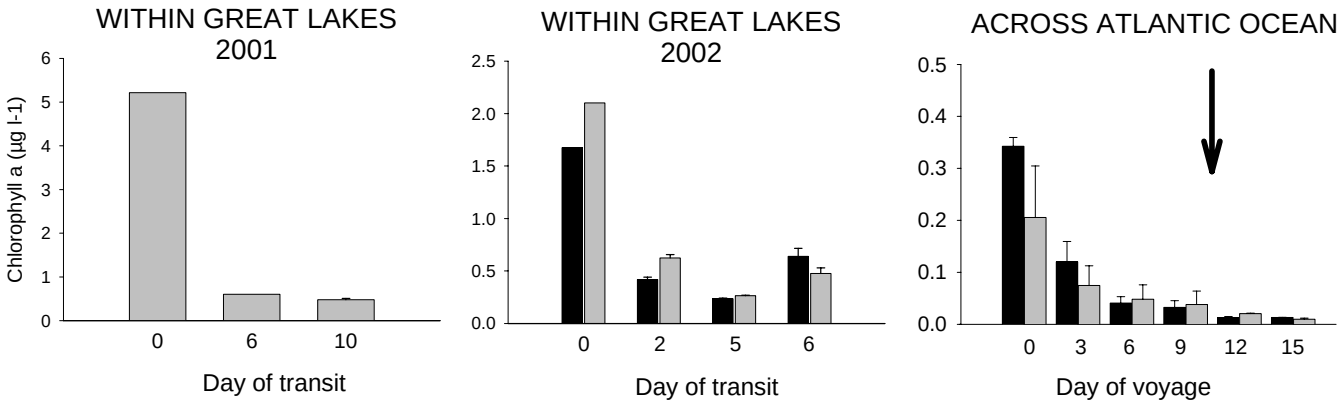


Fig. 4.12: Comparison of chlorophyll-*a* concentration during Experiment 1 (10 days) and Experiment 2 (6 days) and a transoceanic voyage (14 days; data from Drake et al., 2002). There is a consistent exponential decline in chl-*a* in ships transiting the GL and in those crossing the Atlantic Ocean.

Consistent with this, we observed a decrease in the diversity of phytoplankton species during Great Lakes transit (see Fig 4.9), with the potentially harmful blue-green alga, *Microcystis*, being the favored competitor in ballast tanks (Fig. 4.11).

Interestingly, pathogens survive ballast transit within the Great Lakes, but their variable detection suggests low abundance and little potential for predictability.

With respect to composition of microbial populations during vessel transits, our DGGE analyses showed an increase in the abundance of alpha-proteobacteria and gamma-bacteria during Experiment 1 (Fig. 4.4), but there was also a decrease in abundance of an unidentified bacteria group. Likewise, we saw microbial dynamics during Experiment 2 (bands remain to be identified), with the number of bands decreasing during vessel transit (see Fig. 4.5A). An important point to note is that not all microbial components (i.e., of size 0.2 – 1.0 , 1.0 – 5.0, > 5.0 µm) were actively transcribing DNA, as indicated by the absence or very faint bands in RNA lanes (see Fig. 4.5B). This means that there are significant changes in microbial activity during vessel transit.

Overall, our data provide more support for the 'decay' rather than 'incubator' hypothesis for microbes in ballast tanks, with organisms dying in tanks during transit through the Great Lakes.

4.2. Invertebrate Resting Egg Experiments

4.2.1. Introduction

Recent studies designed to identify life history differences between successful and unsuccessful introductions of nonindigenous species (NIS) have focused on the stage of the invasion (e.g. Kolar and Lodge 2001, 2002). These studies recognize that successful invasions encompass a series of different stages including transport, introduction, establishment and spread. Previous studies of invasions by nonindigenous invertebrates to the Great Lakes via ballast water, the leading vector since the St. Lawrence Seaway opened (Holeck et al. 2004), have suggested that the ability of species to form dormant or diapausing life stages should enhance survivability through the transportation stage. Analysis of the invasion history of the Great Lakes since 1959 confirmed that 15 of 19 NIS of crustaceans that invaded successfully possess the capability to produce resting or diapausing stages (Bailey et al. submitted). However, the dense nature of residual ballast sediments, combined with their propensity for accumulating in tanks, suggests that diapausing stages within sediments may not ordinarily be expelled from tanks during normal operation. Thus, the ability of diapausing stages to transition directly from the transportation stage to the introduction stage is probably quite limited. A much more likely mechanism for introduction of NIS from residual sediment consists of hatching of viable resting eggs while the ship is in ballast, followed by discharge of planktonic taxa during deballasting operations. Standard operations of NOBOB vessels may induce hatching of diapausing eggs when ships visit multiple ports on the Great Lakes and carry out a series of ballasting and deballasting operations associated with cargo operations (Bailey et al. 2003). Bailey et al. (2003) proposed that the uptake of oxygenated freshwater may stimulate diapausing eggs in ballast sediments to hatch, facilitating release of planktonic taxa when the mixed ballast water was discharged at the last port-of-call, typically in Lake Superior (Colautti et al. 2004).

4.2.2. Methods

We initially proposed to conduct Task 2 experiments by experimentally filling a NOBOB tank and then repeatedly sampling the ballast water as the ship traveled through the Great Lakes. The first experiment, conducted in October 2001, highlighted the difficulties associated with our original approach. We learned that regular access to ballast tanks, especially double bottom tanks, is very difficult to maintain on an operating vessel, making our original plan to obtain daily samples untenable. In addition, the port-to-port schedule of these vessels is subject to change at the last minute, complicating our ability to run a well-planned experiment. Finally, it became problematic how to find organisms originating from the ballast residuals in the experimental tank against the background of the biota already contained in the local ballast water used to flood the tanks.

As a result of these difficulties, we modified our experimental design for the remainder of the study and added a new and novel approach involving the use of “Incubator-Emergence traps”. These trap experiments were designed to test for zooplankton hatching from resting eggs present in residual sediment in situ and under normal ship operating conditions. The traps address the difficulty of trying to detect the presence of newly hatched organisms against the huge background of organisms present in the incoming water as the tanks are filled, and the limitation of sampling due to tank access and the efficiency of using limited net tows.

Incubator-Emergence traps

Simple, low-cost incubator-emergence traps (IETraps) were constructed from standard 6“ diameter PVC plumbing components (Figure 4.13)to monitor *in situ* hatching of diapause eggs from residual ballast sediments. IETraps were designed to allow ballast water to flow through each experimental chamber, while excluding organisms present in the surrounding water and retaining any organisms hatched inside traps. Each IETrap was built from a 15cm cleanout body and end cap glued together with PVC cement. The cleanout body was threaded on one side to allow for a threaded lid. Stainless steel bolts were put through the bottom of the pipe cap to secure the trap to a rectangular PVC platform. Twelve holes of 2-4cm diameter were drilled through the lid and wall of each trap, and were subsequently covered with Nitex plankton mesh (mesh sizes given below). Mesh was attached to the trap housing using clear PVC cement and then sealed with silicone glue. After construction, traps were left to cure for 48h and were subsequently rinsed in tap water to remove any coarse debris and soluble compounds left by the glues. The traps were mounted on 28in. x 12in. x ¾ in. thick PVC sheets, 3 to a sheet. Each sheet has two 1 in. square strips of PVC stock attached to the bottom along the length of the sheet for further stability inside the tank.

Figure 4.13. Incubator-emergence traps designed for Task 2. IETraps are 16.5 cm high by 17 cm diameter. Holes to allow exchange of water are 3.8 cm in diameter and the interior is lined with 53-micron Nitex mesh. Comment: metric or imperial measurements??? use only one throughout document

Each trap is then seeded with a pre-measured amount of previously collected residual sediment with a known density and viability of resting eggs. Two *in-situ* hatching trials were conducted on each ship voyage; each trial used different sediment that had been selected on the basis of high egg density to maximize opportunities for hatching of diapausing eggs. Sub-samples of each sediment were removed to characterize the density, diversity and viability (hatch rate) of diapausing eggs in the laboratory before usage of sediments in emergence trap experiments (see Bailey et al. in press for laboratory methods). Sediments were stored in the dark at 4°C until the onset of *in situ* trap experiments.

On the initial ship, traps were constructed using 34µm Nitex mesh. Six traps were used for each of two sediment types. One trial consisted of five experimental replicates (precharacterized sediment), and a negative control trap (autoclaved sediment) to monitor for introduction of species from the ship's ballast water. The second trial had four experimental replicates, and negative and live control traps. The latter trap contained the same non-autoclaved sediment as

was used for experimental replicates, to which 40 *Lumbriculus* oligochaetes and 40 *Hyalella* amphipods (both organisms are prevalent in the Great Lakes) were added. The experimental design was modified slightly for the final three trap experiments in an effort to improve water flow through traps, and to increase statistical power. These experiments used traps with 53µm mesh, and six experimental replicates per trial. Each trial had its own negative and live controls, the latter containing 20 *Lumbriculus* and 20 *Hyalella.* In addition, a positive control was included for each trial, consisting of diapausing eggs isolated from 500g sediment using a sugar flotation protocol (Bailey *et al*., 2003); this control served to assess whether hatching and survival of taxa were similar with and without sediment present.

Traps were moored in the designated ballast tank, sediment added (Figure 4.14), traps closed, and the tank flooded for the duration of the cruise. At each sampling port we profiled the water column in the ballast tank for temperature, conductivity/salinity and turbidity. No evidence of stratification was ever found. We then collected water from just above the surface of the traps. Lastly, we conducted 3 full water column net tows, using a 0.25m diameter 30-µm net. Each net sample was later scanned under a dissecting microscope and representative taxa were identified with a compound microscope at up to 1000x magnification. In addition, temperature readings were measured near tank bottom at all ports where net samples were drawn using a Hydrolab DataSonde 4a.

Figure 4.14: NOBOB Team member Sarah Bailey as she prepares one of the IETrap experiments conducted during 2003

In addition to samples for characterizing the water in the ballast tank, net tows and water samples were taken in the water column adjacent to the vessel at the first ports of the field experiment. These samples will be used to compare the chemistry and biota of the ballast tank water to the local water that was pumped in at the first port.

Traps were recovered at the terminal port-of-call after ballast water had been discharged. Approximately 450ml of water, which remained inside traps below the drainage holes, was collected by large-mouth pipette and filtered through 30µm mesh. The filtrate was preserved using 95% ethanol for later enumeration and identification of invertebrate taxa. Sediment was subsequently recovered from each trap and preserved in 95% ethanol; taxa associated with the sediment were isolated for enumeration and identification using a colloidal silica Ludox® HS40 protocol (Burgess 2001). Negative and positive controls were recovered and analyzed in the same manner, except that positive controls consisted solely of a water sample and thus the Ludox method was not required. After recovery, all emergence traps were inspected for integrity; four experimental replicates were excluded from analysis due to visible tears in plankton mesh. Live traps were surveyed to determine the number of oligochaetes and amphipods that remained alive.

For enumeration of hatched taxa, any taxa recovered from trap sediments that do not possess diapausing stages (e.g., bdelloid rotifers, bivalves) or mature forms that could not have developed within the transit timeframe (e.g., copepodids) were excluded from analyses since these organisms were most likely introduced with the sediment at the onset of the experiment. In addition, analysis of negative controls indicated that some organisms present in the Great Lakes ballast water had infiltrated the plankton mesh on emergence traps. As a result, all taxa present in either negative control for each ship were subtracted from results of experimental replicates. Furthermore, to be conservative, any recovered taxa not identified during laboratory experiments with the same sediments, under ideal growth conditions, were excluded from analysis. Our analyses are therefore based on conservative estimates of *in situ* hatch rates and may underestimate actual richness and abundance of hatched taxa. Total abundance of organisms hatched from *in situ* experimental replicates was compared to that of laboratory characterization experiments using a Mann-Whitney U-test (Systat 8.0, SPSS, Inc., 1998). Since laboratory experiments were conducted using only 40g (as opposed to 500g) sediment replicates, total abundances of hatched species were extrapolated to 500g before analysis. We compared species richness of laboratory and in situ hatch rates using a Mann-Whitney U-test.

4.2.3. Results

In all, five Task 2 experiments were completed during the project (Table 4.1). IETrap experiments were conducted during the last four experimental voyages (Table 4.7), covering two summer experiments and two early fall experiments. Emergence traps remained submerged for 6 to 11 days, depending on ship schedule. Average water temperature near the bottom of ballast tanks ranged between 16.5 and 20.6°C for the four experiments (Table 4.7).

Table 4.7. Summary of transit dates and locations for on-board ship experiments. Two trials were run inside the same tank of each ship. Average temperature ± SE, as measured near tank bottom, was calculated from 3-4 points of time across voyage. Tank types: FP – forepeak; UW – upper wing tank; DB – double bottom.

Voyage	Start location	End location	Tank Type	Average temp (°C)	Sediment	# replicates
2	Windsor, ON Oct 5, 2002	Milwaukee, WI Oct 11, 2002	FP	16.5 ± 0.9	A B	5 4
3	Hamilton, ON July 2, 2003	Thunder Bay, ON July 13, 2003	UW	20.6 ± 1.3	A C	4 5
4	Hamilton, ON July 14, 2003	Milwaukee, WI July 24, 2003	UW	20.4 ± 1.2	B C	6 6
5	Cleveland, OH Sept 15, 2003	Duluth, MN Sept 26, 2003	DB	18.4 ± 2.7	D E	6 5

All live control animals were recovered alive, indicating that environmental conditions within traps could support life for the duration of each voyage. In total, 19 individuals were hatched from 41 experimental replicates, producing an average hatching abundance of 0.5 individuals per 500g replicate (Table 4.8). Hatching occurred in six of eight trials. Both trials without hatching occurred on separate ships, thus hatching occurred in at least one experimental replicate on every

ship. Species that hatched included the rotifers *Brachionus calyciflorus*, *Cephalodella catellina*, *Keratella tecta*, *Synchaeta oblonga* and *Trichocerca pusilla*, and copepod nauplii (Table 4.8). None of the individuals recovered from experimental replicates were observed in a reproductive condition.

Table 4.8: Summary of hatching results for *in situ* emergence trap experiments. Egg density is average density of diapausing eggs calculated per 500g replicate. Positive control lists number of individuals present in control for each species, while experimental replicates column provides mean number of individuals hatched per replicate with number of traps having hatching given in parentheses. * denotes occurrence of parthenogenetic reproduction.

Voyage	Species hatched	Egg Density	Positive control	Experimental Replicates
1	**sediment A**	1114		
	Brachionus calyciflorus		n/a	1 (1)
	copepod nauplii		n/a	1 (2)
	sediment B	1840		
	no hatching		n/a	0
2	**sediment A**	1114		
	B. angularis		1	0
	B. budapestinensis		1	0
	B. calyciflorus		9	1 (1)
	sediment C	538		
	B. budapestinensis		1	0
	B. calyciflorus		13	0
	B. diversicornis		1	0
3	**sediment B**	1840		
	B. angularis		38*	0
	B. calyciflorus		51*	0
	Cephalodella catellina		0	1 (1)
	Synchaeta oblonga		0	1 (3)
	Trichocerca pusilla		0	1 (2)
	sediment C	538		
	copepod nauplii		9	1 (1)
	Trichocerca stylata		2	0
4	**sediment D**	5838		
	B. angularis		10	0
	B. budapestinensis		1	0
	B. diversicornis		1	0
	B. calyciflorus		1073*	5 (1)
	sediment E	1605		
	B. angularis		59*	0
	B. calyciflorus		289*	0
	Keratella tecta		2	2 (1)
	Total Number Hatched		**1561**	**19 (13)**

Diapausing eggs were not as likely to hatch *in situ* as under laboratory conditions. Both total abundance and species richness of organisms hatched was significantly lower *in situ* than in laboratory characterization trials (Mann-Whitney *U*-test, $p<0.001$). In addition, the effect of burial appeared to have a significant impact on the number of eggs that hatched (Mann-Whitney *U*-test, $p<0.001$; Fig. 4), although this could only be tested for the laboratory experiments since positive controls on ships were not replicated. Hatching also occurred in all six positive controls, supporting the hypothesis that abiotic conditions in ballast tanks were favorable and that hatching was inhibited in experimental replicates by some factor associated with the sediment. In

total, 1561 individuals were recovered from positive controls, although this number likely included parthenogenetic offspring (see Table 4.8). Species hatched in positive controls include the rotifers *Brachionus angularis*, *B. budapestinensis*, *B. calyciflorus*, *B. diversicornis*, *Keratella tecta* and *Trichocerca stylata,* and copepod nauplii (Table 4.8).

Estimation of inoculum size

The rate of hatching recorded during this study ranged from 0.01-0.04% per sediment. Extrapolations based on sediment volume and surface area suggest the sediments used in these experiments could release approximately 7000 to 10000 individuals per ship under similar environmental conditions (typically June – October). However, previous work suggests that approximately 2.5% of resting stages transported in residual sediments are from species nonindigenous to the Great Lakes (Bailey et al. submitted). Consequently, the inoculum size of NIS introduced via this vector is probably much lower than the total number estimated by this study, at 175-375 nonindigenous individuals hatched per ship. Furthermore, since ships with nonindigenous taxa present typically carry only one or two NIS each (S.A. Bailey, unpublished), this may result in an inoculum size of 87-375 individuals per taxon.

Live-Animal Controls

Two live-animal controls were included in each trial. Animals were sampled at the end of each field experiment (~10 days long), by removing the control traps as soon as possible and immediately screening the contents to enumerate live and dead animals. For the first two experiments we used a 300-micron screen. Animals of both species were found alive in each of the control traps after completion of each field experiment, but in the case of *Lumbriculus* we recovered less than the original 20 animals placed in the traps (Table 4.14). After close inspection of the individual control traps for openings that could have allowed the animals to escape, we concluded that the most likely explanation is that the individuals were lost during the post-experiment processing.

During processing, the sediment in the traps from the first two experiments was sieved through a 300-micron brass sieve and the animals are picked out by hand. However, due to the physical proportions of *L. variegatus*, we believe that some were able to slip through the sieve if they were oriented correctly. As a result, on the last experiment we switched to a 250-micron sieve, which proved much more successful and we retrieved all organisms of both species from the traps. Identification of live vs. dead animals was based on observation of movement with and without stimulation (gentle poking with a spatula).

Table 4.14. Results of live-animal controls by experiment

Experiment #2: Oct 5-11, 2002

TRAP	Live H	Dead H	Not Found	Total
C	24	11	5	40

TRAP	Live L	Dead L	Not Found	Total
D	28	8	4	40

Experiment #3: Jul 2-13, 2003

TRAP	Live H	Dead H	Not Found	Total
B4	16	4	0	20
A9	16	2	0	20

TRAP	Live L	Dead L	Not Found	Total
B4	7	10	3	20
A9	16	2	2	20

Experiment #4: Jul 14-24, 2003

TRAP	Live H	Dead H	Not Found	Total
C7	16	2	2	20
D3	18	2	0	20

TRAP	Live L	Dead L	Not Found	Total
C7	8	2	12	20
D3	8	3	9	20

Experiment 5: Sept 15-26, 2003

TRAP	Live H	Dead H	Not Found	Total
A7	20	0	0	20
B2	20	0	0	20

TRAP	Live L	Dead L	Not Found	Total
A7	20	0	0	20
B2	20	0	0	20

4.2.4. Discussion

Previous studies estimate that only a small proportion (~10%) of invaders will survive passage from the transportation stage to the introduction stage, with most organisms dying in transit (Williamson and Fitter 1996; Kolar and Lodge 2001). However, diapausing eggs of invertebrates likely enhance survivability during transportation, with up to 92% of eggs collected from residual sediments being viable in laboratory studies (Bailey et al. 2003). But does this mechanism that increases survival during the transportation stage also facilitate successful introduction? Through the use of *in situ* emergence trap experiments, we were able to demonstrate that diapausing eggs can hatch from sediments inside ballast tanks of operational ships, albeit at low rates.

Although all of the species hatched in these *in situ* trials are cosmopolitan and appear to pose little or no invasion risk, these species could pose a risk for genetic invasion, as many plankton species differ genetically on different continents. However, the NIS *Brachionus diversicornis* was hatched from positive controls on two voyages, confirming that NIS are present as diapausing eggs although in extremely low abundance (see Bailey et al. in press). The fact that *B. diversicornis* was not hatched from experimental replicates of residual sediment may be a result of the small volumes of sediment used. Conversely, burial in sediment may have precluded hatching cues from inducing hatching in this species.

Only 0.5 diapausing eggs hatched per 500g of replicate sediment during these experiments. This value is less than 0.05% of the number of resting stages present in the experimental sediments. Despite the fact that NOBOB vessels each typically carry 15 tonnes of sediment, the probability that NIS will be present and receive hatching cues is small, giving an estimated inoculum size of 87-375 individuals per taxa per ship. Furthermore, as the sediments used in this study were selected for high egg density, this is likely a greater inoculum than that presented by most ships entering the system. Propagule pressure is based not only on the inoculum size, but also the frequency of inoculations. Approximately 250 NOBOB vessels conduct multi-port operations on the Great Lakes each year that may provide conditions for hatching and introduction of resting stages (Colautti et al. 2004). It has been estimated that approximately 32% of these vessels will carry resting stages of NIS (Bailey et al. in press), providing a frequency of ~80 inoculations per year. This translates to approximately 5.7×10^3 to 3.0×10^4 nonindigenous individuals being introduced via hatching from the residual sediment vector per year.

Since the prolonged existence of temporal zooplankton is dependent on the formation of a sexually-produced diapausing egg bank, Allee effects (i.e., zero or negative growth of small populations owing to density-dependent population dynamics) may impact establishment success of nonindigenous zooplankton in the Great Lakes. In a modelling exercise, Drake (2004) estimated that inoculum sizes as small as ten parthenogenetic individuals may result in successful establishment if given enough time to produce a large population before the onset of sexual reproduction. Activities of NOBOB vessels seem to fit the optimum release strategies calculated for terrestrial biological control insects by participating in numerous, small-sized release events (as discussed in Grevstad 1999). Furthermore, since ballast-mediated introduction events are spread out over both time and space, risks associated with environmental stochasticity will be reduced.

During the course of this study, we recorded one individual of the NIS *Brachionus leydigi* from the Great Lakes' ballast water loaded on voyage two. This species has been observed as a rare component of diapausing egg fauna in residual ballast sediments of previous studies (Bailey et al. in press). However, since residual sediments generally do not accumulate in upper wing tanks, this individual probably did not hatch from sediments within the tank but may be the result of a previous introduction to Hamilton harbor from ballast discharge by a transoceanic vessel. As only one individual was recovered from plankton samples, we cannot determine whether the species has established in Hamilton harbor, but this finding may be an indication that introductions of NIS of rotifers may already have occurred.

We have not adjusted inoculum size for increases due to reproduction since there was no evidence of reproduction by the individuals hatched in experimental replicates during this study.

Reproduction rates of parthenogenetic taxa are affected by numerous factors, such as temperature, food quantity and quality, and genetic composition (Wallace and Snell 2001). Assuming exponential growth (Taylor 1988) and published life history parameters for *Brachionus calyciflorus* at 16-20°C (Pourriot and Rougier 1997; Wallace and Snell 2001), we determined that 15-45 individuals may have been the founding (hatching) population size for the 1073 individuals recovered from the positive control during voyage four. The occurrence of reproduction in positive control replicates during voyages three and four suggest that ballast tanks can provide suitable conditions for parthenogenetic reproduction, at least during warmer months. Time may have been a constraining factor for reproduction in experimental replicates as eggs isolated from sediments probably hatched a number of days earlier than those buried in sediments. Alternatively, toxicity or hypoxia caused by high biological oxygen demand of ballast sediments may have prevented reproduction in experimental replicates as the plankton mesh may have prevented adequate water flow and oxygen renewal (see below). If this is the case, this study may underestimate inoculum sizes since hatched organisms inside ballast tanks may initiate reproduction under conditions of higher oxygen levels.

This study provides empirical support for the hypothesis that different life history characteristics may be beneficial during various stages of the invasion process. While dormancy is a characteristic enabling enhanced survival during transportation, it becomes a hindrance for the introduction stage as less than 0.05% of individuals will likely pass from the transportation stage to the introduction stage under conditions experienced in ships' ballast tanks. In contrast, live planktonic animals probably have low survivability in ballast tanks but high opportunity for introduction with deballasting of water (see MacIsaac et al. 2002). As environmental and demographic stochasticity will further reduce the number of individuals successfully transitioning from the introduction stage to the establishment stage of the invasion process, the risk of invasion via diapausing eggs in residual ballast sediments appears to be very low. However, the assumption that sediments are not being deposited directly into the Great Lakes, either during regular deballasting or tank cleaning operations, must be validated to ensure that this risk is not underestimated. Dry-dock cleaning of sediments from ballast tanks should be carefully managed, since it can provide a direct route for discharge of diapausing eggs into adjacent waterbodies.

4.3 Instrumented Emergence Trap

4.3.1. Introduction

One of the concerns related to using IETraps for ballast tank hatching trials is how well the conditions inside the traps represent the conditions in the surrounding ballast water in the tank. Conditions inside the traps are likely strongly dependent on the effective residence time of the water in the traps and the oxygen demand of the test sediment added to the traps. The maximum surface area available for water to exchange between the inside and outside of the trap was 120 cm^2 for a standard IETrap. However, if the mesh lining the traps (53 micron openings) becomes partially or fully clogged by particulate material, it is likely that exchange between water inside the traps and the surrounding ballast water will be restricted during the experiment. Holes were drilled in the lid and sides of the trap and lined with 53-micron Nitex mesh. Poor exchange with surrounding ballast water combined with the oxygen demand of the test sediment inside the traps

may result in development of low oxygen concentrations inside the traps relative to conditions outside the traps.

We developed two approaches to evaluate the conditions inside the traps during in-tank experiments. First, we added a live-animal control to each Task 2 trial (see above). Second, we instrumented a single trap to obtain a continuous record of basic parameters inside and outside a trap for comparison.

4.3.2. Methods

In order to test how well the traps mimicked the surrounding conditions, we procured two multiparameter (In-Situ Corp., MP Troll 9000) off-the-shelf instruments with sensors for temperature, conductivity and dissolved oxygen. A single IETrap was modified to allow the sensor end of one MP Troll 9000 to be embedded into the middle of the trap, resulting in a slight reduction of the total maximum surface area available for water exchange to 108 cm^2. The second MP Troll 9000 was mounted immediately adjacent to the instrumented IETrap. Each instrument had a self-contained battery pack and data logger set to record readings every 30 minutes (Fig. 4.15).

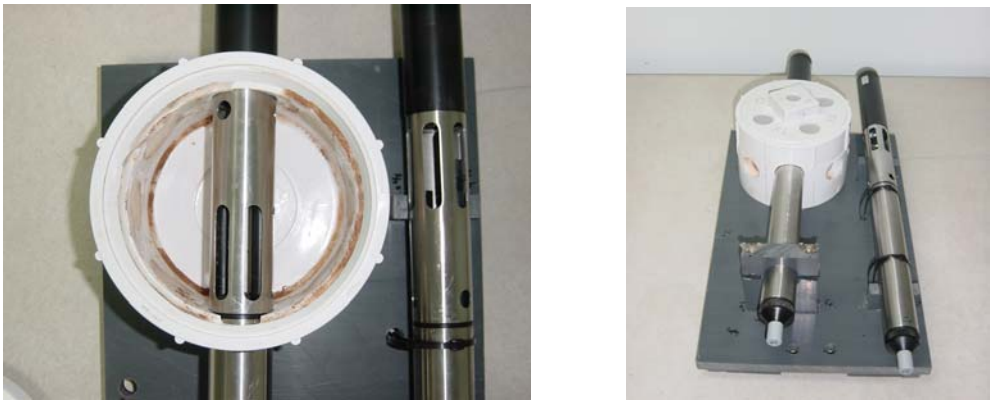


Figure 4.15. Instrumented IE Trap with external instrument fastened on PVC sheet.

We deployed the instrumented trap package at the bottom of a side ballast tank during our final field experiment. A 500g sample of the sediments used in an accompanying hatching experiment was added, the sensor was sealed inside the trap (Silicone II Household Glue, GE Sealants and Adhesives, Stock # GE280), and the trap was closed and moored in the ballast tank. The tank was ballasted with Hamilton, Ontario harbor water within an hour. When ballasting was completed, the instrumented trap was submerged under ~8.5 m of water.

4.3.3. Results

Data for temperature, conductivity, and dissolved oxygen recorded by the internal and external sondes over the entire experiment are shown in Figures 4.16. As seen by the internal sonde, a number of events were recorded during the voyage, and all appear to coincide with changes in the ship's motion. For example, the small reoxygenation event early on day 4 coincides with the

ship's passage through western Lake Erie, where it turned from a westbound to a northbound direction. Winds were blowing from the east. This would have put the ship in a direction parallel to waves driven by the east winds, resulting in increased rolling. Prior to this change in direction the ship was transiting Lake Erie in a westbound direction with following winds, resulting in little roll. The major reoxygenation event during days 5 and 6 started shortly after the ship departed the port of Detroit (Detroit River) and coincided with the transit of lakes Huron and Michigan. The ship logged force 4 winds all the way up Lake Huron, which would have produced a considerable amount of roll, and mixing and flow in the ballast tank. The period immediately thereafter, during which oxygen declined to nearly zero coincided with the ship at berth in Burns Harbor, Indiana (southern Lake Michigan).

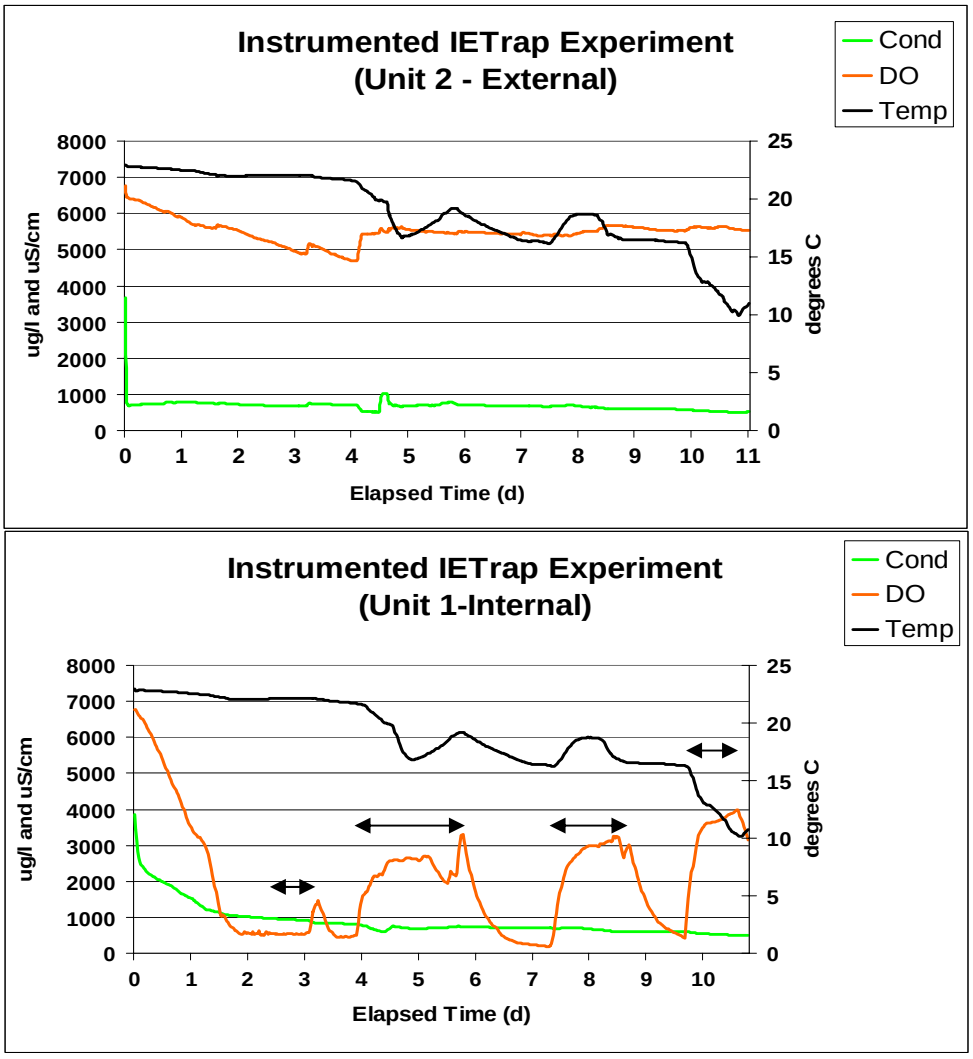


Figure 4.16. Time series of temperature, conductivity, and dissolved oxygen of an instrumented incubator-emergence trap deployed during experiment #5, September 15-23, 2003. Upper panel represents data from the external sonde and the lower panel represents data from the sonde placed within the IETrap.

The second major reoxygenation event occurred during days 8-9, starting shortly after the ship departed Burns Harbor and lasting until the ship anchored above the locks at Sioux Ste. Marie on the St. Mary's River to wait out severe weather. The final period of reoxygenation started late on

day 10 when the ship weighed anchor and began its transit of Lake Superior. The ship reported strong NW winds all the way across the lake. The reoxygenation event continued until the ship arrived at Duluth Harbor on day 11, where the experiments were terminated.

The record from inside the IETrap clearly reveals a significant oxygen demand not observed in the ballast tank. Oxygen declined to less than 600 µg/L within two days and remained at that level through day 3. The effect of a small reoxygenation event during day 4 quickly disappeared, but was followed by major reoxygenation events during days 5-6, 8-9, and 10-11, each of which was followed by rapid decreases in oxygen. As noted above, the latter three periods coincided with ship transits of the open lakes. We hypothesize that 1) the development of hypoxia inside the trap was due to high oxygen demand by the test sediment and is an indication that the traps are NOT significantly exchanging water with the ballast tank in the absence of sustained water flow, and 2) the observed reoxygenation events were a direct result of temporary water flow in the ballast tank caused by ship motion, which varied from gentle to severe as the ship encountered weather fronts and storms.

The conductivity record from inside the trap also provides evidence that the traps did not readily exchange with surrounding ballast water. At the beginning of the experiment the conductivity inside the trap took 3 to 4 days to decrease to the same level as recorded for the surrounding ballast water. Laboratory experiments on same sediment indicated that it held a residual salt content. We hypothesize that the conductivity inside the trap was initially raised when the incoming water mixed with and/or dissolved residual salt in the sediment. The small internal trap volume combined with a very slow rate of exchange with external ballast water during a period of relative calm resulted in gradual dilution until the conductivity was equal to that in the surrounding ballast water. No effect on conductivity was seen during the later reoxygenation events because there was no conductivity differential between the trap water and the surrounding ballast water.

4.3.4. Discussion

In spite of strong evidence from this one instrumentation experiment that the traps may go hypoxic or anoxic due to biochemical oxygen demand associated with the sediment, hatching of diapausing eggs did occur inside IETraps during shipboard experiments. Furthermore, live-animal control results also suggest that conditions were sufficient to maintain both *L. variegatus,* which can survive under low oxygen conditions, and *H. azteca*, which is known to be particularly sensitive to poor ambient conditions. If oxygen demand associated with sediment inside IETraps during *in situ* experiments is causing anoxia, hatching results from trap experiments should be viewed with caution and may underestimate the hatching potential of diapausing eggs in ballast tanks. Redesign and further testing of the IETraps is necessary if they are to be routinely used for *in situ* hatching experiments that include sediment. We recognize that the instrumented trap was used on only one ship without replication, and there may be unaccounted factors that caused the oxygen levels inside the instrumented trap to have been lower than in experimental replicates used for our hatching studies. Certainly, a larger mesh size would help, but would also increase the risk of organisms escaping as well as intruding from the surrounding water. Increasing the vented opening space should be tested.

4.4 Live Invertebrate Analyses for Filled Ballast Tanks

4.4.1. Methods

Five separate up-bound Great Lakes voyages, over three years on different foreign and domestic transoceanic vessels, were examined to assess invertebrate composition and abundance within ballast tanks during transits that ranged from six to eleven days. Three replicate plankton net samples (30-µm, 0.25m-diameter) were taken from port of ballast uptake and from experimental ballast tanks instantly after ballasting, and at either one or two subsequent ports, as well as at the final port-of-call before ballast discharge (see Table 4.1). However, due to loss of the plankton net during voyage five, the port of ballast uptake and sampling of the experimental tank on day one was sampled with a 30-µm, 0.13m-diameter net. Forepeak ballast tanks sampled included three replicate net hauls on each of the port and starboard sides of the tank to examine zooplankton distribution. One replicate tow sample was lost for both voyage two and voyage four during the collection in Detroit, thus only two were enumerated. Total amount of ballast water filtered through the nets ranged from depths of ~1.0 - 8.5m and all samples were preserved with 95% ethanol. Retained animals were examined under a dissecting microscope (Leica MZ75), and all taxa enumerated into major zooplankton groups and identified using a compound microscope (Leica DME) up to 1000x magnification. Owing to the high abundance of organisms during voyage 5, the port of ballast uptake and sampling of the experimental tank on days zero and four were sub-sampled three times with replacement and final numbers adjusted to reflect whole sample counts. In addition to plankton samples, physical-chemical data were collected with either a Hydrolab DataSonde 4a or a YSI meter (Model 30) at typically 0.5m depth intervals from ballast tank surface to bottom.

All taxa were standardized to abundance$\cdot L^{-1}$ by dividing total abundance by volume of water sampled through the net and data checked for normality before analyses. To examine zooplankton distribution on the port and starboard sides of forepeak tanks, densities of taxa from voyages one and two were analyzed in relation to tank side using one-way ANOVA (Systat 8.0, SPSS, Inc., 1998). If significant differences were detected on a sampling date, a two-group t-test ($\alpha = 0.05$) was utilized on proportion of taxa to explore the differences. Separate one-way MANOVA's were conducted on each voyage, to assess taxa abundance$\cdot L^{-1}$ in ports-of-call from initial propagule loads after ballast uptake, as well as from each subsequent port-of-call to final port of ballast discharge. If significant differences were detected, a post-hoc Tukey multiple comparisons test ($\alpha = 0.05$) was utilized.

4.4.2. Results

Voyage 1

Zooplankton densities did not differ between the port and starboard sides of the forepeak tank across all sampling dates ($P > 0.061$), thus samples were combined for analysis. Densities of the major taxa, except nauplii, did fluctuate from harbor abundances, initial ballast load and during the voyage (Fig. 4.17; Table 4.15). Daphnia were the only taxa to have different abundances after ballasting, which were lower inside the tank than from the harbor ($P = 0.003$, Tukey test). Cyclopoid copepods ($P = 0.016$, Tukey test) and the 'other' combined taxa ($P < 0.001$, Tukey

test) decreased from day zero to day six, while the Calanoid copepods (P = 0.023, Tukey test), copepodids (P = 0.004, Tukey test) and Daphnia (P = 0.006, Tukey test) each decreased from day six to day ten of the voyage (see Fig.4.17). Conversely, Bosmina (P < 0.001, Tukey test) and Rotifera (P = 0.016, Tukey test) each increased in abundance from day zero to day six, while the former decreased from day six to ten (P = 0.008, Tukey test) and the latter increased, but not significantly (P = 0.182, Tukey test; Fig. 4.17).

Table 4.15: Results from MANOVA's conducted on NOBOB vessel transits in the Great Lakes examining zooplankton abundance through time. Taxa: Biv – bivalve larvae, Dap – Daphnia, Bos – Bosmina, Cal – Calanoida, Cyc – Cyclopoida, cop – copepodids, nap – Nauplii, Rot – Rotifera, oth – other taxa.

Voyage	Biv	Dap	Bos	Cal	Cyc	cop	Nap	Rot	oth	
1		18.957	12.213	4.688	6.283	6.058	1.581	22.622	68.519	$F_{3,17}$
		<0.001	<0.001	0.015	0.005	0.005	0.231	<0.001	<0.001	P
2	50.837		8.483	18.605	12.899	7.99	17.617	59.397	5.181	$F_{4,21}$
	<0.001		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.005	P
3		10.060	14.834	20.388	17.580	10.118	22.889	9.591	41.615	$F_{4,10}$
		0.002	<0.001	<0.001	<0.001	0.002	<0.001	0.002	<0.001	P
4		33.144	758.139	6.840	108.269	42.776	17.281	29.333	24.109	$F_{3,7}$
		<0.001	<0.001	0.017	<0.001	<0.001	0.001	<0.001	<0.001	P
5	13.272	6.085	12.110	7.980	13.239	7.409		9.723	4.891	$F_{4,10}$
	0.001	0.010	0.001	0.004	0.001	0.005		0.002	0.019	P

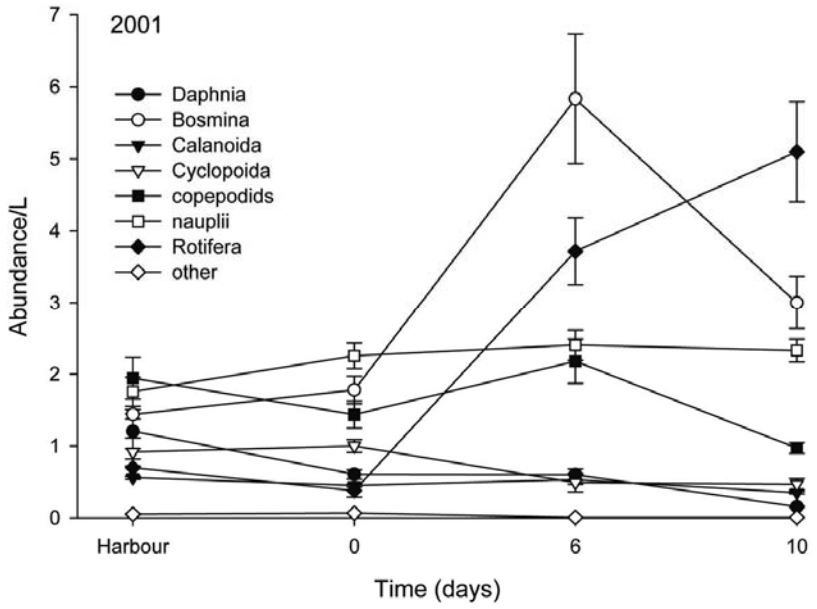


Fig. 4.17: Zooplankton densities over time for Voyage 1.

Voyage 2

Zooplankton abundance did not differ between the port and starboard sides of the forepeak tank on days zero, five and six, ($P > 0.149$), however, they did vary on day two of the voyage ($P = 0.003$). Proportional abundances of Calanoid copepods ($P = 0.046$) where higher on the starboard- than on the port-side of the ballast tank, while the other taxa did not differ on day two ($P > 0.213$). After samples were combined for analysis, abundances of all the major taxa were found to have fluctuated from the harbor, initial ballast loads and during the voyage (Fig. 4.18; Table 4.15). However, the combined 'other' taxa did not display any day to day changes in abundance ($P > 0.062$, Tukey test). Calanoid copepods ($P = 0.003$, Tukey test) and Bosmina had a higher abundance in the ballast tank than which was detected from the harbor samples, but the latter was not significant. Calanoid copepods ($P = 0.007$, Tukey test) and copepodids ($P = 0.001$, Tukey test) were the only taxa to decrease in abundance over the six day voyage, which occurred between initial ballast uptake and day two, while Nauplii and bivalve larvae ($P < 0.001$, Tukey test) where the only taxa to increase in abundance from day two to five. Bosmina, nauplii, Rotifera, bivalve larvae, and Calanoid and cyclopoid copepods all increased in abundance from day five to six ($P < 0.006$, Tukey test; Fig. 4.18).

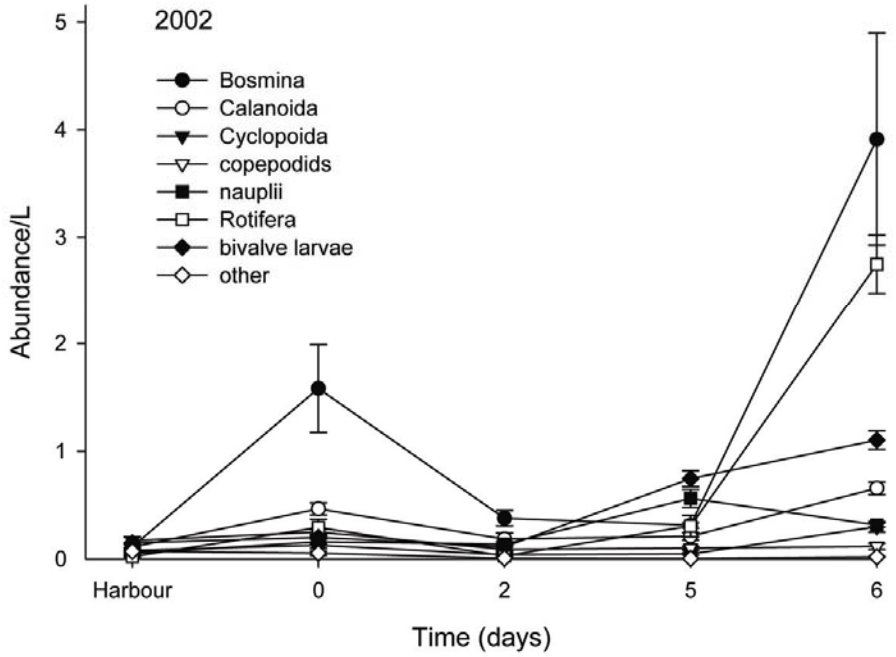


Fig. 4.18: Zooplankton abundance over time for Voyage 2.

Voyage 3

Densities of the taxa fluctuated from harbor abundances, initial ballast load and during the voyage (Fig. 4.19; Table 4.15). Daphnia, Bosmina, Rotifera, and Calanoid and Cyclopoid copepods all had higher densities inside the tank than from the harbor after ballasting ($P < 0.026$, Tukey test). On the other hand, combined 'other' taxa were the only group to display a lower abundance in the upper wing tank after ballasting than from the harbor ($P < 0.001$, Tukey test). Following ballast uptake, there was a drastic decrease in Bosmina, Daphnia and Calanoid copepod densities ($P < 0.003$, Tukey test), while copepodid density increased up to day four ($P = 0.009$, Tukey test). Similarly, copepodid and Cyclopoid copepod density decreased from day four to eight ($P < 0.007$, Tukey test), while nauplii density increased ($P = 0.020$, Tukey test). During the last two days of the voyage, nauplii tended to increase while Rotifera decreased, albeit not significantly (Fig. 4.19)

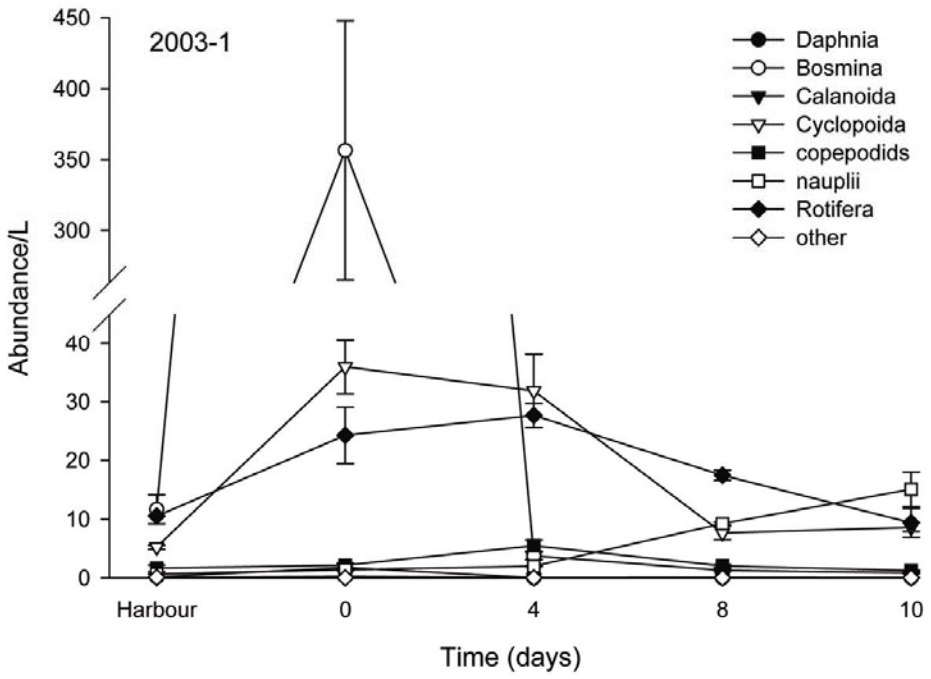


Fig. 4.19: Zooplankton density over time for Voyage 3.

Voyage 4

Abundances of all the major taxa were found to have fluctuated from the harbor, initial ballast loads and during the voyage from Hamilton to Milwaukee (Fig. 4.20; Table 4.15). Daphnia and Bosmina occurred in higher abundances after ballast uptake ($P < 0.001$, Tukey test), while Rotifera and 'other' taxa were lower when compared to harbor abundances ($P = 0.001$, Tukey test). Furthermore, after ballast uptake, Daphnia, Bosmina and Cyclopoid copepods had a drastic decline in abundance up to day three ($P < 0.007$, Tukey test). Bosmina continued its decrease in abundance from day three to day ten ($P = 0.043$, Tukey test), however, Cyclopoid copepods, copepodids and nauplii increased over this seven day time period ($P < 0.004$, Tukey test). Calanoid copepods did not display any changes in abundance in between intermediate ports-of-call, however, there was a significant increase from the initial ballast loading event to the final port of discharge ($P = 0.030$, Tukey test; Fig. 4.20).

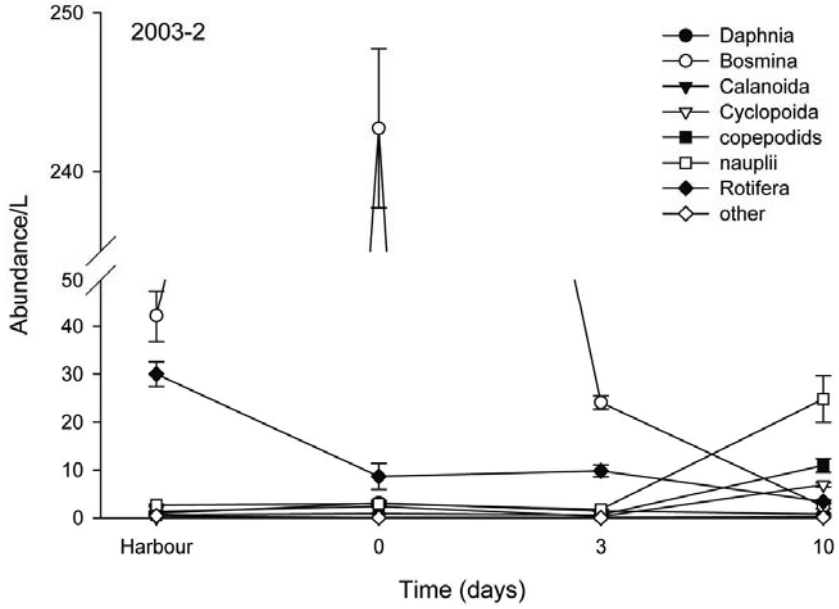


Fig. 4.20: Zooplankton abundance over time for Voyage 4.

Voyage 5

Zooplankton densities from the major taxa fluctuated from the initial port-of-call, ballast uptake and during the voyage (Fig. 4.21; Table 4.15). Daphnia, Bosmina, Calanoid and cyclopoid copepods, and the 'other' taxa (which were mostly comprised of Diaphanosoma and Leptadora), all had higher densities in the double bottom tank after ballasting than from samples collected from the harbor ($P < 0.041$, Tukey test). Rotifera were the only taxa to have lower densities in the tank after ballasting ($P = 0.041$, Tukey test). Following ballast uptake, there was a sharp decrease in Bosmina, Daphnia, Cyclopoid copepods, bivalve larvae and 'other' taxa densities four days after the start of the voyage ($P < 0.037$, Tukey test). While Bosmina, from day four to day six, where the only taxa to increase in abundance over the journey ($P = 0.027$, Tukey test). During the last five days of the voyage, Bosmina and bivalve larvae densities decreased within the ballast tank, but not significantly ($P > 0.072$, Tukey test). Nauplii densities did not display any significant decreases in density from initial ballast loads to day eleven ($P > 0.071$, Tukey test), however, Calanoid copepods increased over this time period ($P = 0.009$, Tukey test; Fig. 4.21).

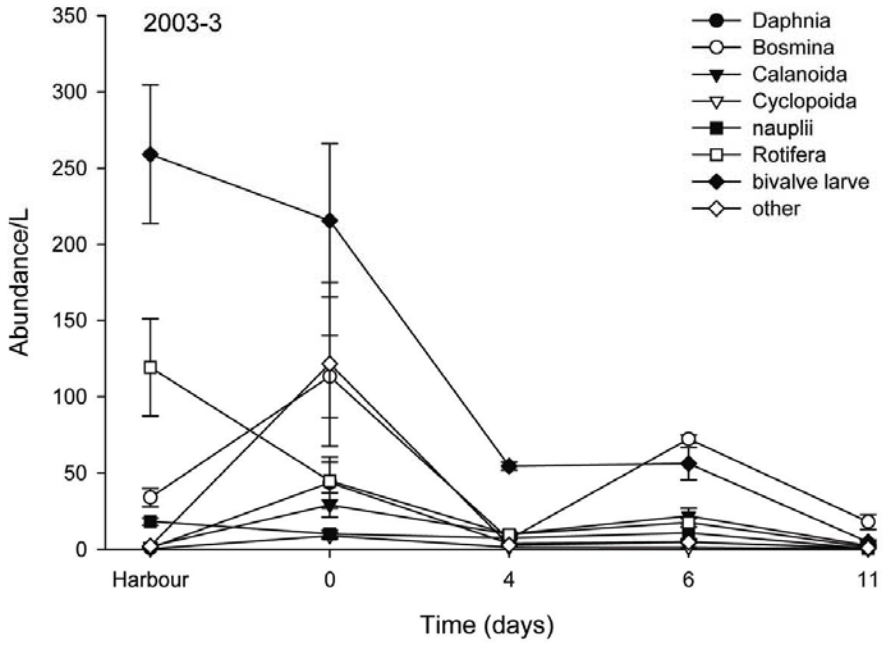


Fig. 4.21: Zooplankton densities over time for Voyage 5.

4.4.3. Discussion

Zooplankton densities for all of the taxa across all voyages tended to decrease as voyage length increased. This trend has been thought to occur as abiotic conditions decline in enclosed ballast tanks as compared to natural systems (MacIsaac et al. 2002); however, this was not the case during every voyage. During voyages 1, 3 and 5, *Bosmina* or Rotifera increased in abundance as the voyage progressed to the upper Lakes. Conditions in the tanks during this time may have been advantageous due to enhanced food sources or a lack of predation. Indeed, it is the former that was responsible for an increase in abundance of a harpacticoid copepod in a transit from the Indian Ocean to the North Sea (Gollasch et al. 2000).

Several NIS were detected in the Great Lakes water loaded as ballast during voyages 1, 2, 3 and 4. The calanoid copepod *Eurytemora affinis* (voyages 1-4), the fishhook waterflea *Cercopagis pengoi* (voyage 2), and the amphipod *Echinogammarus ischnus* (voyage 2). Although these organisms are already present in, and likely originated from, the Great Lakes, inclusion of these species in ballast tanks could pose a risk for invasions to the upper lakes. This finding is also the first record of *Cercopagis* being detected inside a ballast tank, providing the first support for the hypothesis of a ballast-mediated introduction for this species (Cristescu et al. 2001).

Two NIS rotifers that are currently not found in the Great Lakes were also detected in ballast water samples; *Brachionus diversicornis* (voyage 4) and *B. leydigi* (voyage 3). The former was also detected in harbor samples during the same voyage and may constitute a new invasion by this species (as discussed above).

Zooplankton densities decreased with voyage length for most of the taxa detected except on a few occasions. Thus, ballast water tanks may serve as incubators for certain species during favorable conditions. Moreover, NOBOB vessels, along with the domestic fleet, may spread NIS to the upper lakes from populations in the lower lakes.

Chapter 5: Low-Salinity Ballast Water Exchange

The primary goal of Task 3 was to obtain quantitative measures of the efficacy of mid-ocean ballast water exchange (BWE) in reducing abundances of fresh- or brackish-water organisms in ballast tanks.

5.1. Overview

Collaborative research teams from Smithsonian Environmental Research Center (SERC) and Old Dominion University (ODU) conducted shipboard ballast water experiments for these purposes aboard three commercial vessels, including two bulk carriers and one tanker. The first of these vessels, *Berge Nord* (Bergesen d.y. ASA) sailed from Rotterdam, The Netherlands to Sept Iles, Canada in July 2002 (8-day voyage). The second vessel, *Federal Progress* (Canarctic Shipping, a subsidiary of Fednav), sailed from Port Alfred, Canada to Port Esquivel, Jamaica in October 2003 (7-day voyage). The third and final vessel, *Kenai* (Alaska Tanker Company), a double-hulled oil tanker, undertook a 6-day voyage from Benicia, CA (upper San Francisco Bay) to Valdez, AK in June 2004. A summary comparison of these vessels can be found in Table 5.1.

Ballast water exchange is used by commercial vessels in a variety of ways and for a variety of purposes. Ships that are transiting in ballast (*i.e.*, with full ballast tanks) often exchange coastal ballast water in the open ocean, in order to remove coastal biota and sediments that can accumulate. Ships entering the Great Lakes with ballast water are required to have conducted this type of exchange, using either an Empty-Refill or Flow-Through Method, prior to discharge of this ballast water into the Lakes. A similar approach used on "No Ballast on Board" vessels (NOBOBs) is "saltwater flushing" whereby a small amount of ocean water is introduced into empty ballast tanks, allowed to slosh around and resuspend accumulated materials, and then discharged to remove these sediments and organisms.

BWE and saltwater flushing can reduce the concentration of coastal biota in ballast tanks, and the chances of subsequent invasions, in two ways. First, these methods physically remove many of the coastal organisms, replacing these with oceanic organisms that are considered unlikely to colonize coastal ecosystems and especially the Great Lakes. Second, for organisms that originate in low salinity waters, both treatment methods result in a rapid change in salinity that can be fatal. Such "salinity shock" (i.e., toxic effects of salinity) should serve to further reduce the concentration of living/viable organisms delivered by ballast tanks to the Great Lakes from low salinity source ports.

We implemented two approaches to examine the efficacy of ballast water exchange on organisms derived from low salinity sources. (1) We conducted experiments aboard commercial vessels, whereby the effect of BWE on the concentration of coastal organisms was measured, comparing changes in abundance within exchanged ballast tanks to those in paired unexchanged (control) tanks on the same voyage. (2) We compared survivorship of a range of coastal zooplankton, which were exposed to water in simulated (exchange) conditions versus control (unexchanged) conditions, to measure effects of "salinity shock". These experiments consisted of both a shipboard trial and laboratory experiments.

The undertaking of laboratory experiments, which were initiated here, allow us to expand the evaluation of salinity shock to a broader range of organisms. On any one voyage, the organisms available for experiments and the specific salinities experienced are constrained, representing only a small subset of taxonomic groups, species, and environmental (exposure) conditions of interest. Importantly, salinities at brackish water ports are variable and unpredictable, confounding repeated measures of BWE efficacy for low-salinity biota. Further, our experimental shipboard measures indicate that ballast water exchange is highly effective at removing most organisms, making it difficult to measure effects of "salinity shock" for rare (i.e. low density) taxa. In a follow-up study, also funded by the Great Lakes Protection Fund, we are conducting an extensive series of laboratory-based experiments to characterize the effects of exposure to a wide range of organisms from low-salinity source ports to high salinity waters, simulating the effects of ballast water exchange.

Table 5.1: Comparison of ships used in Task 3 Ballast Water Exchange (BWE) experiments. *Hadera* (from a previously funded study; see Drake et al. 2002) is included for the purposes of comparison. ND = not determined.

	Berge Nord	*Federal Progress*	*Kenai*	*Hadera*
Ship type	Motor bulk carrier	Motor bulk carrier	Oil tanker	Motor bulk carrier
Ship specifications	305 m long; gross tonnage = 107,512 MT	177 m long; gross tonnage = 21,469 MT	265 m long; gross tonnage = 60,385 MT	290 m long; gross tonnage = 93,052 MT
Route	Rotterdam, The Netherlands to Sept Iles, Canada	La Baie, Quebec, Canada to Port Esquivel, Jamaica	Benicia, California to Valdez, Alaska, USA	Haifa, Israel to Baltimore, MarylandUSA
Date	2 – 10 July 2002	23 – 30 October 2003	23 – 29 June 2004	23 July – 10 August 1999
Voyage length	8 days	7 days	6 days	19 days
Experiment set up	3 control, 3 exchange	2 control, 2 exchange	2 control, 2 exchange	2 control, 2 exchange
Ballast tank capacity (MT)	8,763 – 11,665	2084 – 2294	2744 – 3005	21,560 – 21,780
Exchange (day #)	3	5	2	10
Exchange procedure	Tank height dropped 20%, followed by 3-volume flow-through replacement	Empty-refill	Empty-refill	Tank overflow for 13h (approx. 1.5-volume flow-through replacement)
Starting salinity (ppt)	31 – 32	11 – 19	15.5	40.2
Incoming ballast salinity (ppt)	35	38	32.4	ND
Final salinity in exchange tanks (ppt)	30 – 35	34	31.5	39
Exchange efficacy (salinity-based)	95-100%	80-82%	94-95%	ND
Exchange efficacy (tracer dye-based)	86-95%	98-99%	91-92%	ND

5.2. Methods

5.2.1. Ballast Water Exchange Experiments

Experimental Design

The purpose of these ballast water exchange experiments was to test the efficacy of ballast water exchange methods (flow-through and/or empty-refill) in removing the original low-salinity coastal water mass and the associated entrained organisms. To conduct these experiments, we first identified commercial vessels that would voluntarily accommodate a research team and allow access to a designated set of tanks during the course of a normal voyage. On each ship, at least two pairs of segregated ballast tanks were identified for sampling. One tank of each pair was designated as the "exchange" tank and underwent a ballast water exchange according to the ship's usual methods during the voyage. The second tank of each pair was the "control" tank. The water in this tank was held for the duration of the voyage. The control tank serves as the baseline against which the exchange tank can be compared. Each tank was sampled twice during the voyage, once before and once after exchange of the designated exchange tanks.

Two different measures were used in these experiments to determine the efficacy of ballast water exchange: (1) The proportion of the original coastal/estuarine **water mass** removed from the tank by exchange, and (2) the proportion of coastal/estuarine **organisms** removed from the tank by exchange. Both these measures are used because the proportion of plankton (virio-, bacterio-, phyto- and zoo-) removed from the tank may differ from the proportion of water removed from the tank. This can result from the behavior of some plankton (e.g. avoiding the pump intake during deballasting) and/or the population dynamics of the plankton in the tanks before and after exchange.

The proportion of the original water mass removed by exchange was estimated through the use of two different physical tracers, including Rhodamine WT dye and salinity. The tracer dye was added to the ballast tanks prior to ballasting and allowed to mix with the ballast water during and after uptake. Samples to measure dye concentration and salinity were collected from all of the designated tanks before and after exchange. Comparisons of post-exchange concentrations of these tracers to the baseline concentrations allowed us to determine the proportion of the water mass removed from the tank by exchange.

The second measure, proportion of coastal organisms removed by exchange, was achieved by sampling the entrained plankton community before and after exchange in both the control and exchange tanks. By comparing coastal plankton densities before and after exchange, and in exchanged vs. unexchanged tanks, it is possible to calculate the proportional change in abundance due to exchange. The more specific methods used to achieve these results are detailed below.

Ship Selection

We identified candidate ships based on a set of pre-determined criteria. The most important of the criteria for ship selection were that the vessel (1) start in a low salinity port and (2) be

engaged in a voyage 5 days in length at a minimum, longer being ideal. Priority was given to ships traveling from a low salinity port of origin (≤5 ppt) to a low salinity destination port in order to most closely mimic conditions of a Great Lakes voyage; however, the salinity of the destination port was ranked as a less important factor given that the hypotheses being tested focused only on the effect of mid-ocean exchange on low salinity water. Criteria were as follows:

- Low Salinity Port. The ship must take on ballast at a low salinity port (salinity reliably less than or equal to 5 ppt).
- Voyage Duration. The voyage must be of sufficient duration to allow complete exchange and sampling. Ideally, this should be 5-10 days.
- Ballast Tanks. The ship must have a minimum of two relatively large ballast tanks, which will be filled at the source port and remain filled for a significant part of the voyage.
- Access to Tanks. The ballast tanks (a) should have multiple access points and (b) must allow access at sea (to permit sampling throughout the voyage). Ideally, this access is from the deck.
- Tank Depth. Tanks should be relatively deep (at least 10 m) and allow access to sample the entire water column with nets, bottles, etc. (i.e., without obstructions), ideally from multiple access points --- see "Access to Tanks" above.
- Exchange. The ship must conduct ballast water exchange during the target voyage, allowing us to stage the exchange schedule (to maintain unexchanged, control tank(s) until near the end of the voyage).
- Research Personnel. The ship will allow a minimum of 3-4 research personnel aboard the ship, sampling at the end points and during the voyage (for safety, we require a minimum of 2 personnel on deck sampling at any one time).
- Repeat visitor. It is critical to identify a ship on a somewhat regular route, allowing us to visit the vessel to help plan the experiment, and to load equipment/supplies in a predictable fashion.
- Ports. Ideally, the participating vessels will be departing a low-salinity European port, destined for the Great Lakes, or vice-versa; however, a vessel departing any low-salinity port and destined for another port on a voyage of sufficient duration to allow complete exchange and sampling will be considered.

Candidate ships were located through the use of multiple sources including maritime exchange reports, the National Ballast Information Clearinghouse (NBIC) database, the U.S. Maritime Administration (MARAD) database, and various colleagues and shipping industry contacts, including shipping companies with whom SERC has previously collaborated on ballast water experiments.

Two primary methods were used to obtain permission to work on candidate vessels. (1) Once an appropriate vessel was identified, SERC staff contacted the appropriate shipping company and requested its participation in targeting the specific vessel(s). (2) SERC staff contacted shipping companies with whom there was already an established working relationship and made a general request for participation. SERC has used both of these methods successfully for this and previous ballast water experiments.

Coordination and Experimental Planning

Coordination with vessel***:*** Upon boarding each ship, the research team met with the captain and ship's officers to finalize which tanks would be used, determine tank access, become familiar with the ship's operations and coordinate the sampling/exchange schedule. This coordination period was not just essential for laying out the sampling schedule, but was also important in providing the captain and officers an opportunity to become fully informed as to what the researchers were doing. Coordination was ongoing with the ship's officers and crew throughout the voyage as well.

Tank Designations***:*** On each ship, 2-3 pairs of segregated ballast tanks were selected for sampling. The appropriate tanks were selected in consultation with the ship's captain and officers. The selected tanks were divided into 2 groups: (1) "Treatment tanks" that underwent ballast water exchange when the vessel entered waters over 200 nautical miles offshore and over 2000 m deep, and (2) "Control tanks" that remained unexchanged until the end of the voyage, or until the last possible moment prior to arrival if exchange was required to enter a port. Every effort was made to ensure that exchange tanks were identical to control tanks with regards to capacity and internal architecture. Details of the tank pairs utilized on all three ships for the ballast water exchange experiments are given in Table 5.2.

Table 5.2. Tank pairs utilized on each ship for ballast water exchange experiments, including the type of tank, location of tank, tank number and side of ship (port or starboard), whether the tank was designated as an exchange or control tank, and the tank capacity (m^3) if completely full.

Ship Name	Tank Type	Tank Pair	Tank Location	Treatment	Tank Capacity (m^3)
Berge Nord	Topside	1 of 2	3 port	Exchange	11,665
			3 starboard	Control	11,665
		2 of 2	5 port	Control	11,648
			5 starboard	Exchange	11,648
Federal Progress	Triangular topside connected via trunk to double bottom	1 of 2	1 port	Exchange	2,294
			3 port	Control	2,084
		2 of 2	1 starboard	Exchange	2,294
			3 starboard	Control	2,084
Kenai	Wing tank, Double hull	1 of 2	2 port	Control	3,052
			6 port	Exchange	2,946
		2 of 2	2 starboard	Control	3,052
			6 starboard	Exchange	2,946

Sampling Locations

Sampling locations (or access points) for a given tank were determined by tank structure and the ease with which equipment could be raised and lowered to a sufficient depth without obstruction. In the case of the *Berge Nord*, this limited us to one location per tank. Two locations per tank were possible on the remaining voyages. Manholes were our preferred type of tank access, however, inclement weather occasionally denied us access to the manholes on the *Federal Progress*, forcing us to use ullage pipes as one of the access points. The number and type of sampling locations used for each ship are presented in Table 5.3.

Table 5.3. Number of sampling locations per tank for each ship and the type of access for each.

Ship	Sampling locations/tank	Type of access
Berge Nord	1	Manhole
Federal Progress	2	Manhole and ullage pipe
Kenai	2	*Tanks 2P & 2S*: 2 Manholes *Tanks 6P & 6S*: 1 Manhole and 1 hatch

Sampling Schedule

Sampling occurred at 2 time points: (1) The pre-exchange time point (T0), as soon after initial ballast water uptake as possible and (2) the post-exchange time point (T1), as soon after ballast water exchange as possible. Tanks were sampled consecutively in pairs, with each tank pair consisting of one treatment tank and one control tank. The control tank served as the reference against which the treatment tank was compared. The control tank and exchange tank of each tank pair were sampled consecutively at each time point. Due to time constraints, different tank pairs were not always sampled on the same day. For example, T0 for Tank Pair A might be sampled on Day 1 of the voyage, while T0 for Tank Pair B would be sampled on Day 2 of the voyage.

Ballast Water Exchange

On all ships, ballast water exchange was conducted once during the voyage in each of the designated exchange tanks using the routine method for that particular vessel. The *Berge Nord* utilized a flow-through exchange method for which tanks were first gravity discharged by 20%, then overflowed until 3 tank volumes had been pumped through the tanks. The *Federal Progress* and *Kenai* both utilized the empty-refill method of exchange. Generally all three vessels adhered to the protocol of conducting ballast water exchange in ocean waters at least 2000 m depth. Two of the three vessels conducted exchange >200 NM offshore. However, the exchange conducted by the *Kenai* occurred closer than usual to the coastal zone (~50 NM offshore) because it was required to bunker in Port Angeles, Washington en route to Alaska and was not able to detour further from shore. Table 5.4 provides details of these exchanges on each ship.

Table 5.4: Details of ballast water exchange by ship, including the type of exchange (flow-through vs. empty-refill), how many days into voyage the exchange occurred, approximate location of the exchange, number of tanks exchanged, and the duration of the exchange from start of the first tank(s) to finish of the last tank(s).

Ship	Type of exchange	Voyage Day #	Location of exchange	# of tanks exchanged	Duration of exchange
Berge Nord	Flow-through	3	~200 NM SW of British Isles	4	24 hours
Federal Progress	Empty-refill	5	~525 NM due west of the South Carolina coast, USA	2	10 hours
Kenai	Empty-refill	2	~50 NM west of the southern Oregon coast, USA.	2	4 hours

Sample Collection and Analysis

Physical Water Tracers*:* Rhodamine WT dye and salinity measurements were both used as physical tracers to measure efficacy of exchange. The quantity of rhodamine dye added to each tank was calculated to result in a target concentration (100 µg/L), such that the dilution caused by a highly effective ballast exchange (i.e. 90% or greater) still produced a measurable end concentration (lower detection limit = ~0.1 µg/L). Calculations were based on individual ballast tank capacity volumes. The dye was added to the ballast tanks in several locations just prior to ballast uptake, to promote mixing throughout the tank. Two replicate whole water samples were collected at T0 and T1 using a 2.2L Niskin bottle at surface, mid-, and/or deep depths to measure concentrations of rhodamine dye. [The overall depth of the tank determined exact depths. A "deep" depth was determined to be either as deep as the equipment could reach or, if the tank bottom was accessible, to within 1 m of the bottom.] Samples were analyzed in SERC's lab using a Turner Designs 10-AU Fluorometer fitted with appropriate excitation and emission filters, employing standard methods (Wilson et al. 1986). Percent removal of the original water mass was then calculated from the concentrations obtained from these analyses (see "Calculating Exchange Efficacy" below).

Vertical salinity and temperature profiles of the water were measured at multiple locations in each tank for each sampling event. Profiles were collected by lowering a YSI 85/100 FT water quality meter probe from the top to the bottom of the tank. These profiles allowed assessment of stratification of ballast water within the tank. On the *Federal Progress*, it was not possible to lower the YSI probe into the ullage pipe due to the pressure of water up the pipe; therefore, water quality profiles were not possible for these locations. Instead, a salinity and temperature reading was taken at the ullage pipe locations using a handheld refractometer and handheld thermometer, respectively.

Salinity of the mid-ocean exchange source water was also measured by collecting samples of incoming ballast water from an intake pipe or on-deck fire extinguisher valve during ballast water exchange. This measurement was generally taken using a hand-held refractometer. Comparison of coastal and mid-ocean source water salinities against the final salinity of the

ballast water in the exchanged tanks also permitted a second method of calculating exchange efficacy (see below).

Zooplankton: Plankton net samples were collected at both time points (T0 and T1) to determine the abundance of coastal organisms in the tanks before and after exchange. These samples were collected using one of two methods:

(1) Vertical net tows -- On the *Berge Nord* and *Kenai*, 3 vertical net tows were conducted with a plankton net (0.30 m diameter, 80-μm mesh) at each location during each sampling event. The net was lowered to within 1 m of the tank bottom, or as low as possible without encountering obstruction, and pulled vertically through the water column. This protocol resulted in 6-m net tows on the *Berge Nord* and 12-m net tows on the Kenai.

(2) Pneumatic pump -- On the *Federal Progress,* tank structure and tank access prevented the execution of vertical net tows. As a result, zooplankton samples were collected using a pneumatic pump fitted with 1" inner-diameter reinforced hose. A weighted length of hose was extended into the tank to a depth of at least 1 m off the bottom (1 m in the manholes, 3.4 m in the ullage pipe) and water pumped from the tank through the hose for a period of 8 minutes per replicate. Three replicate samples were collected at each location at each time point. In the case of manhole access, the outflow passed through a second length of hose into an 80-μm plankton net suspended in the surface waters of the tank. In the case of ullage pipe access, the outflow passed through a second length of hose into an 80-μm plankton net submerged in a large plastic bucket.

Regardless of the collection method, each zooplankton sample was transferred from the cod end to a 125 ml Nalgene bottle and preserved with 10% formalin in seawater. All zooplankton samples were transferred to 70% ethanol within one month of return to the SERC laboratory. The samples were then sorted and enumerated by taxa using either a folsom plankton splitter or Stempel pipette, and a zoom stereo microscope. Density (# individuals/m^3) was then determined for the various taxa.

From the above analyses, it is possible to measure zooplankton abundance in the tanks across time, as well as the change in concentration and percent removal of representative coastal zooplankton during exchange. For the purposes of this report, the representative coastal zooplankton will be referred to as target taxa. Target taxa were selected based on the following criteria:

(1) They are easily identifiable.

(2) They are not found in the mid-ocean regions where the exchange took place. Focusing analyses on coastal forms of zooplankton reduces the likelihood of compensatory changes in abundance (i.e. introducing organisms) during the course of the exchange process.

(3) They occur at pre-exchange densities of ≥ 20 individuals/m^3. Concentrating on the most abundant forms provides increased resolution to measure changes in species abundance as a result of exchange

Phytoplankton*:* Water samples for phytoplankton analyses were collected from tanks using a 2.2L Niskin bottle. The Niskin bottle was lowered to 1 m below the water's surface, then triggered to capture a whole water sample. [Note: Tank structure on the *Kenai* allowed collection of samples at 1-2 additional depths per location, 7 m and 12-15 m.] This procedure was repeated for a minimum of two locations within each tank for each time point (T0 and T1). After collection, water samples were stored in the dark at 4°C until processing.

A number of approaches were used to examine phytoplankton dynamics within ballast tanks:

(1) Chlorophyll-*a* was used as a general indicator of total population biomass. This parameter is commonly used during oceanographic studies to assess phytoplankton dynamics. The immediate degradation products of chlorophyll-*a* (phaeophytin-*a* and phaeophorbide-*a*), collectively known as phaeopigments, were also used to assess the relative proportion of living to dead phytoplankton cells.

(2) HPLC-pigments were measured to assess class (or group)-specific phytoplankton dynamics.

(3) Phytoplankton species were enumerated to assess individual phytoplankton dynamics.

The specific methods for these analyses are outlined below:

Chlorophyll *a* and phaeopigment determination. Chlorophyll *a* (chl *a*) samples were collected by filtering 300-1000 ml of ballast water onto 47 mm-diameter glass fiber filters (GF/F Whatman) at a vacuum pressure of 100 mm Hg. Filters were wrapped in foil and stored in liquid nitrogen for the duration of the voyage; upon return to the laboratory, filters were stored at −85°C until the chl *a* on the filters was extracted in acetone and measured fluorometrically (Parsons et al. 1984). Phaeopigment concentration was quantified by acidifying the chl *a* samples with 5% hydrochloric acid and again determining the sample's fluorescence.

HPLC pigment determination (Dr. G. DiTullio, Hollings Laboratory, South Carolina). Seawater aliquots (300-1000 ml) from ballast tanks were filtered onto Whatman GF/F filters and frozen in liquid nitrogen until they were processed in the laboratory. Filters were cut up and homogenized with 1.5 ml 90% acetone and were allowed to extract for 2-4 hr at -20^{o} C. The extracts were then centrifuged at -4^{o} C and filtered. Samples were injected into an HP-1050 Liquid Chromatograph using an autosampler. The system was equipped with photodiode array and fluorescence detectors. The gradient elution program was a modification of the ammonium acetate pairing method (Wright et al. 1991). Details on the method were described elsewhere (DiTullio and Geesey, 2002). Standards were prepared from algal cultures grown in Dr. G. DiTullio's laboratory. The coefficient of variation from replicate standard injections was < 3%. Peak spectra from each eluted peak were compared to the stored library spectra to confirm peak identity and determine relative peak purity.

Phytoplankton Species identification (Dr. H. Marshall, Old Dominion University; Dr. R. Lacouture, National Academy of Sciences). Ballast water samples (250 ml) were preserved with acid Lugols solution and passed through a series of settling/siphoning steps to produce a 40 ml concentrate. From each concentrate, sub-samples of known volumes were removed for subsequent analysis with an inverted plankton microscope (Zeiss). These sub-samples were placed in the inverted plankton microscope settling chambers, allowed to settle for 48 hours, and then examined with light microscopy. Ten randomly selected fields were examined for

phytoplankton initially at 315X magnification, and at higher magnification if necessary (e.g. 500X). The phytoplankton composition and abundance were determined, with a minimum cell count of 200 cells. If 200 cells weren't recorded after examining 10 fields, additional fields were examined until this level was reached. Taxa were determined to the lowest taxonomic level feasible with light microscopy. This combination of random fields and minimum cell count was estimated to result in a level of 85% accuracy estimate of abundance.

Bacteria and viruses: Water samples for analysis of microorganisms (bacterio- and virio-plankton) were collected together with phytoplankton samples (see above). Because of the difficulty in identifying these organisms to species, several 'bulk' microbial metrics were used to assess population dynamics within ballast tanks. They include total bacteria abundance, total virus-like-particle (VLP) abundance and microbial biomass. The specific methods for these analyses are outlined below:

Bacteria enumeration. Bacteria samples were fixed in formaldehyde solution (final concentration 2.7%) and stored in the dark at 4°C until they were enumerated via flow cytometry. Analyses were done using a Becton Dickinson FACScan flow cytometer equipped with a 15 mW, 488 nm, air-cooled Argon ion laser. Simultaneous measurements of forward light scatter, 90degree light scatter, and green fluorescence were made on all samples. PicoGreen (Molecular Probes, Inc., Eugene, Oregon), a DNA-specific probe that emits in the green wavelengths when excited with 488 nm light, was used to detect and enumerate bacteria. Detectors (photomultiplier tubes) were in log mode and signal peak integrals were measured. The volume of sample analyzed by the FACScan was determined gravimetrically using an A-160 electronic balance (Denver Instruments Co.) whereby each sample was weighed prior to analysis and immediately after the analysis was terminated. All samples were run at a low flow rate setting (approximately 20 $\mu l\ min^{-1}$).

Virus-like particle enumeration. Virus-like particle (VLP) samples were fixed in formaldehyde solution (final concentration 2.7%) and stored in the dark at 4°C until they were counted using the method of Noble and Fuhrman (1998). Upon return to the laboratory, samples were diluted with 0.1 μm-filtered distilled, deionized water. Next, diluted samples were filtered onto 0.02 μm-pore size Anodisc filters (Whatman International Ltd., Maidstone, England) and stained in the dark for 20 min at room temperature with a working solution of the nucleic acid stain Syber Green™ (Molecular Probes, Inc., Eugene, Oregon). Filters were dried on absorbent paper, placed on microscope slides with antifade solution, and stored in the dark at −85°C until the VLPs were counted. Filters were randomly chosen (in groups of two), thawed in the dark at room temperature for ca. 5 minutes, and VLPs were counted using an Olympus BX50 System Microscope with a BX-FLA epifluorescence attachment. For each set of filters prepared, two control filters were prepared using only 0.02 μm-filtered distilled, deionized water and their average VLP count was subtracted from values determined in field samples.

Microbial biomass. One liter of seawater was filtered onto 47 mm-diameter glass fiber filters (GF/F Whatman) at a vacuum pressure of 100 mm Hg. Filters were wrapped in aluminum foil and stored in liquid nitrogen on the ship; upon return to the laboratory, filters were stored at −80°C until they were placed into a modified (White et al. 1979) Bligh and Dyer (1959) solution (methanol-chloroform-buffer) to extract lipids. From an aliquot of the extracted bulk lipid,

microbial biomass was determined by oxidizing the phosphorus-containing cell-membrane lipids, thus releasing inorganic phosphate, then performing an inorganic phosphate determination (Dobbs & Findlay 1993). Finally, phosphate concentrations were converted to carbon equivalents assuming 100 μmoles of P per gram of C (Dobbs & Findlay 1993).

Calculating Exchange Efficacy: Exchange efficacy for all measures (physical and biological) was calculated as the change in concentration that occurred only as a result of the exchange process. This was calculated by comparing the change in the exchange tank with that in the control tank (Fig. 5.1). This method is the most useful means of comparing the exchange efficacies among different measures (i.e. physical tracer vs. zooplankton) when units of measure make comparison difficult (i.e. ppt vs. μg/L vs. individuals/m^3). It is also useful for comparing results among different voyages where variations in starting concentrations can complicate analyses.

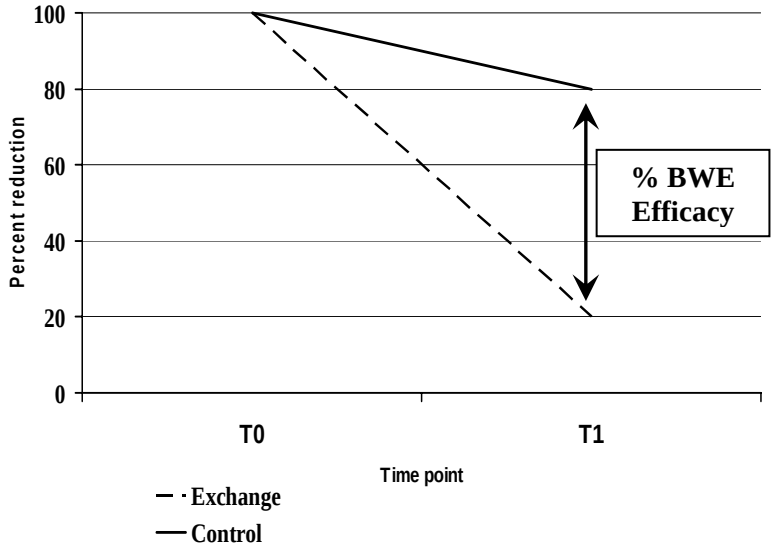


Fig. 5.1: Graphic representation of the calculation of ballast water exchange (BWE) efficacy for biological and physical tracers. Efficacy is estimated as a function of change in concentration over time (T0 toT1) in the exchange tank relative to that in the observed in the control tank.

Exchange Efficiency for Physical Tracers

Salinity

To calculate exchange efficacy based on changes in salinity, the expected change in salinity ($\Delta S_{Expected}$) if the ship achieved 100% exchange efficacy was first determined:

$$\Delta S_{Expected} = S_{mid\text{-}ocean} - S0;$$

where S0 = salinity in the tank at T0 and

$S_{mid\text{-}ocean}$ = salinity of incoming mid-ocean water during exchange.

The observed change in salinity following exchange ($\Delta S_{Observed}$), is then calculated as follows:

$$\Delta S_{Observed} = S1 - S0;$$
where S1 = salinity in the tank at T1 (i.e. after exchange)

The actual percent change in salinity, which will also serve as the exchange efficacy or $S_{Effic,}$ is then calculated as:

$$S_{Effic} = (\Delta S_{Observed} / \Delta S_{Expected}) * 100$$

Rhodamine Tracer Dye

The exchange efficacy based on concentrations of rhodamine tracer dye is calculated as the percent change in the dye concentration at T1. Only T1 measurements are used for this calculation to allow that the dye had sufficient time to fully mix. Since the quantity of dye added to each tank was calculated to achieve the same target concentration for all (~100ug/L), each tank was assumed to begin the experiment with an equal concentration of rhodamine dye. The equation is as follows:

$$R_{Effic} = [(R_{C1} - R_{Ex1}) / R_{C1}] \times 100;$$
where R_{C1} = the concentration of rhodamine in the control tank at T1 and
R_{Ex1} = the concentration of rhodamine in the exchange tank at T1 (i.e. after exchange).

Exchange Efficiency for Biological Tracers

Percent change

For target taxa of coastal zooplankton and phytoplankton, the percent change in concentration (Δ) within a tank is calculated as follows:

$$\Delta = [(T1-T0)/T0]*100$$
where T0 = the initial concentration and
T1 = the concentration following exchange.

This calculation is performed for each tank in a tank pair (exchange and control) to gain a relative measure of the changes that have occurred in each tank by T1. If a very large decrease in concentration occurs in the control tank by T1, it is simply not possible to assess effects at exchange, because there is not a sufficient margin left in the control to estimate additional decline due to exchange. For the purposes of this study, a percent change less than or equal to -85% for a target taxon ($\Delta C \leq -85\%$) was selected as the threshold for calculating efficacy (as below).

Ballast Water Exchange Efficiency

If the percent change in the control tank was above the threshold of -85% ($\Delta C > -85\%$), the ballast water exchange efficacy (Ex_{Effic}) was calculated as relative change for the exchanged tank as follows:

$$\% r = (T1/T0)*100;$$

where % r = percent of target taxa density remaining in tank at T1,
T0 = the initial concentration, and
T1 = the concentration following exchange.

$$Ex_{Effic} = [(C_{\% r} - X_{\% r})/(C_{\% r})]*100;$$

where $X_{\% r}$ = fraction remaining in the exchange tank and
$C_{\% r}$ = fraction remaining in the companion control tank.

Ballast water exchange efficacy is not calculated for target taxa which do not exceed the -85% threshold in the control tank.

5.2.2. Salinity Tolerance Experiments

Shipboard Experiment

A shipboard salinity tolerance experiment was designed to test the relative effects of salinity stress (mortality associated with the exposure of organisms to high salinity water during mid-ocean ballast exchange) on a target organism. Without being able to isolate organisms in the tanks and observe them pre- and post-exchange, it is not possible to tell whether declines in their abundance are due to natural attrition, removal by exchange, or mortality due to salinity stress. For this experiment we collected a number of specimens of a target organism from a tank containing the original, coastal ballast water; isolated these specimens in plankton "cages"; and placed an equal number of "cages" in one control/exchange tank pair following exchange. The cages were removed after 24 hours for assessment of survivorship/mortality of the target organism. From the resulting data, it is possible to determine the proportion of individual organisms surviving the salinity increase in the exchange tank vs. the proportion surviving the control tank conditions. Details of the methods of this experiment are provided below.

The shipboard experiment was conducted aboard the *Berge Nord*. The target organism was selected from supplementary plankton tows conducted in non-experimental tanks. Target selection was based on size (the largest possible) and abundance (at least 100-200 organisms, to provide 5-10 organisms per cage for a minimum of 20 cages).

Zooplankton samples were kept in clean plastic buckets aerated with aquarium air pumps for the duration of the sorting process. Potential target organisms were evaluated and sorted on board the ship using a stereo dissecting microscope. The final target, a mysid shrimp, was sorted into Toby Teaboy tea infusers, which served as "cages" for the zooplankton. Each cage held 5 to 6 organisms.

Cages were wired shut with fine gauge wire to prevent their coming open in the ballast tanks. They were then distributed evenly between 2 depths (1m and 3m) in each tank of a single tank pair (1 exchange tank, 1 control tank). The number of cages per tank was 13. Cages were secured in each tank by a line fixed to a wooden pole (so that the rope would not wrap around the ladder and prevent removal of the cages from the ballast tanks). Cages were deployed in the tanks immediately following exchange and were removed for observation 24 hours later.

When removed from the tanks, the plankton cages were placed in a bucket of ballast water from their respective tanks and aerated with an aquarium air pump. Individual cages were sufficiently watertight to temporarily hold water when removed from a bucket. The wires were cut off the cages, which were then turned on one end and sprayed from the outside (using a squirt bottle filled with ballast water) to collect organisms at the bottom. The bottom half of each cage was then placed in a 4 oz plastic container containing ballast water. From this set-up, the organisms were pipetted into a petri dish for microscopic examination and enumeration of the number alive, dead and/or moribund. These specimens were then transferred into trays of individual wells labeled with cage numbers and later preserved in 70% ethanol in glass shell vials as voucher specimens for taxonomic identification in the SERC lab.

5.2.3. Laboratory Experiments

Laboratory-based salinity tolerance experiments were conducted to examine the effect of simulated flow-through and empty-refill methods of ballast water exchange on the survival of a wide range of zooplankton species found in low salinity waters. These experiments provided an opportunity to evaluate the response to salinity stress across a broader range of organisms than is possible in shipboard trials. They also allowed us to simultaneously mimic both of the common methods of mid-ocean exchange for a given organism, thus greatly expanding the amount of information that can be obtained from a single trial.

Each trial involved a target zooplankton species collected from a low salinity habitat in the upper Chesapeake Bay. There were two treatments (flow-through and empty-refill) plus a control for each target. All treatments involved exposure to 34 ppt seawater, and survivorship was measured every hour for the first three hours and, following this, at 24 hour intervals. Each trial ran for a period of 48 hours.

While it is known that there is a wide range of salinity tolerance among taxa in the coastal zooplankton communities, it remains unclear with what frequency species from low salinity waters are killed by exposure to high salinity. The results of these experiments will help us begin to fill in the gaps in this regard.

Zooplankton species were collected from low salinity waters (0-10 ppt) at the Port of Baltimore, Maryland, U.S.A. and surrounding low salinity/freshwater sources. Target species were selected from the samples based on abundance and size, and one species was examined per trial. An organism was considered to be a suitable target if it was a non-resting stage of ample size and abundance. A total of 120 organisms was required per trial (5-10 organisms per replicate).

The experiment consisted of two treatments as follows:

1. To simulate empty-refill ballast water exchange and saltwater flushing, salinity was raised directly from the original (source) concentration to 34 ppt in a single step; and
2. To simulate flow-through ballast water exchange, salinity concentrations were elevated systematically over time (e.g. 10 ppt every hour to 34 ppt).

A control was also conducted, in which organisms were exposed to the same handling as those in the treatments, but were maintained at the ambient salinity for the duration of the trial. Four replicates were conducted for each treatment and for the control, with between five and ten individuals being placed into each replicate dish.

Details of the treatments and the timeline of the experiment are presented in Table 5.5. Experiments were begun by transferring all replicates into clean filtered water of the appropriate salinity. Salinity 'step-ups' were carried out for the flow-through treatment to gradually elevate the salinity to 34 ppt. This required transferring the flow-through replicates into new water at regular intervals. In order to insure all replicates underwent the same degree of handling, replicates from the empty-refill treatment and the control were also transferred into clean filtered water of their respective salinities until the step-ups were completed.

Replicates were maintained in an incubator in individual culture dishes at constant temperature (the ambient temperature at which organisms were collected), on a 24 hour dark cycle. The trials were kept in the dark to mimic the conditions in a ballast tank. However, this darkness was necessarily interrupted during periods of observation to record survivorship.

Survivorship was noted at each transfer time and one hour following the final transfer. All replicates were again observed at 24 hours and 48 hours after the initiation of the trial. The trial was terminated after 48 hours and specimens vouchered in shell vials in 70% ethanol.

Table 5.5. Salinity tolerance laboratory experiment timeline and treatments, and the protocol for each treatment at each time point. All experiments were 48 hours long.

Day	Time point	Treatment		
		Control	Empty-Refill	Flow-Through
0	**T0** *Start*	• Organisms picked from source water; • Transferred to culture dishes containing filtered source water at ambient salinity.	• Organisms picked from source water; • Transferred to culture dishes containing filtered source water at 34 ppt.	• Organisms picked from source water; • Transferred to culture dishes containing filtered source water at 14 ppt.
0	**T1** *=T0 + 1 hour*	• Survivorship assessed; • Organisms transferred into clean filtered source water at ambient salinity.	• Survivorship assessed; • Organisms transferred into clean filtered source water raised to 34 ppt.	• Survivorship assessed; • Organisms transferred into clean filtered source water raised to 24 ppt.
0	**T2** *=T1+ 1 hour*	• Survivorship assessed; • Organisms transferred into clean filtered source water at ambient salinity.	• Survivorship assessed; • Organisms transferred into clean filtered source water raised to 34 ppt.	• Survivorship assessed; • Organisms transferred into clean filtered source water raised to 34 ppt
0	**T3** *=T2 + 1 hour*	• Survivorship assessed; • Dead removed; • No transfers.	• Survivorship assessed; • Dead removed; • No transfers.	• Survivorship assessed; • Dead removed; • No transfers.
1	**T24** *=T0 + 24 hours*	• Same as T3	• Same as T3	• Same as T3
2	**T48** *=T0 + 48 hours*	• Survivorship assessed; • Remaining organisms vouchered in 70% ethanol.	• Survivorship assessed; • Remaining organisms vouchered in 70% ethanol.	• Survivorship assessed; • Remaining organisms vouchered in 70% ethanol.

5.3. Results

5.3.1. Ballast Water Exchange Experiments

Voyage 1: ***Berge Nord*** (Bergesen d.y. ASA), Rotterdam, the Netherlands to Sept Isles, Canada, 1 – 10 July 2002.

Physical Water Tracers

Temperature and Salinity Profiles. Initial measurements for both of these parameters were comparable between control and treatment tanks at all depths (Fig. 5.2a and b) and were similar to Port of Rotterdam water (at ballast intake salinity was much higher than anticipated at 27 ppt and temperature was 17 ^{o}C). Measurements recorded post-exchange indicate that salinity remained virtually the same in the control tanks (3 starboard and 5 port), but increased approximately 5 ppt with open-ocean exchange in the treatment tanks (3 port and 5 starboard). Temperatures measured post-exchange decreased approximately 1.0^{o}C -1.5^{o}C in the control tanks, reflecting colder ocean temperatures in the North Atlantic. Post-exchange temperatures in the exchanged tanks were approximately 2.5 ^{o}C colder than temperatures measured in the control tanks. There were no signs of stratification in the tank to depths of 6 m in either tank pair. Tank configuration presented obstructions that prevented the lowering of sampling equipment deeper than 6 m.

A sample of incoming seawater was obtained from the engine room during the ballast exchange procedure. The salinity of this ballast water was 35.4 ppt. Exchange efficacy based on salinity alone was calculated by comparing final salinity measurements in the exchanged tanks to the salinity of the incoming ballast. These calculations yield an exchange efficacy of 94.7% for Tank #3P and 100.1% for Tank #5S.

Rhodamine Dye Concentrations. The inert tracer dye Rhodamine WT was added to the ballast tanks (#3 port, #3 starboard, #5 port, and #5 starboard) prior to ballast uptake in Rotterdam. At T1, dye concentrations in the control tanks were near the expected concentration of Rhodamine dye (mean expected concentration = 66.9 ± 0.01 μg/l, calculated based on actual volume of ballast in tank and volume of dye added); Tank 3 = 67.5 ± 0.8 μg/l; Tank 5 = 63.7 ± 0.8 μg/l) and the dye concentrations in the treatment tanks dropped to 9.2 ± 13.0 μg/l (#3 port) and 3.0 ± 0.5 μg/l (#5 starboard) (Fig. 5.3). This is equivalent to ballast water exchange efficacies of 86.4% and 95.3%, respectively.

Biological Tracers

Zooplankton Analyses. Table 5.6 shows total zooplankton densities for all taxa identified in pre- and post-exchange samples. Pre-exchange samples were dominated by the calanoid copepod *Temora longicornis.* Overall, the density of organisms decreased following exchange. The primary exceptions to this were the Cyclopoida and the harpacticoid copepod *Microsetella* sp., both of which increased in the exchange tanks at T1.

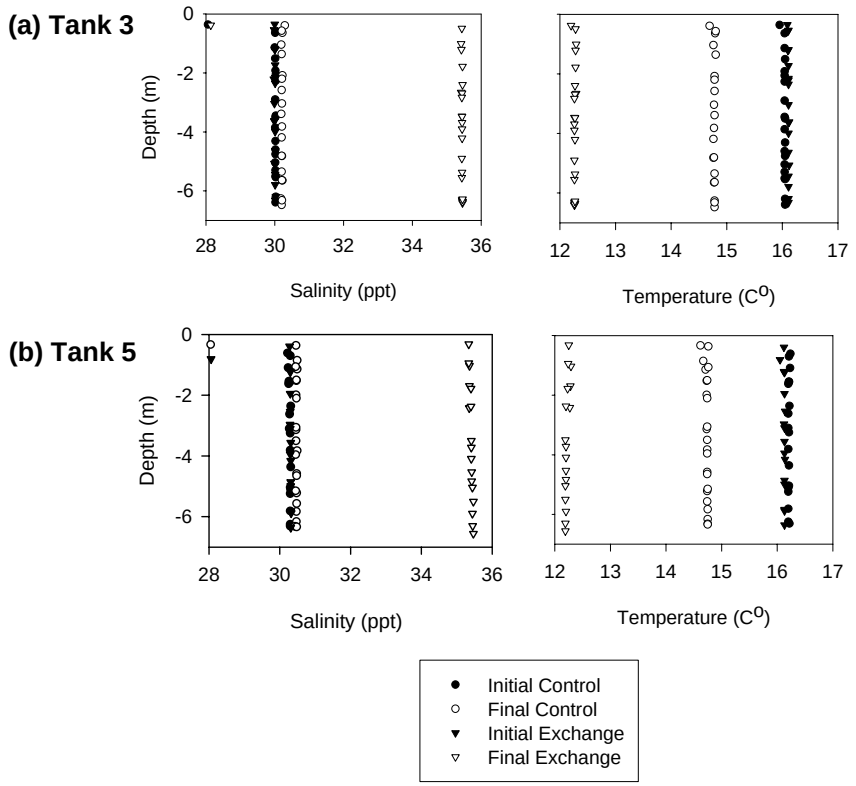


Fig. 5.2: Vertical profiles for salinity (ppt) and temperature (°C) in the *Berge Nord*'s (a) Tanks 3 port (exchange) and 3 starboard (control) and (b) Tanks 5 port (control) and 5 starboard (exchange).

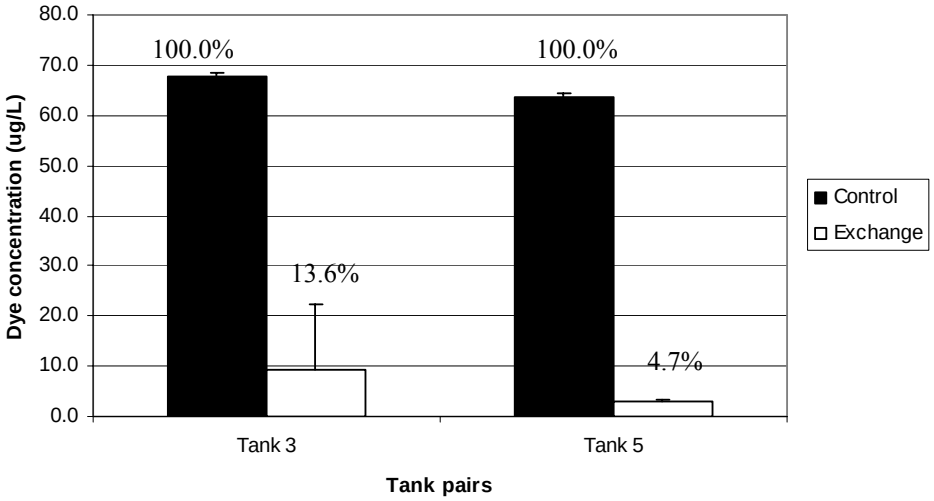


Fig. 5.3: Concentration of rhodamine dye remaining in *Berge Nord* Tank pairs 3 and 5 at T1 (post-exchange). Percent values shown above bars represent the percent dye remaining in the tanks at T1. This equates to ballast water exchange efficacy values of: Tank 3 = 86.4%, Tank 5 = 95.3%. Error bars are one standard deviation.

Target taxa identified for Tank 3 were *Temora longicornis,* the harpacticoid copepod *Euterpina acutifrons*, barnacle nauplii, cyprids, Cumacea, decapod zoea, Mysidaceae, and spionid

polychaetes. Target taxa identified for Tank 5 were *Temora longicornis*, *Euterpina acutifrons*, barnacle nauplii, cyprids, and spionid polychaetes.

Final densities were similar between control and exchange tanks for all targets, with the exception of mysids (Tank 3 only), and *Euterpina acutifrons* and spionid polychaetes (Tanks 3 and 5). T1 densities of *Euterpina acutifrons* and spionids were actually higher than T0 densities in the Tank 5 control. This same result occurred for spionids and mysids in the Tank 3 control.

Figures 5.4(a) and 5.5(a) illustrate the comparative percent change in target taxa density that occurred in the control tank versus the exchange tank of each tank pair at T1. These graphs indicate a large percent change (range: -65% to -100%) for all targets in the exchanged tanks. However, it should also be noted that a number of the taxa in the Tank 3 control (barnacle nauplii, cumaceans, and decapod zoea) fell below the -85% threshold for being able to reliably determine ballast water exchange efficacy. Figures 5.4(b) and 5.5(b) illustrate the associated ballast water exchange efficacies for each target taxon in each of the exchange tanks. Exchange efficacies for all targets were high (range: 84.1% to 100%).

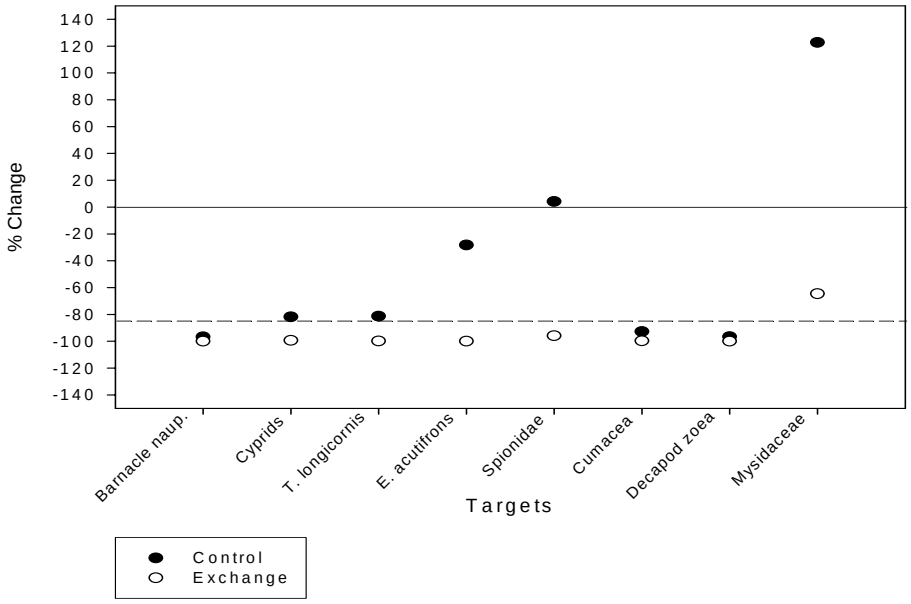
Table 5.6. Total zooplankton densities [individuals/m^3], per tank, per time point for the *Berge Nord*. Data presented as follows: density (standard deviation) ; "n"= number of replicate samples. Target (coastal) taxa are identified with an asterisk (*) following the taxon name: * = *Target organism, Tanks 3 and 5*, ** = *Target organism, Tank 3 only.*

Taxon	Tank 3 Control		Tank 3 Exchange		Tank 5 Control		Tank 5 Exchange	
	T0	T1	T0	T1	T0	T1	T0	T1
Copepoda	**n=3**	**n=3**	**n=3**	**n=3**	**n=3**	**n=3**	**n=3**	**n=3**
Calanoida - *Temora longicornis****	2921 (1522.6)	546 (194.7)	6542 (5433.7)	10 (2.3)	631 (207.8)	550 (163)	1725 (584.1)	14 (9)
Other Calanoida	1089 (501.7)	797 (139.1)	1629 (504.9)	1023 (231.4)	1064 (6.9)	835 (126.5)	917 (71.7)	935 (261.9)
Caligidae (parasitic cop)	0 (0.3)		0 (0.3)					
Copepod nauplii	699 (335.7)	364 (90.1)	1140 (190.2)	1946 (588)	401 (107)	339 (27.3)	217 (24)	2001 (917)
Cyclopoida	46 (25.3)	21 (17.8)	45 (32)	1420 (444.6)	19 (17.9)	25 (7.5)	4 (2)	1644 (107.6)
Harpacticoida - *Euterpina acutifrons**	58 (32.9)	42 (25.2)	130 (146.3)		18 (3.8)	38 (4.7)	31 (9.1)	
Harpacticoida – *Microsetella* sp.			9 (15.7)	948 (171.3)	4 (6.3)			1142 (508.2)
Other Harpacticoida	15 (2.8)	2 (2.8)	11 (9.5)		8 (12.5)	7 (7.1)	4 (1.4)	
Poecilostomatoida	0 (0.3)	0 (0.3)	1 (0.3)	2 (2.3)	3 (4)	2 (1.6)	1 (0.5)	2 (1.7)
Cirrepedia								
Barnacle nauplii*	207 (128.2)	7 (2.7)	765 (380.5)		32 (23.4)	5 (0.9)	43 (14.2)	
Cyprids*	189 (74.3)	34 (9.8)	368 (125)	2 (2.1)	37 (6.3)	28 (9.1)	99 (15.4)	9 (1.7)
Other Crustaceans								
Caprellidae	1 (0)		2 (1.4)					
Gammaridae	4 (2.8)	0 (0.3)	5 (2.5)	0 (0.3)	0 (0.3)	1 (0.9)	2 (0.5)	1 (0.5)
Hyperidae				3 (1.6)				5 (2.2)
Caridean	0 (0.3)	1 (0.9)	1 (0.9)	1 (0.8)	2 (3.1)		1 (0.9)	1 (1.1)
Cladocera	4 (3)		6 (4.1)	6 (2.1)	1 (0.5)		1 (0.5)	27 (4.7)
Cumacea**	55 (17.8)	4 (1.1)	122 (33.1)	0 (0.6)	9 (3.3)	3 (2)	11 (3.5)	1 (0.3)
Decapoda	9 (5.3)	5 (2.8)	17 (6)	0 (0.3)	10 (1.3)	7 (3.4)	6 (3.1)	2 (1.1)
Decapoda zoea**	27 (18.5)	1 (1.1)	22 (3.8)		2 (0.6)	2 (1.4)	4 (1.9)	1 (0.8)
Mysid**	20 (9.7)	44 (14.2)	14 (2.8)	5 (4.4)	13 (3.1)	18 (3.1)	13 (5.7)	3 (1.4)
Ostracoda				1 (1.6)				
Mollusca								
Gastropoda	1 (0.9)	0 (0.6)	0 (0.3)	3 (2.5)	0 (0.3)	0 (0.6)	1 (1.6)	1 (0)

Table 5.6. -- continued. Total zooplankton densities [individuals/m^3], per tank, per time point for the *Berge Nord*. Data presented as follows: density (standard deviation); "n"= number of replicate samples. Target (coastal) taxa are identified with an asterisk (*) following the taxon name: * = *Target organism, Tanks 3 and 5, ** = Target organism, Tank 3 only*.

Taxon	Tank 3 Control		Tank 3 Exchange		Tank 5 Control		Tank 5 Exchange	
	T0	T1	T0	T1	T0	T1	T0	T1
Annelida	**n=3**	**n=3**	**n=3**	**n=3**	**n=3**	**n=3**	**n=3**	**n=3**
Polychaeta-Spionidae*	26 (25.2)	27 (4.9)	40 (14.1)	2 (0.5)	35 (13.9)	37 (9)	22 (2.2)	1 (0.5)
Other Polychaeta	1 (0.9)		1 (1.1)	0 (0.3)	0 (0.6)		1 (0.9)	
Other Taxa								
Chaetognatha - *Sagitta* spp	6 (2.8)	1 (0.6)	9 (2.7)		2 (1.7)		5 (2.1)	0 (0.6)
Ctenophore	2 (1.3)	3 (0.8)	1 (0.8)		4 (2.2)	4 (3.3)	6 (1.4)	
Medusae	5 (2.6)	0 (0.3)	2 (1.1)	0 (0.3)	7 (5.5)	1 (0.6)	4 (1.7)	0 (0.3)
Fish Larvae	0 (0.6)		0 (0.3)					

(a)



(b)

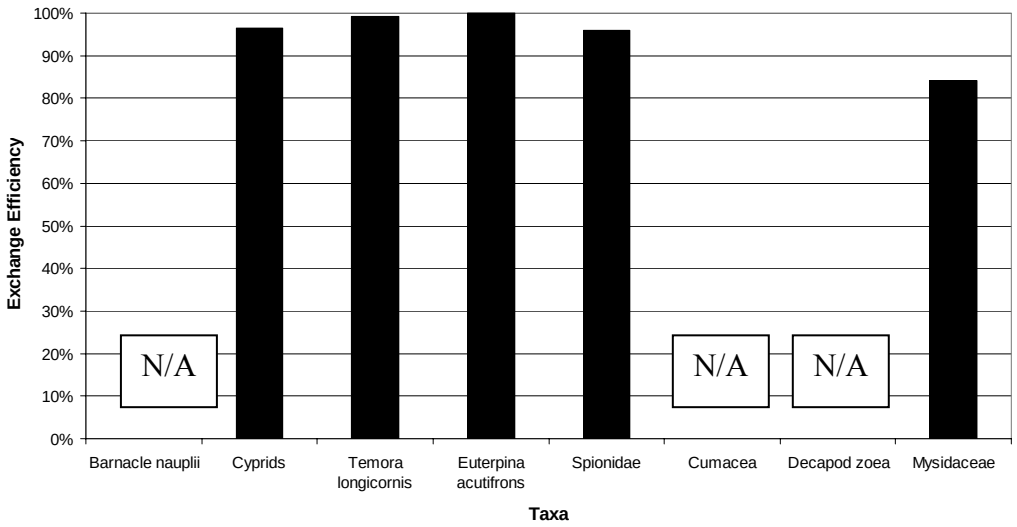
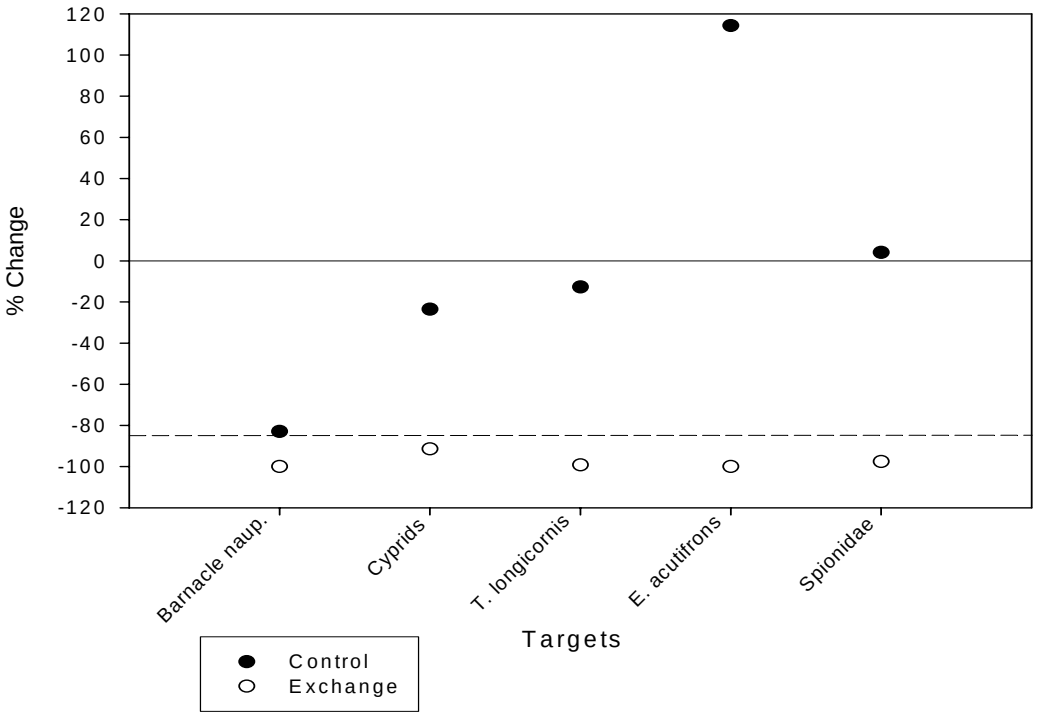


Fig. 5.4: (a) Percent change in density of target zooplankton taxa following exchange (T1) for Tank 3 control vs Tank 3 exchange on the *Berge Nord*. The dashed line represents the -85% threshold for target taxa in the control tank. Ballast water exchange efficacy is not calculated for a target if $\Delta C \leq -85\%$. (b) Ballast water exchange efficacies (%) for target zooplankton taxa on the *Berge Nord* , for Tank 3 exchange. "N/A" indicates taxa for which the percent change in density in the control tank did not meet the -85% threshold (i.e. the taxa decreased in abundance by >85% in the control tank).

(a)



(b)

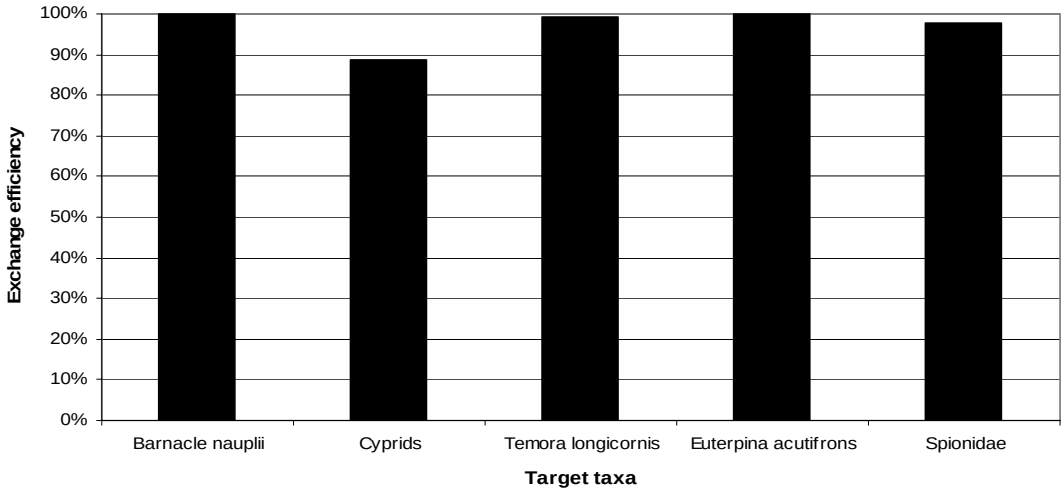


Fig. 5.5: (a) Percent change in density of target zooplankton taxa following exchange (T1) for Tank 5 control vs Tank 5 exchange on the *Berge Nord*. The dashed line represents the -85% threshold for target taxa in the control tank. Ballast water exchange efficacy is not calculated for a target if $\Delta C \leq -85\%$. (b) Ballast water exchange efficacies (%) for target zooplankton taxa on the *Berge Nord* , for Tank 5 exchange.

Phytoplankton Analyses

Chlorophyll-*a* and bulk indicators of microbial biomass: Chlorophyll-*a* concentration declined in both control and exchange tanks, however chl-*a* was noticeably higher in exchange tanks compared to control tanks following exchange (Fig 5.6d). In contrast, microbial biomass remained constant in both control and exchange tanks during the voyage (i.e. there was no difference between T0 and T1 (Fig 5.6c)).

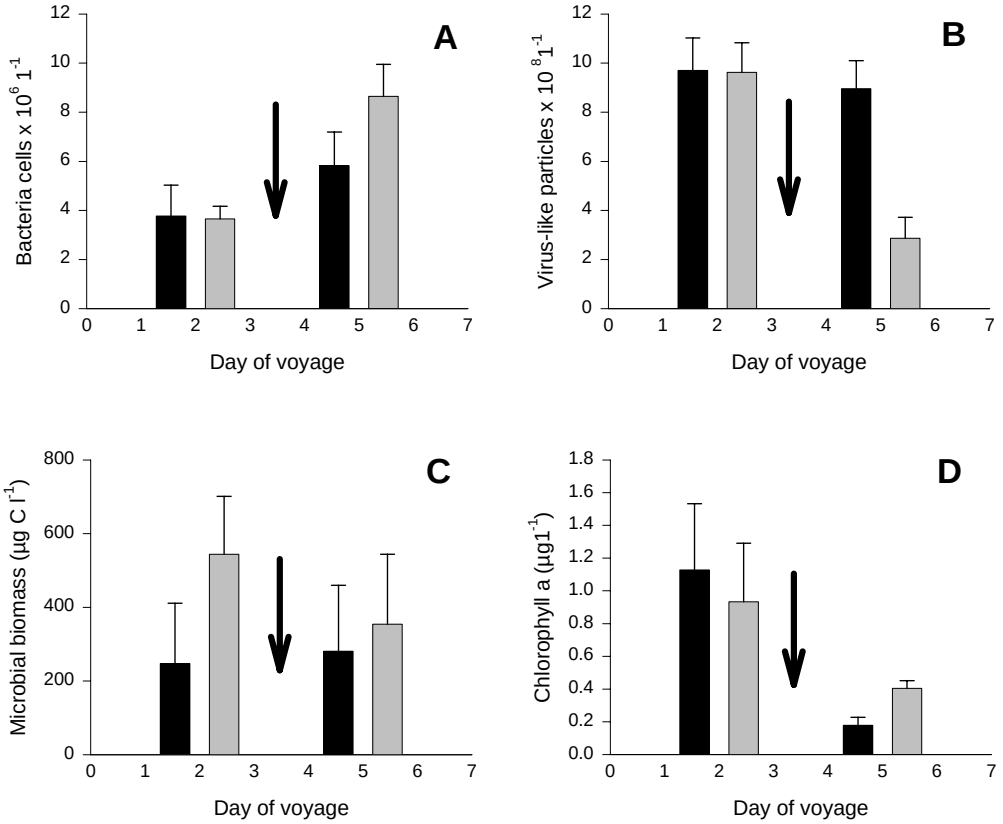


Fig. 5.6: Microbial metrics in ballast water from the *Berge Nord* voyage. Black bars represent samples from Control tanks; grey bars represent samples from Exchange tanks. (A) bacteria abundance; (B) virus-like particle abundance; (C) microbial biomass; (D) chlorophyll-a concentration. Arrows indicate Day 3, when Exchange tanks were exchanged in the open ocean. Data are mean values (n = 3, with a minimum of 2 subsamples per replicate); error bars are one standard deviation.

HPLC pigments: The concentration of degraded pigments (phaeophytin) declined after exchange, due to the uptake of 'fresh' phytoplankton that displaced degraded or dead cells in the original ballast tank population (Fig. 5.7b). However, in comparison to control tanks, exchange caused an increase in chlorophyll-a and other accessory pigments such as 19-hexamide and chl-c2 (Fig. 5.7b). However, the concentration of peridinin (unique indicator of dinoflagellates) and chl-c1 increased in both control and exchange tanks, indicating growth (control and exchange tanks), or uptake (exchange tanks) of cells containing these pigments, or both. In general, these

data show two trends: (1) an increase in abundance of dead or degraded phytoplankton in control tanks; and (2) an increase in diversity of phytoplankton following exchange.

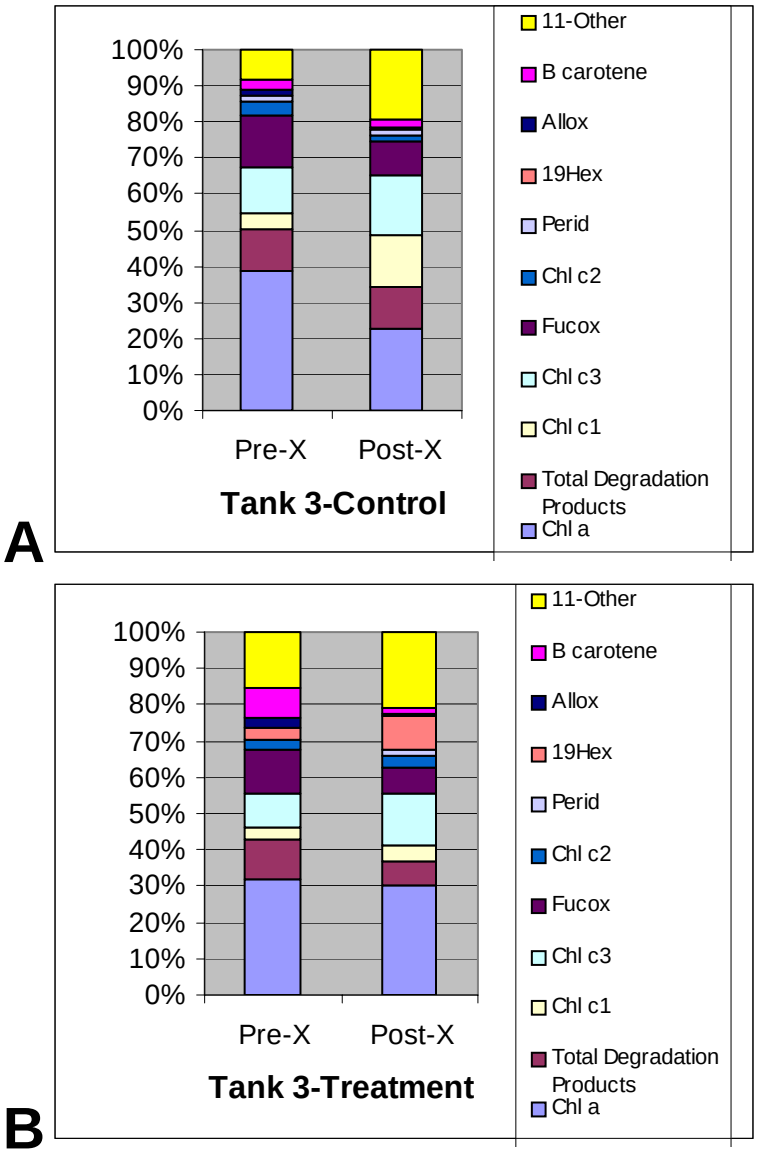
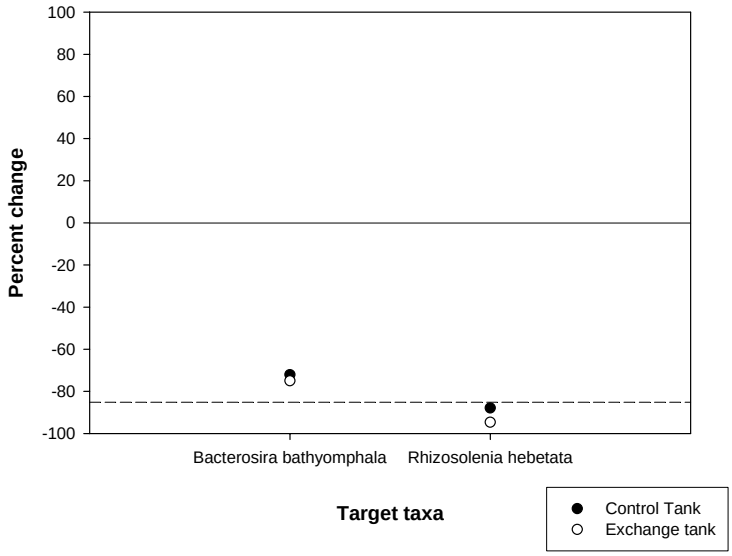


Fig. 5.7: Relative percentage of accessory pigments present in #3 control (A) and #3 exchange (B) ballast tanks on the *Berge Nord*. Other = unspecificed accessory pigments, B carotene = β-carotene, Allox = alloxanthin, 19 Hex = 19-hexamide, Perid = peridinin, Chl c2 = chlorophyll-c2, Fucox = fucoxanthin, Chl c3 = chlorophyll-c3, Chl c1 = chlorophyll-c1, Chl a = chlorophyll-*a*.

Phytoplankton species: Ballast tanks on the *Berge Nord* contained 34 phytoplankton taxa, mainly dominated by centric diatoms and a variety of dinoflagellates (Table 5.7). Because most phytoplankton taxa were species common to the North Atlantic, it was difficult to choose target taxa (i.e. their presence in the open ocean made it impossible to tell if a change in abundance after exchange was the result of growth within ballast tanks or uptake of new cells). However, two diatom species, *Bacterosira bathyomphala* and *Rhizosolenia hebetate*, qualified as coastal

targets. We calculated the percentage change in concentration in each tank pair for these targets using the method described above for zooplankton. The abundance of *B. bathyomphala* declined 40-72% in the #3 and #5 control tanks, and 65-75% in the paired #3 and #5 exchange tanks, indicating little effect of exchange (Fig. 5.8(a) and 5.9(a)). This result was confirmed by the exchange efficacy calculation, which yielded an exchange efficacy of 10.5% in the #3 exchange and 41.7% in the #5 exchange (**Fig**. 5.8(b) and 5.9(b**)**). Likewise, the abundance of *R. hebetata* declined by 80-88% in #3 and #5 control tanks, and 80-95% in #3 and #5 exchange tanks (Fig. 5.8(a) and 5.9(a)). Since *R. hebetata* declined below the -85% threshold in the #3 control tank, no exchange efficacy could be calculated for the #3 exchange tank; however, exchange had little demonstrable effect in the #5 exchange tank, which had an exchange efficacy of –1.6% (Fig. 5.9(b)). Based on this limited data set, ballast water exchange does not appear to greatly decrease the abundance of some phytoplankton species within ballast tanks.

(a).



(b)

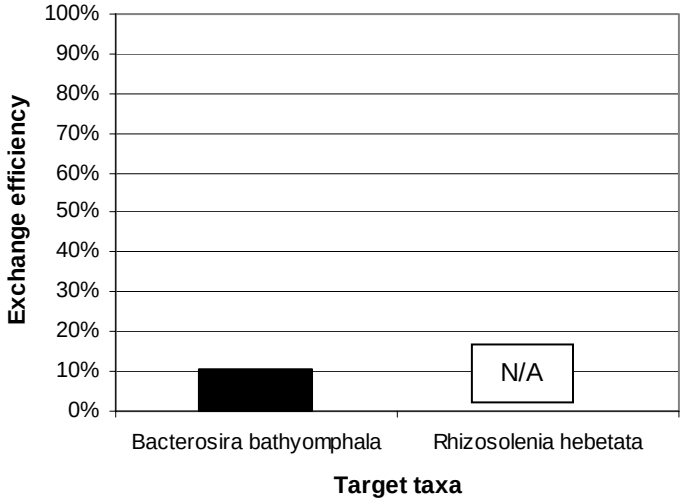
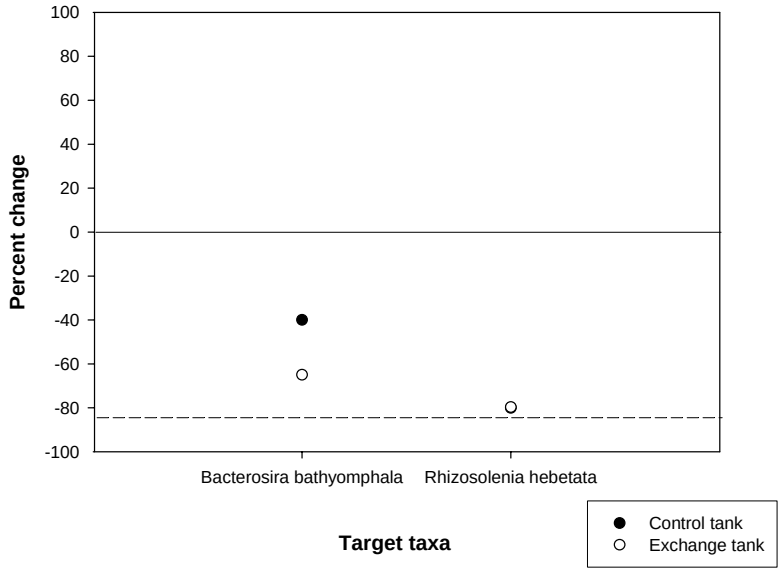


Figure 5.8. (a) Percent change in density of target phytoplankton taxa following exchange (T1) for Tank 3 control vs Tank 3 exchange on the *Berge Nord*. The dashed line represents the -85% threshold for target taxa in the control tank. Ballast water exchange efficacy is not calculated for a target if $\Delta C \leq -85\%$. (b) Ballast water exchange efficacies (%) for target phytoplankton taxa on the *Berge Nord* , for Tank 3 exchange. "N/A" indicates taxa for which the percent change in density in the control tank did not meet the -85% threshold (i.e. the taxa decreased in abundance by >85% in the control tank).

(a)



(b)

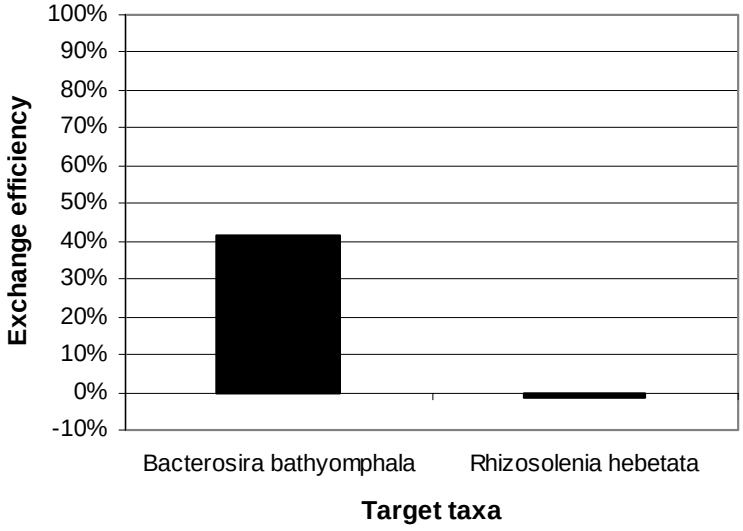


Fig. 5.9: (a) Percent change in density of target phytoplankton taxa following exchange (T1) for Tank 5 control vs Tank 5 exchange on the *Berge Nord*. The dashed line represents the -85% threshold for target taxa in the control tank. Ballast water exchange efficacy is not calculated for a target if $\Delta C \leq -85\%$. (b) Ballast water exchange efficacies (%) for target zooplankton taxa on the *Berge Nord* , for Tank 5 exchange.

Table 5.7. Total phytoplankton densities [cells/L], per tank, per time point for the *Berge Nord*. Data presented as follows: density (standard deviation); "n"= number of replicate samples. Target taxa are identified with an asterisk (*) following the taxon name:

Taxon	Tank 3 Control		Tank 3 Exchange		Tank 5 Control		Tank 5 Exchange	
	T0	T1	T0	T1	T0	T1	T0	T1
Diatoms	(n = 1)	(n = 2)	(n = 1)	(n = 2)	(n = 2)	(n = 2)	(n = 2)	(n = 1)
Bacterosira bathyomphala*	95,800	26,700 (10,300)	56,500	14,100 (12,100)	142,600 (77,900)	85,600 (6,900)	70,600 (5,200)	24,700
Centric diatoms unid.	17,600	3,600 (500)			72,900 (72,100)	7,300 (5,700)	95,700 (76,100)	10,600
Coscinodiscus sp.							27,300 (38,600)	
Cylindrotheca closterium							1,600 (2,300)	
Ditylum brightwellii				1,600 (2,300)				3,500
Eucampia zodiacus				2,800 (3,900)				
Pennate diatoms unid.		1,400 (2,000)	7,600	3,300 (4,600)	1,600 (2,200)	1,600 (2,300)		67,100
Proboscia alata	20,500	1,400 (2,000)			10,900 (15,400)	29,400 (9,400)	74,400 (14,000)	14,100
Pseudo-nitzschia sp.		2,000 (2,800)						
Rhizosolenia hebetata*	160,800	19,400 (27,400)	180,800	9,600 (13,600)	178,600 (36,100)	35,600 (1,100)	139,700 (60,600)	28,300
Rhizosolenia setigera	62,300							
Rhizosolenia styliformis							11,300 (16,000)	20
Thalassionema nitzschioides				4,900 (6,900)				
Dinoflagellates								
Amphidinium sp.	3,500	6,300 (3,300)		2,800 (3,900)	21,800 (4,500)	6,500 (9,100)	6,500 (9,100)	
Ceratium furca			10					110
Ceratium fusus	110		20	100 (100)	30 (42)			190
Ceratium horridum								10
Ceratium lineatum						1,900 (2,700)		
Ceratium minutum								305,000
Ceratium tripos					58,100 (82,100)			
Gymnodinium sp. #1 (G. pigmentosum?)	22,600	22,600 (32,000)	93,200	33,800 (24,900)	27,400 (38,700)	58,100 (82,200)	6,900 (500)	109,600
Gymnodinium sp. #2	3,100	34,200 (44,400)	14,100	25,900 (13,600)	39,200 (24,500)		1,800 (2,500)	
Heterocapsa rotundatum		16,200 (22,800)						
Protoperidinium depressum								80
Protoperidinium sp.				2,800 (3,900)		1,600 (2,300)		
Pyrocystis noctiluca	140	100 (100)	20		60 (100)		100 (100)	50
Unidentified dinoflagellates		5,700 (8,000)		4,200 (5,900)	72,900 (25,700)	29,100 (22,800)	10,900 (15,400)	28,300

Table 5.7 - continued. Total phytoplankton densities [individuals/ml], per tank, per time point for the *Berge Nord*. Data presented as follows: density (standard deviation); "n"= number of replicate samples. Target taxa are identified with an asterisk (*) following the taxon name:

Taxon	Tank 3 Control		Tank 3 Exchange		Tank 5 Control		Tank 5 Exchange	
	T0	T1	T0	T1	T0	T1	T0	T1
Cryptophytes	**(n = 1)**	**(n = 2)**	**(n = 1)**	**(n = 2)**	**(n = 2)**	**(n = 2)**	**(n = 2)**	**(n = 1)**
Cryptomonas sp.		3,800 (200)	11,300		6,300 (8,800)	1,900 (2,700)	7,300 (10,300)	
Hillea fusiformis		19,300 (27,300)			2,000 (2,800)			
Rhinomonas fulva		38,600 (0)						
Silicoflagellates								
Dictyocha staurodon		2,000 (2,800)						
Dictyocha speculum	3,700							

Bacteria and viruses: Total bacteria abundance increased but VLP abundance decreased in exchange tanks between T0 and T1. However VLP abundance remained relatively constant and bacteria abundance increased slightly in control tanks. This result is inconsistent with data from a previous voyage (compare Fig. 5.10(a) versus 5.10(b)), but clearly shows the unique nature (in terms of the bacteria and virus population) of exchanged ballast water on the *Berge Nord*.

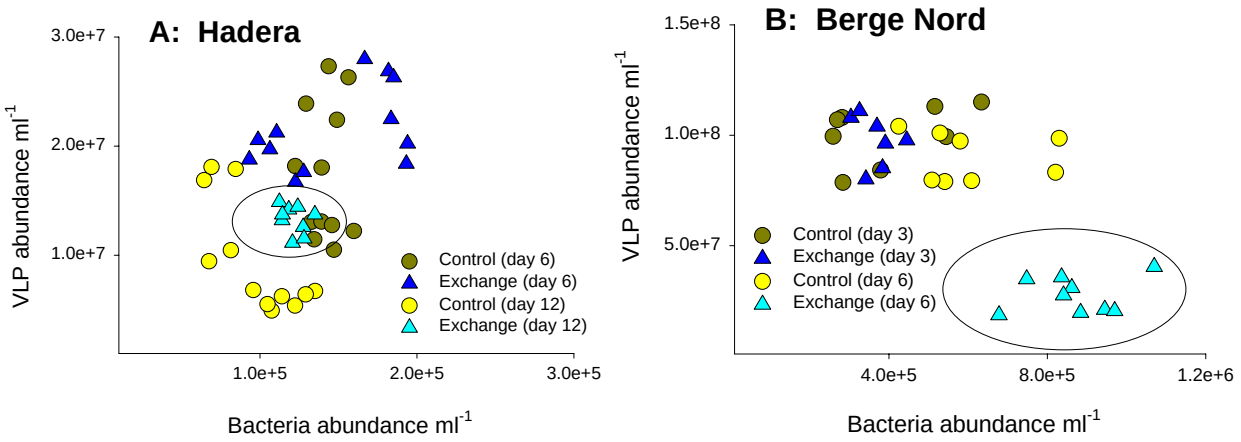


Fig. 5.10: A) Virus and bacteria abundance in control and exchange tanks before and after exchange on the *Hadera (*from a previous study and included for the purposes of comparison; see Drake et al. 2002). Virus abundance decreased as a result of exchange, but bacteria concentrations remained the same; B) As above for the *Berge Nord*. In this case, virus abundance also decreased after exchange, but the abundance of bacteria increased. Ellipses indicate exchanged microbial communities.

Most of these virio- and bacterioplankton species cannot be identified other than through molecular biology. Given that species identification was beyond the proposed scope of work, we instead calculated the percentage change in virus and bacteria abundance in control and exchange tanks using the calculations described above. The results show between-tank variability, but in general demonstrate a greater decrease in the virus to bacteria ratio (VBR) in exchange compared to control tanks (Table 5.8). We present these calculations appreciating that the short generation time of these microbes potentially could confound the results.

Table 5.8: Percentage change in virus-like-particle (VLP) abundance, bacteria abundance and virus to bacteria ratio (VBR) from T0 to T1 (before and after exchange) in ballast tanks on the *Berge Nord*.

	VLP (cells/ml)		Bacteria (cells/ml)		VBR	
Tank	ΔControl	ΔExchange	ΔControl	ΔExchange	ΔControl	ΔExchange
3	-25.2	-58.5	+8.4	+134.2	-44.9	-81.9
5	+5.1	-68.8	+149.3	+163.3	-56.6	-88.3
7	-0.2	-81.6	+46.5	+102.6	-34.0	-91.1

Voyage 2: *Federal Progress* (Canarctic Shipping, a division of Fednav Limited); Port Alfred, Quebec, Canada to Port Esquivel, Jamaica; 23 - 30 October 2003

Physical Water Tracers

Temperature/Salinity Profiles: It was not possible to obtain detailed water quality profiles below 2 m depth on this vessel because of the shallow, sloping bottom at the manhole sampling locations and the fact that the YSI could not be used in the ullage pipes. Presented instead, in Table 5.9, are the average temperature and salinity measured at each location, at each time point. Initial salinities ranged from 11-19 ppt. This was higher than we anticipated, based on previously reported measurements in the nearby Saguenay River. Prior to exchange, salinity was 2-6 ppt higher in the deeper, aft portion of the tanks (sampled via ullage pipe) compared to the shallower, more forward locations (forward manholes).

Table 5.9. Average temperature and salinity for each tank, at each sampling location and time point on the *Federal Progress*. * indicates equipment failure; - - indicates missing data.

Tank	Treatment	Time point	Temp. (C)		Salinity (ppt)	
			Manhole	Ullage pipe	Manhole	Ullage pipe
3S	Control	T0	*	*	15.0	18.0
1S	Exchange	T0	*	*	17.0	19.0
3P	Control	T0	6.6	--	11.0	17.0
1P	Exchange	T0	5.9	5.0	14.8	17.0
3S	Control	T1	18.1	18.0	13.3	15.5
1S	Exchange	T1	24.5	*no access*	34.0	*no access*
3P	Control	T1	21.9	23.0	10.9	12.0
1P	Exchange	T1	26.0	25.5	34.1	38.0

During ballast water exchange, the salinity and temperature of the incoming ballast water was measured from a fire hydrant on the deck using a hand-held thermometer and refractometer. The incoming water had a salinity of 38 ppt and temperature of 24˚ C. Exchange efficacy based on salinity alone was calculated by comparing final salinities in the exchanged tanks to the salinity of the incoming ballast. These calculations yielded an exchange efficacy of 82.4% for Tank #1 port and 80.0% for Tank #1 starboard.

Rhodamine Dye Concentrations: At T1, the dye concentration in the topside portion of the control tank (Tank 3 port) was still below the expected concentration of Rhodamine dye (mean expected concentration = 85.2 ± 2.8 μg/l, calculated based on actual volume of ballast in tank and volume of dye added); Tank #3 port = 54.2 ± 0.5 μg/l). After open-ocean exchange, the dye concentrations in the treatment tanks dropped to 0.97 ± 0.06 μg/l (#1 port) and 0.82 ± 0.1 μg/l (#1 starboard), which is equivalent to a 98.2% and a 98.5% ballast water exchange efficacy, respectively (Fig. 5.11).

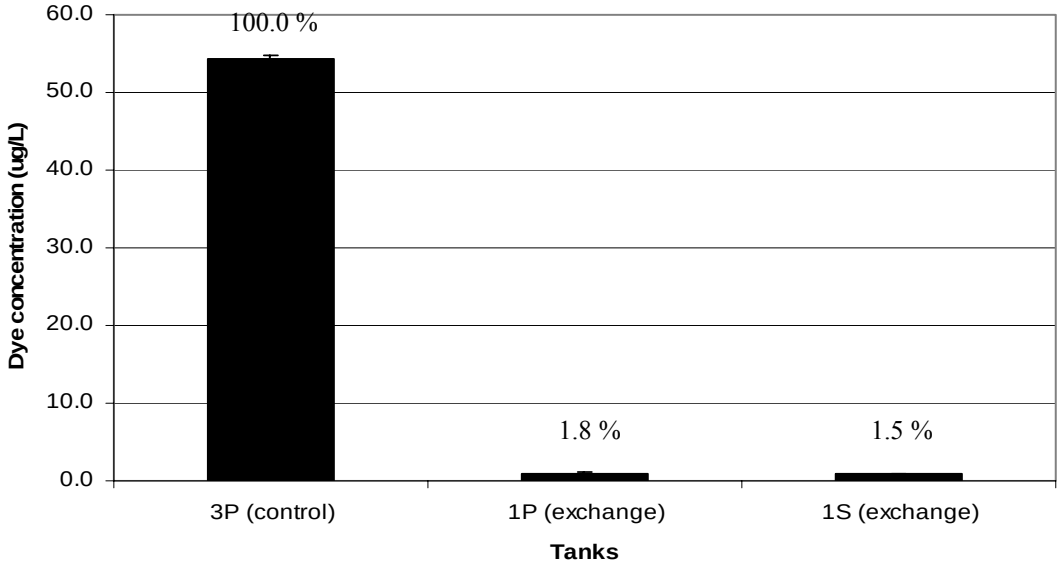


Fig. 5.11: Concentration of rhodamine dye remaining in *Federal Progress* tanks at T1 (post-exchange). Percent values shown above bars represent the percent dye remaining in the tanks at T1. This equates to ballast water exchange efficacy values of: Tank 1P = 98.2%, Tank 1S = 98.5%. Error bars are one standard deviation.

Biological Tracers

Zooplankton Analyses: Inclement weather and sea conditions led to inconsistent sampling on the starboard side of the ship. It was not possible to obtain samples from comparable locations in the starboard control and exchange tank at both time points. For this reason, only the port-side tank pair is being used for zooplankton analysis.

Table 5.10 shows total zooplankton densities for all target taxa identified in pre- and post-exchange samples in the port-side tank pair. Taxa diversity was quite low. Pre-exchange samples were dominated by the calanoid copepod *Eurytemora* sp. and the phylum Rotifera, both of which were identified as the target taxa for this experiment. Overall, the density of all taxa decreased following exchange. The primary exceptions to this were the Calanoida (other than *Eurytemora* sp.), copepod nauplii, and Poecilostomatoida.

Final densities at T1 were similar between control and exchange tanks for each target taxon (two-tailed t-test: *Eurytemora* sp., p=0.17; Rotifera, p=0.36). Furthermore, Table 10 shows that all taxa, with the exception of those that increased due to the uptake of mid-ocean forms, were absent or near absent at T1 in both the control and exchange tanks. Both target taxa underwent a 100% change in density (i.e. 100% removal) in the exchange tanks. Both targets failed to meet the -85% threshold in the control tank (Fig. 5.12), with a 99.94% reduction in density for *Eurytemora* sp. and a 99.98% density reduction for Rotifera; therefore, no ballast water exchange efficacy calculations were performed for the taxa in this experiment.

Table 5.10. Total zooplankton densities [individuals/m^3], per tank, per time point for the *Federal Progress*. Data presented as follows: density (standard deviation); "n"= number of replicate samples. Target (coastal) taxa are identified with an asterisk (*) following the taxon name.

Taxon	Tank 3 Control		Tank 1 Exchange	
	T0	T1	T0	T1
Copepoda	**n=6**	**n=6**	**n=6**	**n=6**
Eurytemora sp.*	1652 (1606.4)	1 (1.1)	65 (38.1)	
Other Calanoida	1675 (1613.2)	9 (9)	102 (46.9)	340 (82.1)
Copepod nauplii	27 (6.6)	6 (6.7)	101 (46.6)	325 (89.5)
Cyclopoida	7 (3.5)	14 (22.8)	57 (50.5)	28 (13.3)
Harpacticoida	9 (4)	6 (4.7)	2 (2)	16 (7.6)
Poecilostomatoida	0 (0.6)	0 (1.1)		73 (30.6)
Other Crustaceans				
Barnacle nauplii	0 (0.6)	0 (1.1)		
Cladocera	11 (12.6)	0 (0.7)		
Ostracoda		0 (0.6)		
Decapoda		0 (0.6)		0 (0.6)
Annelida				
Polychaeta			0 (0.6)	0 (0.7)
Spionidae		0 (0.6)	1 (1.8)	
Other Taxa				
Chaetognatha				1 (1.2)
Cnidaria				1 (1)
Echinodermata				1 (1.2)
Rotifera*	1541 (606.8)	0 (0.6)	53 (21.7)	

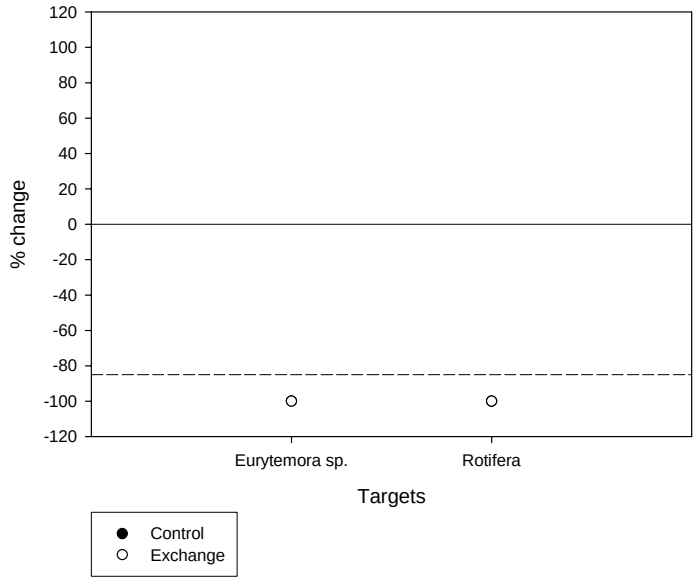


Fig. 5.12. *Federal Progress*: Percent change in density of target zooplankton taxa for control tank (Tank 3P) vs. exchange tank (Tank 1P) at T1. The dashed line represents the -85% threshold for change in the control tank. Values for percent change in the control tank are obscured in this graph by the values for percent change in the exchange tank.

Phytoplankton Analyses.

Chlorophyll-a and bulk indicators of microbial biomass: Between T1 and T0, chlorophyll-a concentration declined in both control and exchange tanks, however there was no significant difference between tank types (i.e., no additional effect of exchange on the total phytoplankton biomass; Fig. 13(A**)**). The incoming ballast water had a relatively high proportion of chlorophyll-a to phaeopigments, resulting in an increase in this ratio in the exchange tanks (Fig. 13(B)).

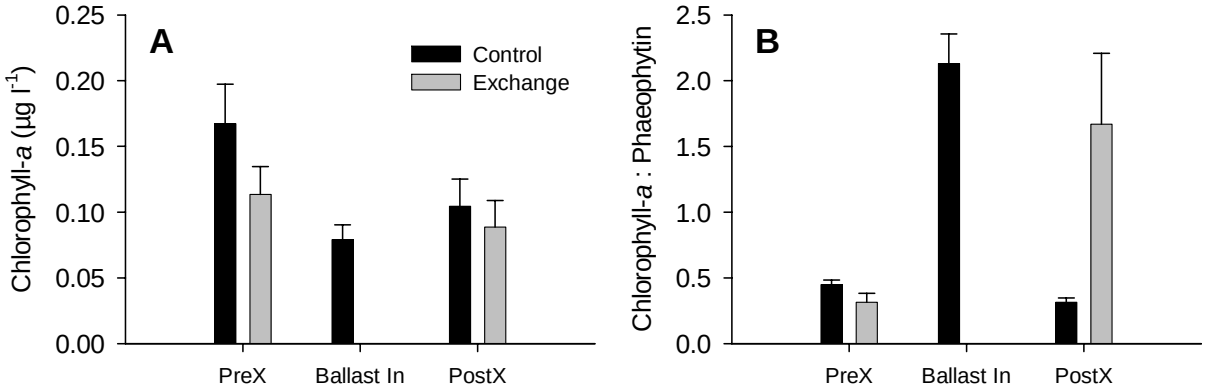


Fig. 5.13: (A) Concentration of chlorophyll-a in control and exchange tanks on the *Federal Progress*. (B) Ratio of chlorophyll-a to phaeopigments (i.e. ratio of living to dead or degraded chlorophyll-a) in control and exchange tanks on the *Federal Progress*.

Phytoplankton species: Ballast tanks on the Federal Progress contained a significantly less diverse and abundant phytoplankton population compared to the Berge Nord. A summary of the species that were present is found in Table 5.11. Unfortunately, none of the taxa could be used as targets for calculating ballast water exchange efficacy.

Table 5.11. Total phytoplankton densities [cells/l], per tank, per time point for the *Federal Progress*. Data presented as follows: density (standard deviation); "n"= number of replicate samples. No taxa qualified as usable target (coastal) taxa due to very low densities.

Taxon	Starboard Control		Starboard Exchange		Port Control		Port Exchange	
	T0	T1	T0	T1	T0	T1	T0	T1
Diatoms	**(n = 2)**	**(n = 2)**	**(n = 2)**	**(n = 1)**	**(n = 2)**	**(n = 2)**	**(n = 2)**	**(n = 2)**
Asterionellopsis glacialis	3,900 (5,400)		17,800 (25,100)		8,500 (12,000)		9,200 (8,400)	
Aulacoseira sp.			45,200 (27,400)					
Bacillaria paradoxa								
Bacterosira bathyomphala							16,200 (22,800)	
Centric diatoms unid.			1,600 (2,300)			7,600 (10,700)	35,800 (50,600)	
Coscinodiscus sp.			1,600 (2,300)					
Cyclotella sp.							5,700 (8,000)	
Diatoma ehrenbergii							1,900 (2,700)	
Nitzschia longissima			1,600 (2,300)					
Pennate diatoms unid.	1,300 (1,800)	3,600 (5,000)	8,100 (2,200)		2,800 (4,000)		1,600 (2,300)	
Pleurosigma sp.							1,900 (2,700)	
Proboscia alata	3,300 (4,600)							
Tabellaria floculosa							1,600 (2,300)	
Thalassionema nitzschioides			9,700 (13,700)				3,200 (4,500)	
Dinoflagellates								
Amphidinium sp.						5,700 (8,000)		
Gymnodinium sp. #3							1,900 (2,700)	2,500 (3,500)
Heterocapsa rotundatum	18,000 (25,400)			3,200			1,900 (2,700)	
Protoperidinium sp.								
Unidentified dinoflagellates	22,900 (10,600)	3,700 (300)	1,600 (2,300)		31,800 (45,000)	3,800 (5,300)	9,700 (13,600)	
Cryptophytes								
Cryptomonas sp.			3,200 (4,500)		2,800 (4,000)	3,800 (5,300)		
Hillea fusiformis				3,200		3,800 (5,300)	5,700 (8,000)	
Rhinomonas fulva							3,800 (5,300)	
Cyanobacteria								
Merismopedia sp.					5,700 (8,000)			

Bacteria and viruses: Interestingly, there was an increase in bacteria and VLP density in the control tanks (perhaps due to the increase in sea surface—and hence ballast water—temperature during the voyage). Following exchange however, there was a significant decline in bacteria and VLP density (Fig. 5.14).

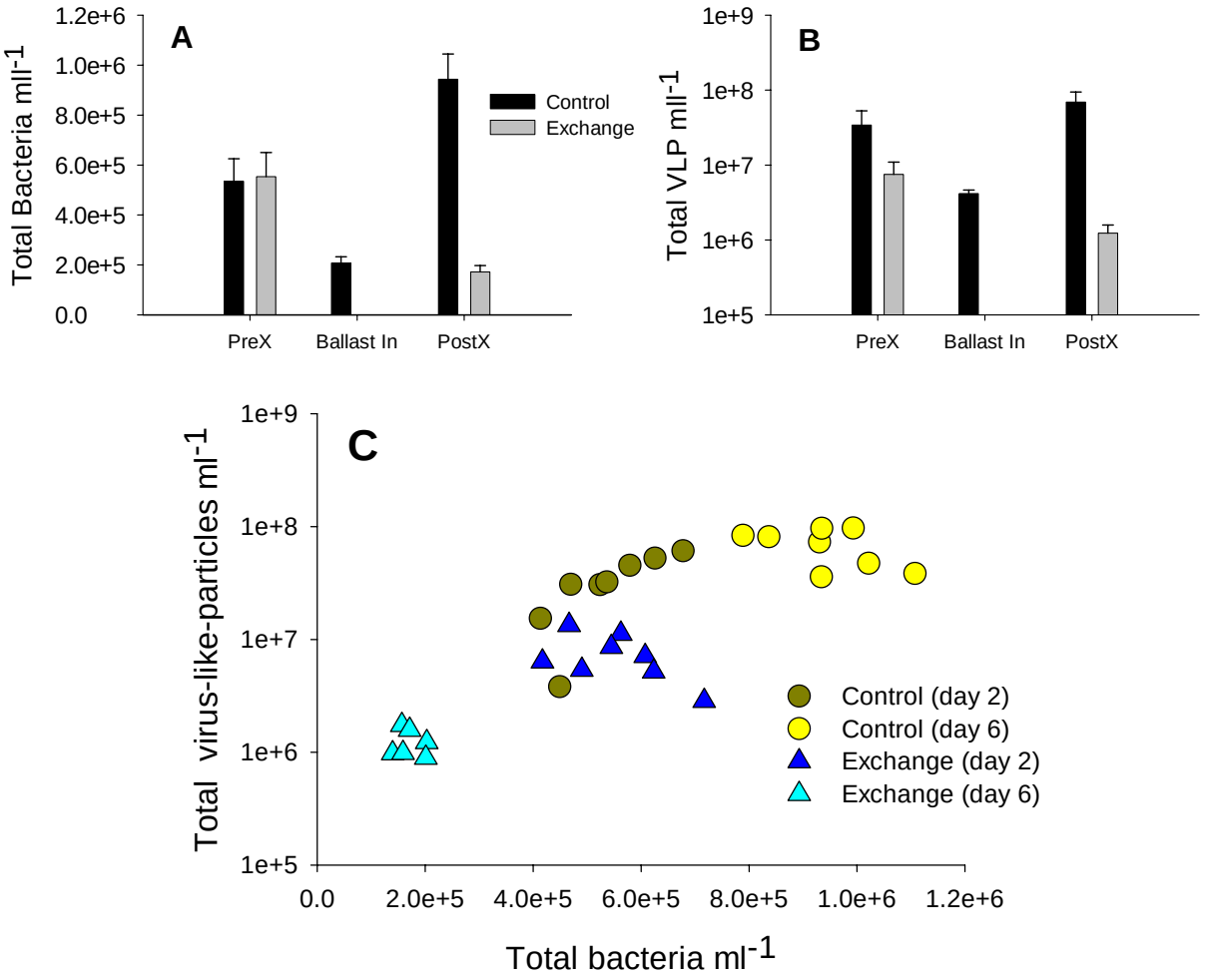


Figure 5.14. Total bacteria (A) and virus-like-particle (B) abundance in control and exchange tanks, as well as incoming ballast on board the *Federal Progress*; (C) Virus and bacteria abundance in control and exchange tanks before and after exchange on the *Federal Progress*, showing unique character of exchange communities.

Table5. 12. Percentage change in virus-like-particle (VLP) abundance, bacteria abundance and virus to bacteria ratio (VBR) from T0 to T1 (before and after exchange) in #1 and #3 ballast tanks on the *Federal Progress*.

	VLP (cells/ml)		Bacteria (cells/ml)		VBR	
Tank	ΔControl	ΔExchange	ΔControl	ΔExchange	ΔControl	ΔExchange
Port	33.9	-77.6	71.5	-70.1	-14.7	-30.30
Starboard	207.2	-85.3	82.6	-67.0	71.4	-54.90

Voyage 3: ***Kenai*** (Alaska Tanker Company); Benicia, California to Valdez, Alaska; 23 – 29 June 2004.

Physical Water Tracers

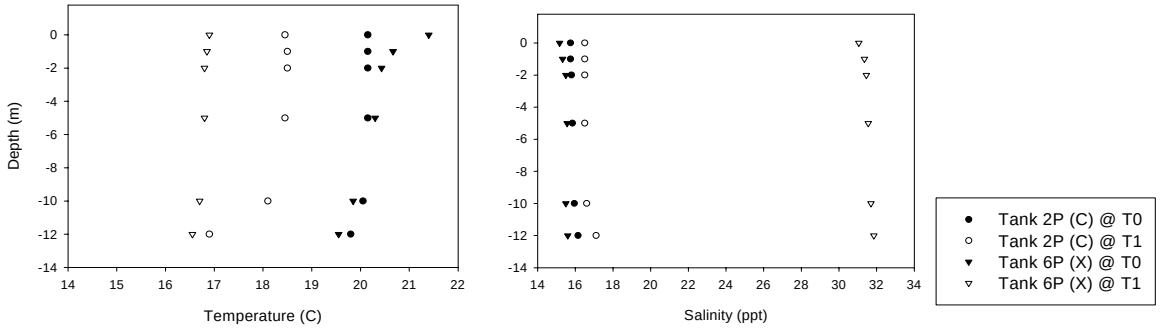
Temperature/Salinity Profiles: Initial measurements for temperature and salinity were comparable between control and treatment tanks at all depths (Fig. 5.15). Temperatures measured post-exchange decreased approximately 1.5°C-2.0° C in the control tanks, reflecting colder ocean temperatures as the vessel traveled northward. Post-exchange temperatures in the exchanged tanks were approximately 2.5° C colder than temperatures measured in the control tanks; although temperatures at around 12 m in the control tanks approached the temperature of the water in the exchanged tanks at this depth.

Slight temperature stratification was evident at all time points in the starboard-side tanks, marked by 1.0-1.5° C cooler temperatures at depths of 10-12 m. This stratification was more pronounced post-exchange than pre-exchange. In the port-side tanks a slight difference in surface temperature (~1° C) was seen at T0 between control and exchange tanks. At T1 slight stratification of the control tank was also evident at 12-m depth with the temperature dropping to approximately that of the exchanged water.

Salinity of the Benicia water (15-17 ppt) was again higher than initially anticipated during the planning phases of this voyage. Measurements recorded post-exchange indicate that salinity increased marginally in the control tanks (Tanks 2 port and 2 starboard), but increased substantially – approximately 15 ppt – with open-ocean exchange in the treatment tanks (6 port and 6 starboard). The salinity of the incoming ocean water at the time of exchange was 32.4 ppt. Exchange efficacy calculations based on salinity alone yield an exchange efficacy of 94.6% for Tank #6 port and 94.1% for Tank #6 starboard.

Rhodamine Dye Concentrations: At T1, the dye concentration in the port-side control tank (Tank 2 port = 119.7 ± 27.5 μg/l;) was near the mean expected concentration of 114.8 ±3.0 μg/l (calculated based on actual volume of ballast in tank and volume of dye added); however, Tank 2 starboard fell below the mean expected concentration at 72.6 ± 7.1 μg/l). T1 dye concentrations for the exchanged tanks were 8.9 ± 0.9 μg/l (Tank 6 port) and 6.8 ± 0.9 μg/l. These reductions in Rhodamine concentration are equivalent to ballast water exchange efficacies of 91.8% (Tank #6 port) and 90.7% (Tank #6 starboard). Figure 5.16 represents the dye concentration for all tanks after open-ocean exchange.

(a) **Port-side tank pair**: Tank 2P (control) and Tank 6P (exchange)



(b) **Starboard-side tank pair**: Tank 2Sb (control) and Tank 6Sb (exchange)

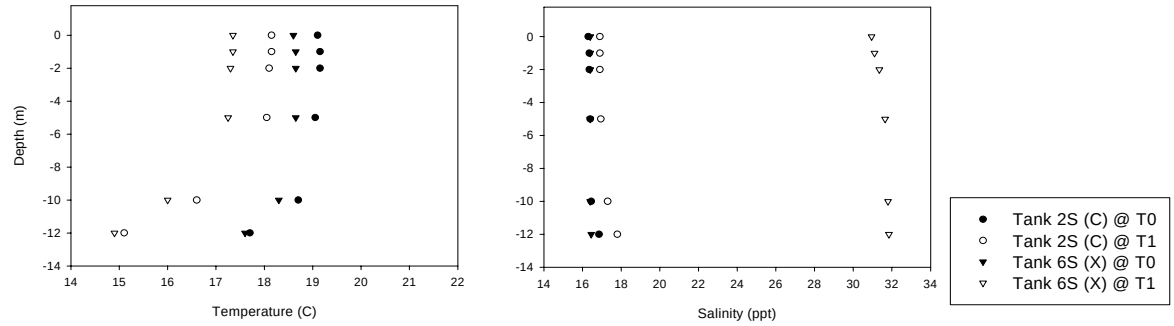


Fig. 5.15: Vertical profiles for salinity (ppt) and temperature (°C) in the *Kenai*'s (a) Tanks 2 port (control) and 6 port (exchange) and (b) Tanks 2 starboard (control) and 6 starboard (exchange).

Biological Tracers

Zooplankton Analyses: Table 13 shows total zooplankton densities for all taxa identified in pre- and post-exchange samples. Pre-exchange samples were dominated by the cyclopoid copepod *Limnoithona* spp. and bryozoan cyphonautes larvae. Overall, the density of organisms decreased following exchange. The primary exceptions to this were the phyla Gastropoda and Polychaeta, and the copepod orders Calanoida and Harpacticoida.

Target taxa identified for this experiment in both the port-side and starboard-side tanks were the cyclopoid copepod *Limnoithona* spp., the calanoid copepod *Tortanus* spp., bryozoan cyphonautes larvae, and the mollusc larvae (Bivalvia and Gastropoda). Additionally, Polychaeta were identified as a target taxon in the starboard-side tanks only, since initial (T0) density was far below our threshold value of 20 individuals/m^3 in the port-side exchange tank for this group.

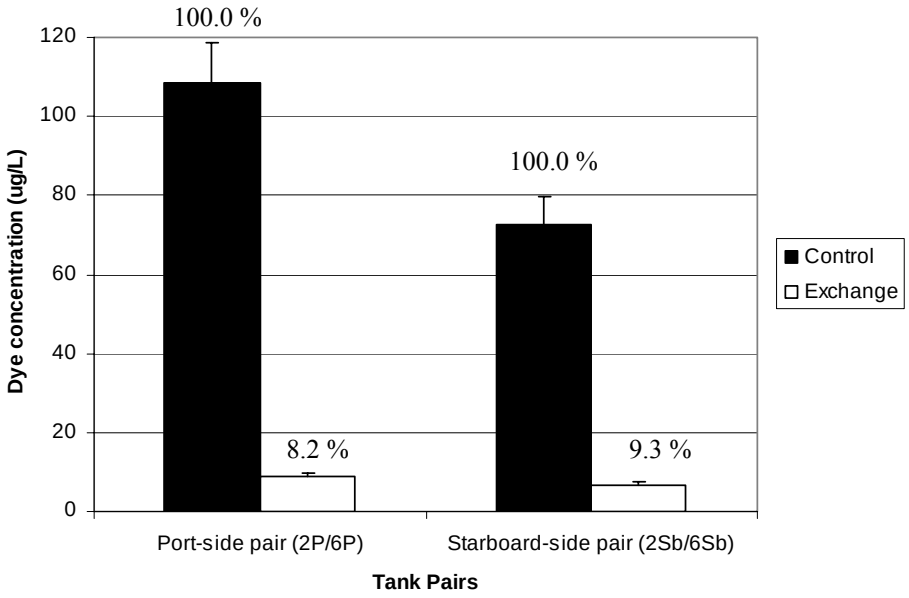


Fig. 5.16. Concentration of rhodamine dye remaining in *Kenai* port-side and starboard-side tank pairs at T1 (post-exchange). Percent values shown above bars represent the percent dye remaining in the tanks at T1. This equates to ballast water exchange efficacy values of: Tank 6 port = 91.8%, Tank 6 starboard = 90.7%. Error bars are one standard deviation.

Figures 5.17(a) and 5.18(a) illustrate the percent change in density that occurred for target taxa following exchange. In general, density decreased with exchange for all targets except Gastropoda in the port-side exchange tank and Polychaeta in the starboard-side exchange tank. Only the latter of these increases was statistically significant (two-tailed t-test: Gastropoda, p = 0.10; Polychaeta, p = 0.03).

With the exception of Gastropoda, the proportion of all target taxa removed in the port-side exchange tank was moderate to high (-53% to -100%). The survivorship of target taxa in the port-side control tank was variable. Cyphonautes increased substantially (55.6%), while Bivalvia and Gastropoda showed little change (9.1% and 2.9%, respectively). In contrast, *Limnoithona* spp. and *Tortanus* spp. both decreased by large proportions (-76% and -64%, respectively).

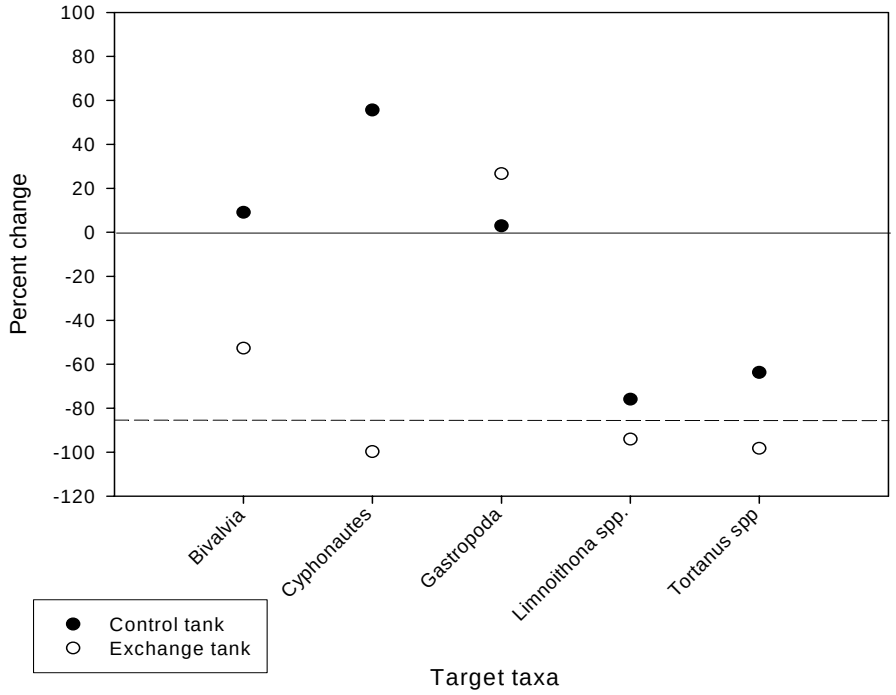
In the starboard-side exchange tank all target taxa but Polychaeta decreased by a large proportion (-76% to -100%). As in the port-side control, survivorship of target taxa in the starboard control tank was variable. Bivalvia increased substantially (74%). All other taxa stayed within +/- 26% of the initial density.

All targets in both control tanks stayed above the -85% threshold. Ballast water exchange efficacies were calculated accordingly (Fig. 5.17(b) and 5.18(b)). Efficacies in the port-side exchange tank were fairly high (range: 56.6% - 99.8%), except for Gastropoda, which increased in both the control and exchange tanks, resulting in a negative ballast water exchange efficacy (-23.0%). Likewise, efficacies in the starboard-side exchange tank were high (range: 78.7% - 100%), except Polychaeta (-76.7%).

Table 5.13. Total zooplankton densities (individuals/m^3), per tank, per time point for the *Kenai*. Data presented as follows: density (standard deviation); "n"= number of replicate samples. Target (coastal) taxa are identified with an asterisk (*) following the taxon name. ** represents target in starboard side tanks only.

Taxon	Tank 2P Control		Tank 6P Exchange		Tank 2S Control		Tank 6S Exchange	
	T0	T1	T0	T1	T0	T1	T0	T1
Copepoda	**n=5**	**n=6**	**n=6**	**n=6**	**n=6**	**n=6**	**n=6**	**n=6**
Calanoida – *Tortanus* spp.*	229(22.4)	83(33.1)	256(131)	5(2.1)	107(13.2)	80(8.3)	171(64.1)	2(3.2)
Total Calanoida	259(29.6)	119(37.9)	349(128)	3,455(498.2)	126(15.4)	99(9.3)	194(75.4)	5,258(1284.4)
Copepod nauplii	2,851(1270.8)	2,763(483.5)	5,398(1,312.3)	9,385(4390.2)	2,060(705.6)	2,198(593)	1,490(880.5)	12,999(2936.8)
Cyclopoida - *Limnoithona* spp.*	7,373(1188.2)	1,775(262.3)	16,850(3,211.8)	1,000(159.4)	2,847(348.4)	3,188(812.6)	3,363(886)	803(130.7)
Total Cyclopoida	7,373(1188.2)	1,775(262.3)	16,850(3,211.8)	1,734(359)	2,847(348.4)	3,188(812.6)	3,363(886)	1,078(179.3)
Harpacticoida	4(1.8)	20(21.3)	22.9(9.5)	4,702(1010.9)	3(2.1)	31(12.8)	2(1.5)	6,599(1501.9)
Copepoda OTHER	31(7.2)	53(13.3)	13(2.2)	112(14.5)	33(8.3)	50(13.1)	22(6.1)	174(30.3)
Cirripedia								
Barnacle Nauplii	14(5.4)	15(4.9)	57(8.6)	6(4.2)	17(7.3)	4(4.3)	40(10.3)	2(1.2)
Cyprids	2(1.2)	0(1.2)			1(0.8)			
Other Crustaceans								
Amphipoda	1(1.8)		1(1.6)		1(0.8)			0(1)
Decapoda	1(0.5)	1(1.4)	1(2)		1(1.1)	0(0.9)	1(1.6)	
Mysidaceae	2(1.7)	1(1.2)	1(1)	1(0.8)	1(0.9)	2(2.2)	3(1.7)	
Mollusca								
Bivalvia*	46(8.6)	50(13.6)	72(72)	34(11.7)	18(8.6)	31(9.5)	128(81.6)	4(5.4)
Gastropoda*	90(16.9)	92(19.7)	35(13.6)	44(12.9)	94(26.9)	69(13.5)	79(14.5)	9(7.3)
Annelida								
Spionidae	7(2.2)	13(5.5)	27(9.6)	2(1.1)	6(5.2)	7(3.8)	7(2.7)	3(3.9)
Polychaete**	20(5.5)	75(13.1)	6(2.7)	46(14.6)	55(10.8)	59(13.9)	34(17.5)	65(18)
Other taxa								
Cyphonautes*	3,817(695.9)	5,937(914.9)	1686(518.7)	5(3.1)	4,472(742.9)	4,975(470.6)	3,780(789.9)	0(0.5)
Cnidaria		0(1.2)		1(1.7)	0(0.5)			1(0.6)
Ctenophora			0(0.4)					0(0.5)
Turbellaria	2(2.5)	4(4.6)	3(2.2)	1(0.9)	2(1.3)	3(1.1)	4(3.2)	1(1.3)
Other Zooplankton	1(1.2)	1(1.2)	2(2.5)	286(34.9)	1(1.1)	2(1.6)	1(1.6)	251(41.2)

(a)



(b)

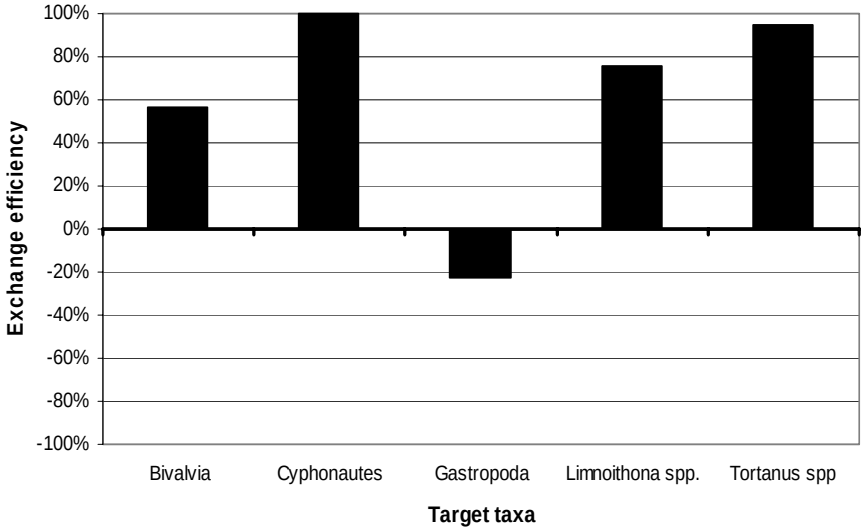
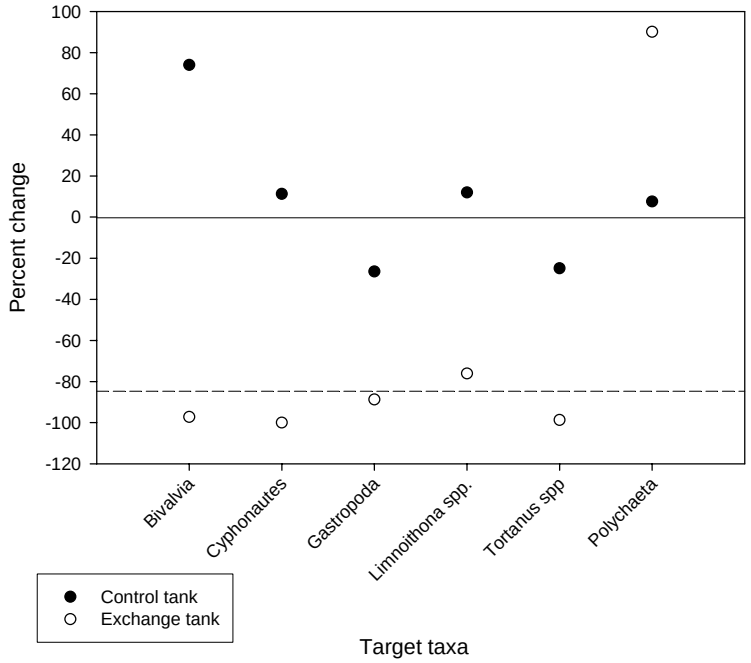


Fig. 5.17: (a) Percent change in density of target zooplankton taxa following exchange (T1) for Tank 2P (control) vs Tank 6P (exchange) on the *Kenai*. The dashed line represents the -85% threshold for target taxa in the control tank. Ballast water exchange efficacy is not calculated for a target if $\Delta C \leq -85\%$. (b) Ballast water exchange efficacies (%) for target zooplankton taxa on the *Berge Nord* , for Tank 6P (exchange).

(a)



(b)

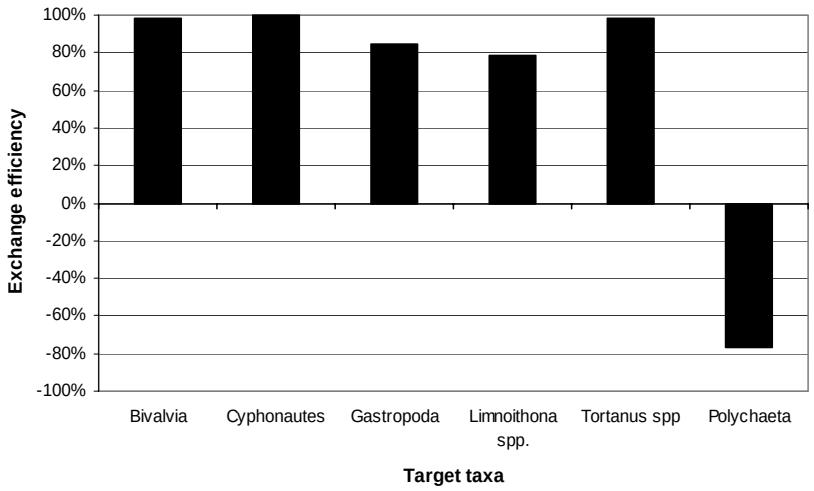


Fig. 5.18: (a) Percent change in density of target zooplankton taxa following exchange (T1) for Tank 2S (control) vs Tank 6S (exchange) on the *Kenai*. The dashed line represents the -85% threshold for target taxa in the control tank. Ballast water exchange efficacy is not calculated for a target if $\Delta C \leq -85\%$. (b) Ballast water exchange efficacies (%) for target zooplankton taxa on the *Berge Nord* , for Tank 6S (exchange).

Phytoplankton Analyses:

Chlorophyll-a: Exchange caused a significant reduction in chlorophyll-a concentration compared to control tanks. However there was a greater decline in phaeopigments, which resulted in an increase of the chlorophyll-a: phaeopigment ratio in exchange tanks (Fig. 5.19; Table 5.14).

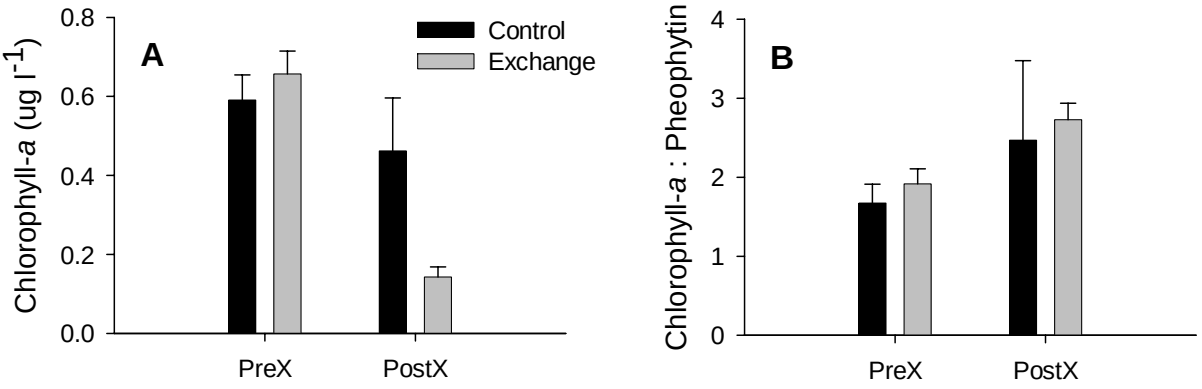


Fig. 5.19: (A) Chlorophyll-*a* concentration and (B) chlorophyll-*a*: phaeopigments in control and exchange tanks on the *Kenai* before and after exchange. Phytoplankton biomass was evenly distributed (i.e. no stratification), so data for mid-depths only are presented.

Table 5.14. Percentage change in the concentration of chlorophyll-*a*, phaeopigments, and chlorophyll-*a*: phaeopigments from T0 to T1 (before and after exchange) in #2 and #6 ballast tanks on the *Kenai*.

	Chlorophyll-*a* (µg/l)		Phaeopigments (µg/l)		Chl-*a*:Phaeo	
Tank	ΔControl	ΔExchange	ΔControl	ΔExchange	ΔControl	ΔExchange
Port	-5.9	-75.3	-55.5	-83.1	114.3	46.4
Starboard	-38.3	-81.3	-32.4	-86.5	-6.2	38.6

Phytoplankton species: Table 5.15 shows total phytoplankton densities for all taxa identified in pre- and post-exchange samples. Pre-exchange samples were dominated by the diatom *Skeletonema costatum,* the micro-phytoflagellate *Cryptomonas sp. #1* (length <10 µm) and unidentified centric diatoms (diameter <10 to 30 µm) and pennate diatoms (length <10 µm). Target taxa identified for this experiment were *Skeletonema costatum* and *Cryptomonas sp. #1.*

Figures 5.20(a) and 5.21(a) illustrate the percent change in density that occurred for target taxa post-exchange in the port-side and starboard-side exchange tanks, respectively. *S. costatum* and *Cryptomonas* sp. #1 both exhibited an overall decline in density following exchange. *Skeletonema costatum* was gone from both exchange tanks following exchange (ΔX = -100%). Change in the control tanks was more variable for this species. In the port-side control, this species experienced only a moderate decline (ΔC = -27%), but fell to the -85% threshold in the starboard-side control. Exchange efficacy could not be calculated for this species in the starboard-side exchange tank due to the large decline; however, calculations for the port-side exchange resulted in a 100% exchange efficacy for *S. costatum* (Fig. 5.20(b)). *Cryptomonas* sp. #1 declined equally in both exchange tanks (-63 % port-side and -60 % starboard-side) and, like

S. costatum, experienced a more variable decline in the two control tanks (-63% port-side and -25% starboard-side). Exchange appeared to have no effect on *Cryptomonas* sp. #1 in the port-side tank (Fig. 20(b), Ex_{Effic} = 0%) and a limited effect on this species in the starboard side tank (Fig. 21(b), Ex_{Effic}=47%).

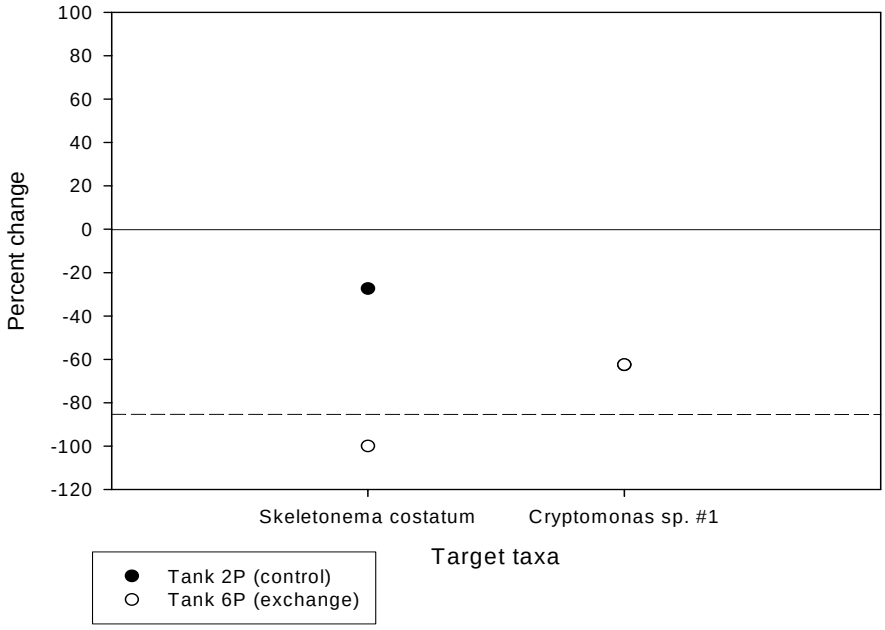
Table 5.15. Total phytoplankton densities [cells/L], per tank, per time point for the *Kenai*. Data presented as follows: density (standard deviation); "n"= number of replicate samples. Target (coastal) taxa are identified with an asterisk (*) following the taxon name.

TAXA	2P		6P		2S		6S	
	T0	T1	T0	T1	T0	T1	T0	T1
BLUE-GREEN	**n=3**	**n=4**	**n=4**	**n=4**	**n=4**	**n=4**	**n=4**	**n=4**
Anabaena sp. 2	30,688(53153.2)							
Oscillatoria cells #1 diam <5um	26,323(45592.5)							
Unid. Blue green trichome (cell) sm blunt								52,170(104339)
DIATOM								
Chaetoceros sp#1 diam <10 microns	6,138(10,630.6)	12,228(8,635.7)	9,026(18,051.8)	67,514(73,480.4)		9,206(10,630.6)		62,910(30,636.8)
Chaetoceros sp#2 diam 10-30 microns				7609(7639.5)				7672(5876.3)
Cylindrotheca closterium				3053(3525.4)			1534(3,068.8)	1534(3,068.8)
Leptocylindrus danicus	4,092(7,087.1)							
Licmophora sp.			1,519(3,037.3)					
Nitzschia sp#1 length <30 microns				3,069(3,543.5)				3,069(3543.5)
Nitzschia sp#2 length 30-70 microns				3,037(6,074.5)			1,519(3,037.3)	
Rhizosolenia delicatula				4,603(5,876.3)			0(0)	3,069(3,543.5)
Rhizosolenia fragilissima							0(0)	1,534(3,068.8)
Rhizosolenia sp.				3,037(3,507.1)			0(0)	1,519(3,037.3)
*Skeletonema costatum**	63,359(39,567.2)	46,000(40,282.2)	32,072(25,257.9)		19,947(39,894.4)	3,037(6,074.5)	26,085(29,381.5)	
Thalassionema nitzschioides				12,275(20,662.2)				9,206(10,630.6)
Unid. Centric diatom diam <10 microns	139,119(51,473)	24,550(7,087.1)	123,083(86,380.4)	23,016(7,723)	32,222(13,609.2)	29,154(30,636.8)	46,032(20,963.9),	39,894(22,689.8)
Unid. Centric diatom diam 10-30 microns	16,367(7,087.1)	4,587(3,058.4)	7,596(5,819.8)	7,640(3,090)	3,053(3,525.4)	1,534(3,068.8)	3,069(6,137.6)	9,191(10,636.8)
Unid. Centric diatom diam 31-60 microns			4,556(5,815.9)					
Unid. Pennate diatom <10 microns length	135,027(75,420.4)	92,064(41,019.3)	103,888(47,176)	153,440(61,986.9)	112,011(91,224.8)	104,339(54,437.1)	148,837(67,861.4)	179,525(13,8095.9),4

Table 15- continued. Total phytoplankton densities [cells/L], per tank, per time point for the Kenai. Data presented as follows: density (standard deviation); "n"= number of replicate samples. Target (coastal) taxa are identified with an asterisk (*) following the taxon name.

TAXA	2P		6P		2S		6S	
	T0	T1	T0	T1	T0	T1	T0	T1
DIATOM-continued	**n=3**	**n=4**	**n=4**	**n=4**	**n=4**	**n=4**	**n=4**	**n=4**
Unid. Pennate diatom 10-30um length		2,278(2,907.9)	3,037(6,074.5)	15,344(19,082.6)	3,053(3,525.4)	1,519(3,037.3)	6,122(5,011.4)	7,656(3,079.5)
Unid. Pennate diatom 31-60um length			1,519(3,037.3)	1,519(3,037.3)				1,519(3,037.3)
DINOFLAGELLATE								
Amphidinium sp. Syn. *Phalacroma* sp.								3,069(3,543.5)
Amphidinium sphenoides				1,534(3,068.8)				
Gymnodinium sp.#1 5-20um w 10-20um l		1,534(3,068.8)			3,435(3,147)	1,534(3,068.8)	1,534(3,068.8)	12,275(11,205.7)
Gymnodinium sp.#2 21-40um w 21-50um l			1,519(3,037.3)	3,069(6,137.6)		3,037(6,074.5)		
Gyrodinium sp#1 5-20um w 10-20um l		3,037(3,507.1)		15,328(10,648.9)	1,519(3,037.3)	1,534(3,068.8)	1,519(3,037.3)	10,741(7,723)
Gyrodinium sp#2 21-40um w 21-50um l			4,556(9,111.8)		1,519(3,037.3)			1,534(3,068.8)
Katodinium rotundatum			1,534(3,068.8)			1,534(3,068.8)		
Protoperidinium sp.#1 10-30w 10-40l			1,519(3,037.3)					
Scrippsiella trochoidea								1,534(3,068.8)
MICRO-PHYTOFLAGELLATE								
Apedinella radians					3,069(6,137.6)			
Cryptomonas sp#1 length <10 microns*	161,624(30,891.9)	60,609(8,814.5)	220,713(90,974.3)	82,858(36,654.7)	115,080(12,653.1)	85,926(50,859.4)	73,651(39,776.4)	29,154(1,6141.6)
Cryptomonas sp#2 length >10 microns		12,275(10,022.7)	24,340(17,785.1)	4,603(5,876.3)	15,312(19,089.4)	10,741(5,876.3)	1,534(3,068.8)	3,069(3,543.5)
Euglena sp.			1,534(3,068.8)				4,587(5,870.9)	
Pyramimonas sp.		1,534(3,068.8)		1,534(3,068.8)		3,069(6,137.6)		
Unid. Micro-phytoflag length <10 microns	118,660(46,066)	72,884(43,136.2)	85,054(62,308.2)	76,720(33,429.7)	47,566(26,694.4)	93,599(45,758.6)	23,016(22,058.4)	133,493(57,439.5)
Unid. Micro-phytoflag length >10 microns	10,229(17,717.7)	2,302(4,603.2)	9,206(7,923.6)		7,672(5,876.3)	3,053(3,525.4)	3,069(6,137.6)	4,603(5,876.3)

(a)



(b)

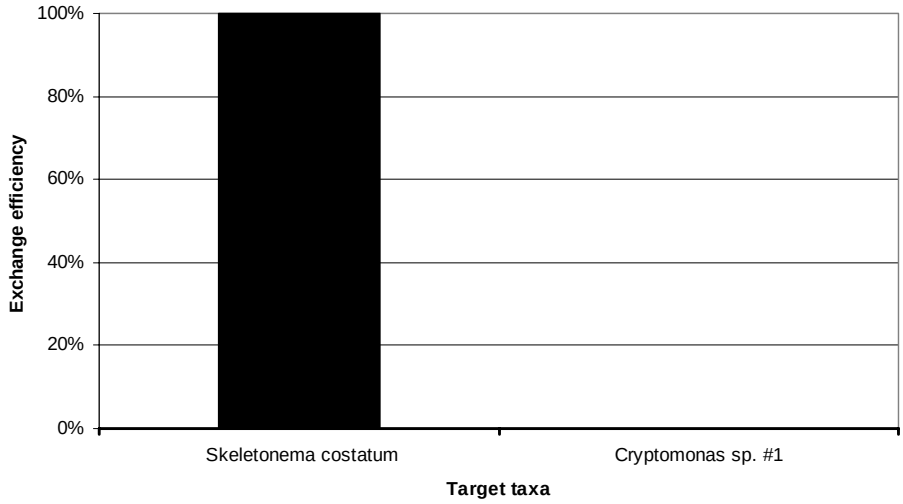
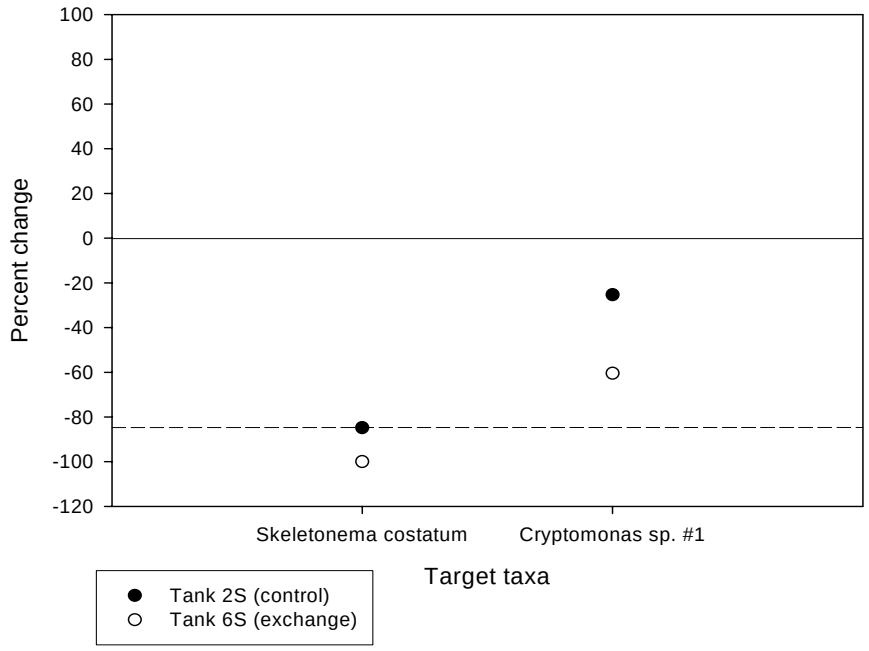


Fig. 5.20: (a) Percent change in density of target zooplankton taxa following exchange (T1) for Tank 2P (control) vs Tank 6P (exchange) on the *Kenai*. The dashed line represents the -85% threshold for target taxa in the control tank. Ballast water exchange efficacy is not calculated for a target if $\Delta C \leq -85\%$. (b) Ballast water exchange efficacies (%) for target zooplankton taxa on the *Berge Nord* , for Tank 6P (exchange).

(a)



(b)

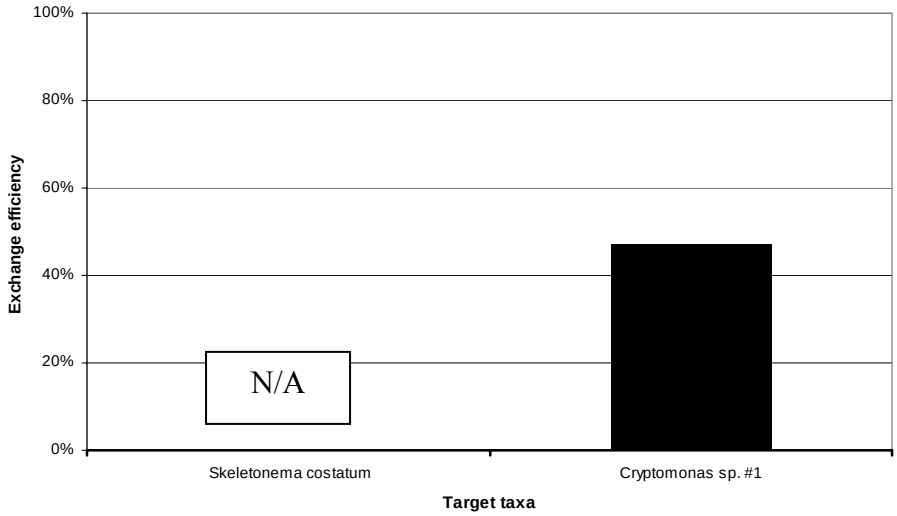


Fig. 5.21: (a) Percent change in density of target phytoplankton taxa following exchange (T1) for Tank 2S (control) vs Tank 6S (exchange) on the *Kenai*. The dashed line represents the -85% threshold for target taxa in the control tank. Ballast water exchange efficacy is not calculated for a target if $\Delta C \leq -85\%$. (b) Ballast water exchange efficacies (%) for target zooplankton taxa on the *Berge Nord* , for Tank 6S (exchange). "N/A" indicates taxa for which the percent change in density in the control tank did not meet the -85% threshold (i.e. the taxa decreased in abundance by >85% in the control tank).

Bacteria and viruses: Bacteria density in the control tanks remained relatively constant but declined following exchange (Fig. 5.22, Table 5.16).

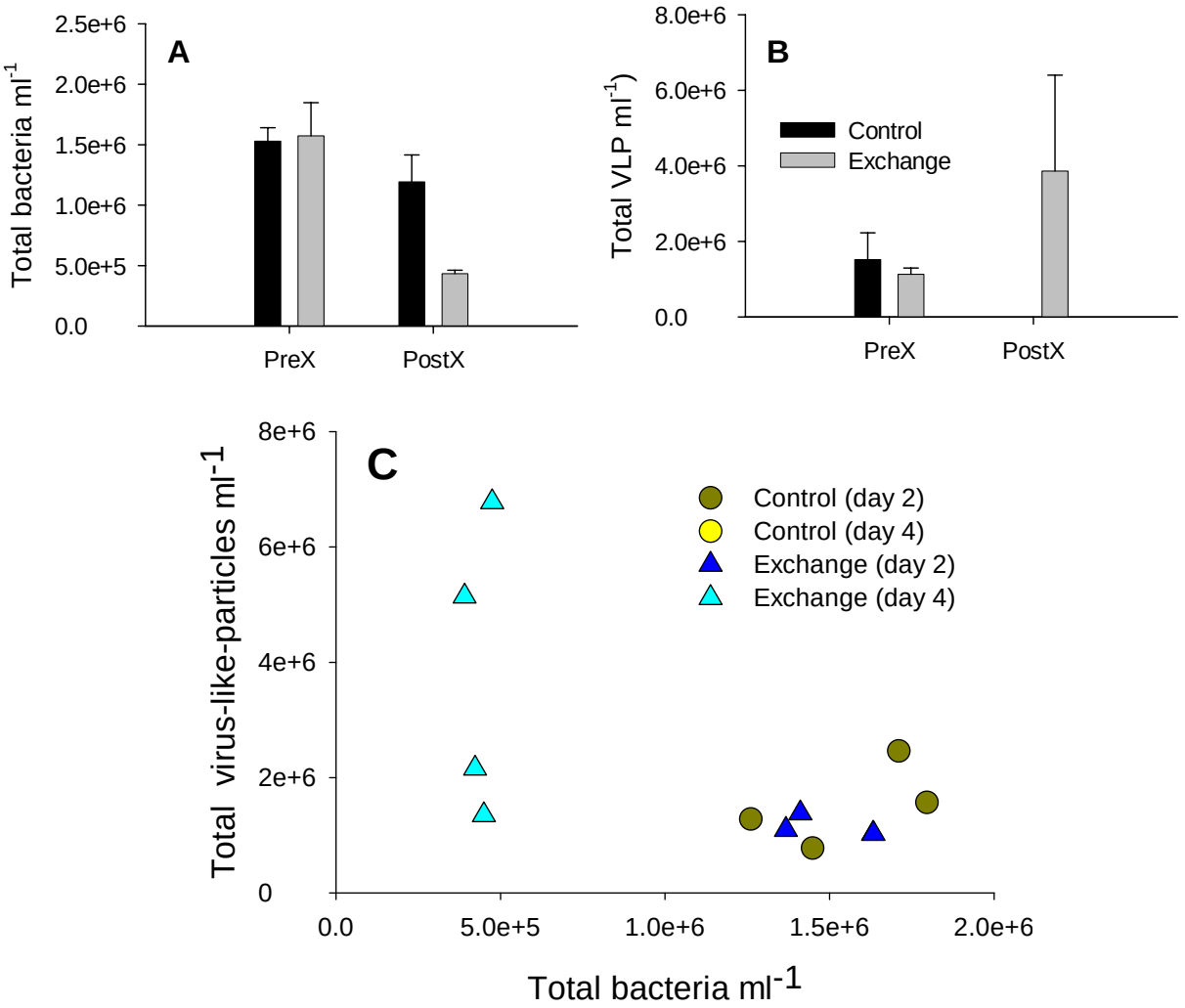


Fig. 5.22. Total bacteria (A) and virus-like-particle (B) abundance in control and exchange tanks on the *Kenai*; (C) Virus and bacteria abundance in control and exchange tanks before and after exchange on the *Kenai*, showing unique character of exchange communities. Note: VLP data for control tanks after exchange are not available.

Table 5.16. Percentage change in virus-like-particle (VLP) abundance, bacteria abundance and virus to bacteria ratio (VBR) from T0 to T1 (before and after exchange) in #1 and #3 ballast tanks on the *Kenai*. N/A: Data not available.

	VLP (cells/ml)		Bacteria (cells/ml)		VBR	
Tank	ΔControl	ΔExchange	ΔControl	ΔExchange	ΔControl	ΔExchange
Port	N/A	+215.6	-16.5	-76.2	N/A	+1182
Starboard	N/A	+260.5	-28.2	-68.0	N/A	+988

5.3.2. Salinity Tolerance Experiments

Shipboard zooplankton caging experiments

SERC tested the effect of salinity shock (approximately 5 ppt increase) associated with ballast water exchange on the survival of a target organism (mysid shrimp) from the Port of Rotterdam. All specimens were recovered from the cages at the end of the experiment. The average survivorship in both tanks was very high (control: 94%, exchange: 91%; Fig. 5.23). Survivorship data were subjected to a two-way ANOVA and did not differ significantly amongst the exchanged and control tanks [$F(1, 22)$, p=0.38], or the two depths [$F(1,22)$, p=0.653]; nor was there a significant interaction between these two factors [$F(1,22)$, p=0.91] (Table 5.17). Consequently, there was no evidence that salinity shock affected the short-term survival of this species on this voyage.

Table 5.17. Results from a two-way ANOVA with replication to evaluate the effect of tanks (control vs. exchange) and depths (1 m vs 3 m) for a shipboard salinity tolerance experiment. No significant effects were found.

Factor	**df**	**SS**	**MS**	***F* ratio**	***P*-value**
Tanks	1	0.05128	0.05128	0.82	0.376
Depth	1	0.01304	0.01304	0.21	0.653
Interaction	1	0.00090	0.00090	0.01	0.906
Error	22	1.37946	0.06270		

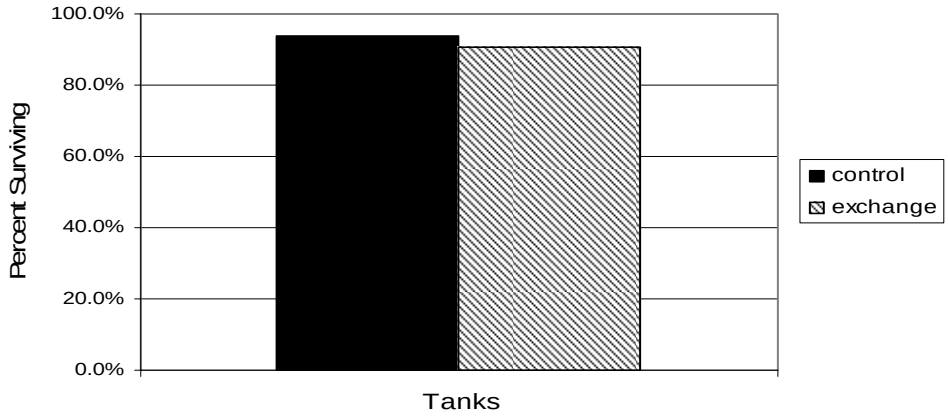


Fig. 5.23: The following graph presents the percent of mysid shrimp surviving in each tank (control vs. exchange) after 24 hours exposure to post-exchange conditions. Survivorship was not significantly different between tanks or between depths at which cages were submerged (1 m vs. 3 m).

Laboratory Salinity Tolerance Experiments

Laboratory-based salinity tolerance experiments were conducted on a total of 14 different taxa collected from low-salinity and freshwater sources in the Upper Chesapeake Bay area (Table 5.18). The resulting data were evaluated for the proportion of taxa surviving each treatment at the end of each trial (48 hours).

A two-way ANOVA with replication was conducted to evaluate the effect of species and treatments (Table 5.19). Since there was a significant species x treatment interaction [$F(26,126)$, $p<0.0001$], each species was then analyzed independently using a one-way ANOVA. ANOVA results and survival for individual species are shown in Fig. 5.24**.**

There were three taxa for which neither treatment (flow-through nor empty-refill) had an effect: *Rhithropanopeus harrisii* zoeae, the harpacticoid copepods, and the barnacle (cirriped) nauplii. To the other extreme, four taxa experienced 100% mortality in both the flow-through and empty-refill treatments. These were Rotifera, Cladocera, and the copepods *Acartia* sp., and *Eurytemora* sp. Three taxa (Polychaetes – general, bivalve veliger larvae, and spionid polychaete larvae) experienced significant reductions in both treatments, with empty-refill being more effective than flow-through. Only the empty-refill treatment had a significant effect on the remaining four taxa (*Corophium* spp., *Gammarus* spp., Platyhelminthes, and *Leptinogaster* sp.). The extent of the effect of the empty-refill treatment varied by species in this group, ranging from near 80% survival for *Gammarus* spp. to 100% mortality for the Platyhelminths.

Table 5.18. Taxa collected for laboratory-based salinity tolerance experiments, and the ambient salinity and temperature at the time of collection.

Taxa	Collection Date	Ambient Salinity (ppt)	Ambient Temperature (Deg C)
Amphipods			
Gammarus spp.	10-May-2004	6.0	22.5
Corophium spp.	10-May-2004	6.0	22.5
Crabs			
Rhithropanopeus harrisii zoea	18-May-2004	4.5	25.2
Copepods			
Harpacticoida	18-May-2004	4.5	25.2
Acartia spp.	26-Jul-2004	5.9	26.0
Eurytemora spp.	9-Aug-2004	0.2	26.5
Leptinogaster major	27-Sep-2004	9.7	23.0
Barnacles			
Cirriped nauplii	17-Aug-2004	7.2	26.2
Water Fleas			
Cladocera	7-Jun-2004	0.1	21.0
Polychaete worms			
Polychaeta - general – late trochophore larvae	12-Jul-2004	9.0	28.0
Spionidae – late trochophore larvae	20-Sep-2004	4.9	23.7
Flatworms			
Platyhelminthes	30-Aug-2004	6.8	27.6
Molluscs			
Bivalve veligers	2-Aug-2004	7.9	29.0
Other			
Rotifera	7-Jun-2004	0.1	21.0

Table 5.19. Results from a two-way ANOVA with replication to evaluate the effect of species and treatments for laboratory salinity tolerance experiments. Results are significant at all levels.

Factor	**df**	**SS**	**MS**	***F* ratio**	***P*-value**
Species	13	28.92202797	2.22477138	47.00	<0.0001
Treatment	2	11.48368916	5.74184458	121.31	<0.0001
Interaction	26	9.34423724	0.35939374	7.59	<0.0001
Error	126	5.96394444	0.04733289		

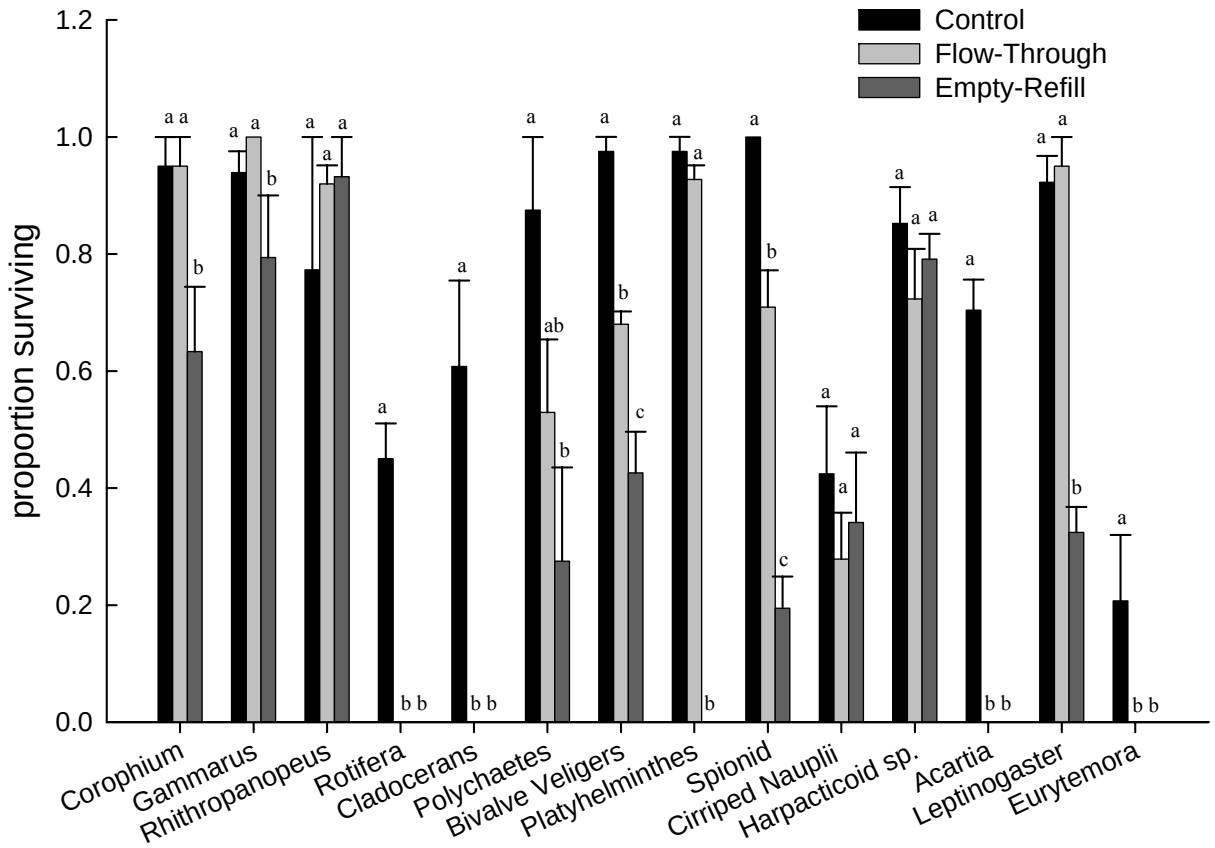


Fig. 5.24: Proportion of each species surviving salinity tolerance experimental trials at the end of each trial (48 hours). Results of one-way ANOVAs are indicated by letters at the top of each bar. Different letters indicate significant differences within each species (*e.g.* "a" & "a" are not statistically different from each other, but "a" & "b" are significantly different). These ANOVA results are not comparable across species.

5.3.3. Summary of Ballast Water Exchange Efficacy Results Across Voyages

Physical tracers

Changes in the concentration of the physical tracers, salinity and rhodamine dye, were used to calculate ballast water exchange efficacy with regards to removing the original water mass from the tanks. For each tracer, high exchange efficacies were calculated for all three vessels. Exchange efficacy based on salinity measurements ranged from 80.0% (*Federal Progress*) to 100.1% (*Berge Nord)* (Fig. 5. 25; Table 5.20). Exchange efficacy based on rhodamine-dye ranged from 86.4% (*Berge Nord*) to 98.5%, (*Federal Progress*) (Fig. 5.26; Table 5.20). Exchange efficacy values for the *Berge Nord* and the *Kenai* were slightly higher when calculated using the changes in salinity than when using the changes in rhodamine; however, the opposite was true for the *Federal Progress* (Table 5.20).

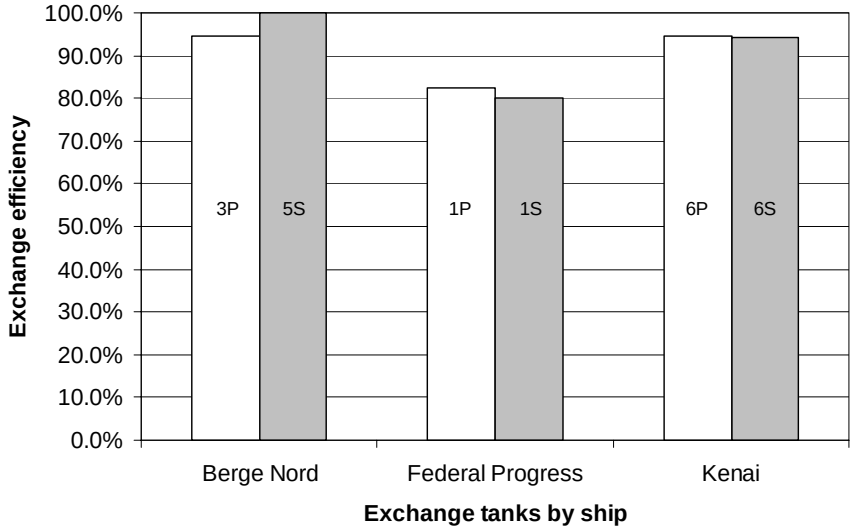


Fig. 5.25. Summary of salinity-based ballast water exchange efficacies across ships.

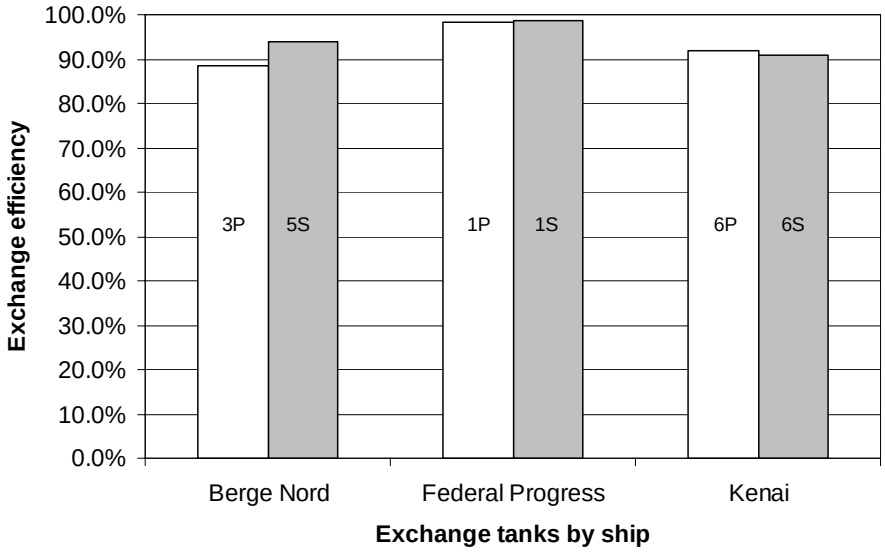


Figure 5.26. Summary of Rhodamine-based ballast water exchange efficacies across ships.

Table 5.20. Exchange efficacies in ballast tanks on the *Berge Nord, Federal Progress* and *Kenai*. +100% is the maximum value and indicates complete removal of the parameter as measured before and after exchange. Negative values indicate an increase in the parameter after exchange. "N/A" indicates target taxa for which exchange efficacies were not calculated due to ≥ 85% density decrease in the control tank. Blank cells represent taxa either not present in a given tank, or not present in high enough densities to be considered as target taxa.

Parameter	Ballast Water Exchange Efficacy (%)					
	Berge Nord		***Federal Progress***		***Kenai***	
	Tank 3	**Tank 5**	**Tank 1P**	**Tank 1S**	**Tank 6P**	**Tank 6S**
Physical Water Tracers						
Salinity	94.7	100.1	82.4	80.0	94.6	94.1
Rhodamine WT tracer dye	86.4	95.3	98.2	98.5	91.8	90.7
Zooplankton Target Taxa						
Calanoida - Eurytemora sp.			N/A			
Calanoida - *Temora longicornis*	99.2	99.1				
Calanoida - *Tortanus* spp.					95.1	98.2
Cyclopoida - *Limnoithona* spp.					75.4	78.7
Harpacticoida - *Euterpina acutifrons*	100.0	100.0				
Barnacle nauplii	N/A	100.0				
Cyprids	96.5	88.8				
Decapod zoea	N/A					
Mysidaceae	84.1					
Cyphonautes					99.8	100.0
Cumacea	N/A					
Bivalvia					56.6	98.4
Gastropoda					-23.0	84.6
Polychaeta						-76.7
Polychaeta - Spionidae	96.0	97.6				
Rotifera			N/A			
Phytoplankton Target Taxa						
Bacterosira bathyomphala	10.5	41.7				
Rhizosolenia hebetata	N/A	-1.6				
Skeletonema costatum					100.0	N/A
Cryptomonas sp. #1					-0.11	47.0

Biological tracers

Zooplankton: A total of 16 zooplankton taxa met the criteria for use as target taxa across all three voyages (Table 5.20). No single taxon qualified as a target on more than one vessel. The majority of these taxa experienced changes in density between -85% and -100% in the exchange tanks (Fig. 5.27). Two taxa, Polychaeta and Gastropoda, actually increased in one of two exchange tanks following exchange on the *Kenai*. These two taxa are represented by the outlier points in the upper right quadrant of the graph in Fig. 5.27. Changes in the control tanks were more variable than those in the exchange tanks, with the majority of targets changing in abundance between -30% to +15%.

Comparison across target taxa indicates that in most cases, ballast water exchange efficacy is >90% (Table 5.20). For five of the targets, exchange efficacy is between 95% and 100% in both tank pairs of their respective vessels (*Temora longicornis*, *Tortanus* sp., *Euterpina acutifrons*, cyphonautes larvae, and spionid polychaetes). Of the targets that were present in more than one tank pair, exchange efficacy values were generally within ± 3% of each other between the tanks of a vessel. The notable exceptions are Cyprids (96.5% vs. 88.8%), Bivalvia (56.6% vs. 98.4%) and Gastropoda (-23.0% vs. 84.6%) [*Note: a negative exchange efficiency is equivalent to an increase in the exchange tank following exchange.*]

Ballast water exchange was least effective for Polychaeta (-76.7%), followed by Gastropoda (-23.0%) and Bivalvia (+56.6%). However, the latter two also had high efficacies in the second exchange tank of the pair, as noted in the previous paragraph. There are five taxa for which ballast water efficacy could not be calculated because they did not meet the -85% threshold for change in the control tanks (Table 5.20). These are *Eurytemora* sp., barnacle nauplii (in 1 of 2 exchange tanks), decapod zoea, Cumacea, and Rotifera.

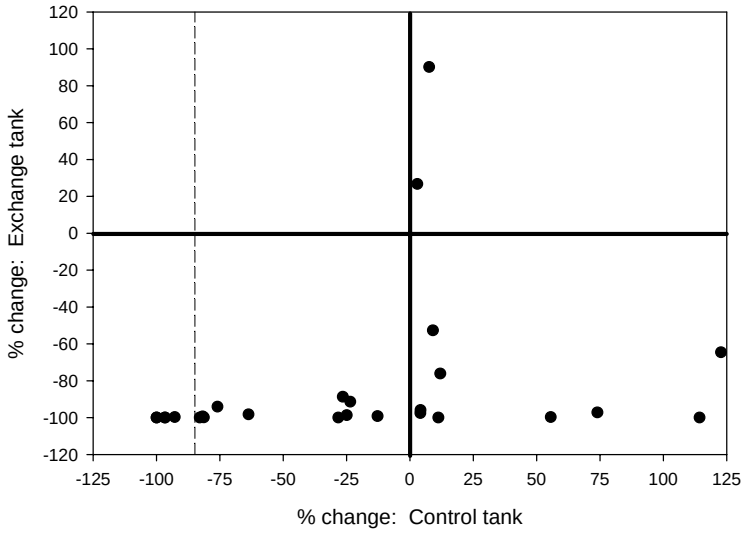


Fig. 5.27: Percent change in zooplankton target taxa density for exchange tanks vs. control tanks across all voyages. This graph includes all target taxa for each tank pair analyzed. The dashed line represents the -85% threshold for target taxa in the control tank. Ballast water exchange efficacy is not calculated for a target if $\Delta C \leq -85\%$.

Phytoplankton: Only four taxa qualified as phytoplankton target taxa across the three voyages (Table 5.20). As with the zooplankton, no single taxon qualified as a target on more than one vessel. All targets declined between 60-100% in the exchange tanks and, unlike the zooplankton, all phytoplankton targets decreased in concentration in the control tanks (Fig. 5.28). As a result, exchange efficacies for phytoplankton were considerably more variable than for zooplankton, ranging from –1.6% (*Rhizosolenia hebetata* in Tank 5, *Berge Nord*) to 100% (*Skeletonema costatum* in Tank 6P, *Federal Progress*) (Table 5.20).

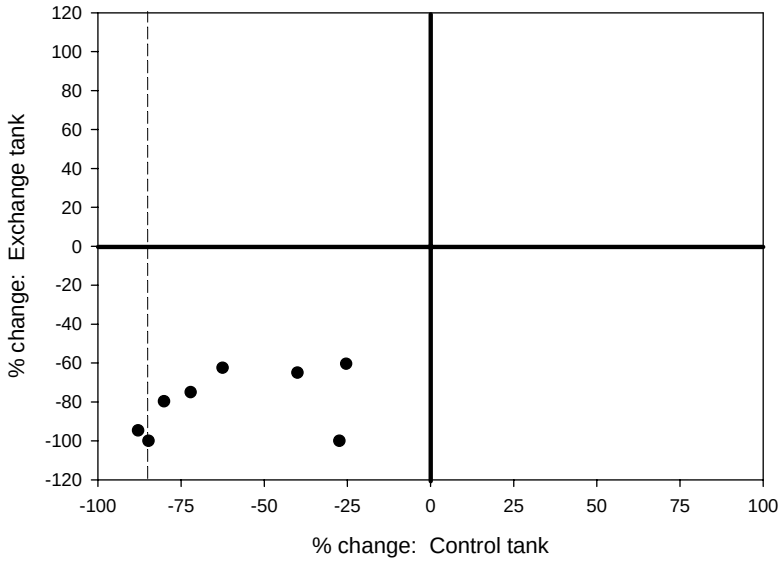


Fig. 5.28. Percent change in phytoplankton target taxa density for exchange tanks vs. control tanks across all voyages. This graph includes all target taxa for each tank pair analyzed. The dashed line represents the -85% threshold for target taxa in the control tank. Ballast water exchange efficacy is not calculated for a target if $\Delta C \leq -85\%$.

Comparing these species-specific results to gross changes in the phytoplankton (using chlorophyll-*a* as an index of biomass), results were mixed across experiments. On the *Berge Nord*, chl-*a* decreased in both control and exchange tanks, but at T1, exchange tanks had higher chl-*a* (Fig. 5.6). On the *Federal Progress*, chl-*a* concentrations in both control and exchange tanks were relatively low, but there was no difference between them after exchange (see Fig. 5.13a). However on the *Kenai*, there was a bigger decline in chl-*a* concentration in exchange versus control tanks (Fig. 5.19a).

Bacteria and viruses: While we didn't identify specific bacterial or viral targets for calculating exchange efficacy, we calculated the percentage change in the total abundance of bacteria and viruses to see if there were any differences as a result of exchange. Table 5.21 shows that for 2 of 3 experiments, bacteria abundance declined in tanks as a result of exchange—only on the *Berge Nord* did it increase. Further, VLP abundance declined in tanks as a result of exchange in 2 of 3 experiments—only on the *Kenai* did it increase. In all cases, the exchanged population had a unique signature of bacteria and VLP abundance (see Figs. 5.10, 5.14, and 5.22).

Table 5.21: Comparison of percentage change in bacteria abundance on the *Berge Nord*, *Federal Progress* and *Kenai* voyages in control and exchange tanks. For the *Berge Nord* experiment, results are listed for #3 (*) and #5 (**) tanks, rather than port and starboard.

	Berge Nord		***Federal Progress***		***Kenai***	
Tank	ΔControl	ΔExchange	ΔControl	ΔExchange	ΔControl	ΔExchange
Port*	+8.4	+134.2	71.5	-70.1	-16.5	-76.2
Starboard**	+149.3	+163.3	82.6	-67.0	-28.2	-68.0

5.4. Discussion

5.4.1. Exchange efficiency

The three ballast water exchange experiments conducted for Task 3 resulted in exchange efficacies of 80% to 100% for the majority of parameters measured. Exchange efficacies based on the removal of the original water mass (as measured by changes in physical tracers) fell consistently within this range. Exchange efficacies for individual biological tracers were somewhat more variable, both among and within the ships tested, but the majority were exchanged at 80% or greater efficacy. Exchange efficacy was not noticeably different between high-salinity ballast water and low-salinity ballast water or between flow-through and empty-refill methods of exchange for the ships compared in this study. Laboratory-based salinity tolerance experiments were deemed necessary to address the question of whether "salinity shock" improves the effectiveness of ballast water exchange with respect to killing organisms from low-salinity environments that remain in the tank following mid-ocean exchange. These laboratory experiments showed a variable response to high salinity exposure across taxa, with empty-refill exchange having the most significant negative effect on survival. This discussion will review the key findings of the Task 3 experiments and expand upon their meaning with regard to the impact of mid-ocean exchange on low-salinity ballast water.

For all three experimental voyages, we initially targeted ships making repeat voyages from low salinity ports in the Baltic/northern Europe region to the U.S. east coast or Great Lakes region. However, it proved exceedingly difficult to identify suitable candidate vessels. Some of the most common difficulties included the fact that vessels were: (1) from high-salinity source ports or berths; (2) traveling fully loaded with cargo and carrying no ballast between Europe and the U.S.; (3) too small to accommodate the research team; and/or (4) fitted with ballast tanks whose physical design (e.g., depths or configurations) prohibited access or were incompatible with required sampling methods. For these reasons, we found it necessary to shift our efforts to locating vessels originating from any low salinity port and engaging in a voyage of at least 5 days length, regardless of the destination port. Locating vessels was quite difficult even using these broadened search criteria. One major constraint was the number of suitable vessels that were operating on a spot market. In this type of market vessels often do not know their destination until the last minute and schedules can shift suddenly as the company works up until the last instant to broker the best cargo deal. In one instance, we were offered a vessel 48 hours before its departure from a low salinity U.S. port, which did not allow us adequate time to

prepare our equipment and make it to the departure point in time; and the vessel still did not know its final destination port at the time it sailed.

The final result of our ship search was three voyages originating from ports in three different geographic areas (Rotterdam, The Netherlands; La Baie, Canada; and Benicia, California). For each of the three ports selected, advance information indicated that it would be a suitable low-salinity port. However, the salinity was higher than anticipated upon the research team's arrival in each location. The discrepancy was the most extreme at the Port of Rotterdam, where source ballast salinity averaged ~30 ppt. The research team chose to continue with this experiment (*Berge Nord*), the results of which provided a measure of comparison between exchange efficacy with high salinity water and exchange efficacy with low salinity water. The second and third voyages (*Federal Progress* and *Kenai*), while starting at higher than optimal salinities, were comparable to each other because they had intersecting ranges of starting salinities (*Federal Progress*: 11-19 ppt; *Kenai*: 15.4-15.8 ppt). While not in the preferred range of 5 ppt or less, the initial salinities for these two voyages were still sufficiently below those typical of the mid-ocean to allow analyses based on changes in salinity.

Both salinity and the tracer dye Rhodamine WT were used as means of judging the effectiveness of ballast water exchange in removing the original coastal water mass from a ballast tank. The differences between the two methods' results were quite small in the *Berge Nord* and the *Kenai*, with salinity-based calculations yielding only slightly higher exchange efficacies (Table 5.20). However, the differences between the two methods were more pronounced for the *Federal Progress*, with rhodamine-based calculations yielding exchange efficacy values in the upper 90th percentile and salinity-based calculations resulting in values in the lower 80th percentile. The differences between the two parameters' results could be related to the degree of dye mixing achieved in the tanks, which in turn can be influenced by density-driven stratification of the seawater in the tanks and/or the tank structure itself. The *Federal Progress* was the only vessel in which salinity stratification was evident within the depths sampled. This may have influenced the distribution of Rhodamine, or it may instead have skewed the salinity-based results. Tank structure varied among all three vessels, with varying degrees of compartmentalization within the tanks. In-tank structure (i.e. horizontal and/or vertical partitions or bottlenecks such as a trunk connecting a double-bottom to a top-side tank) can influence flow dynamics and create restrictions that can prevent complete mixing of the dye.

Overall, exchange efficacy with regard to the water mass was high among all three vessels, and was not noticeably different when comparing type of exchange [flow-through (*Berge Nord*) vs. empty-refill (*Federal Progress* and *Kenai*)] or starting salinity [high (*Berge Nord*) vs. low (*Federal Progress* and *Kenai*)]. The efficacy of exchange in removing biological specimens was more variable, both among and within vessels. This variability may be attributed to a number of different factors.

For all voyages, the density of organisms in the exchanged tanks generally decreased following exchange. There were exceptions to this on every voyage (for example, copepod nauplii and Harpacticoida on the *Kenai*, Table 5.13), most of which may be due to the replacement of coastal specimens of a broad taxonomic group with mid-oceanic specimens from the same group. These organisms were generally not classified as target taxa and so exchange efficacies were not

calculated in these instances. However, in a few instances zooplankton target taxa actually increased in density at T1, leading to a very low (negative) exchange efficacy. This occurred on the *Kenai* for Gastropoda, in Tank 6 port, and for Polychaeta, in Tank 6 starboard, (exchange efficacies of -23.0% and -76.7%, respectively). While ballast water exchange for this vessel occurred only about 50 nautical miles offshore, it is unlikely that this increase in organisms is due to a patch of nearshore water being introduced to the tank. Had that been the case, we would have expected to see similar increases for other coastal taxa in the tanks, which did not occur. Also, Gastropoda was traceable as a target taxon in both exchange tanks on the ship and had a high exchange efficacy (84.6%) in the second tank (Tank 6 starboard, the same tank in which Polychaeta increased). Thus, it seems unlikely that this organism was strongly avoiding exchange. The increases of these coastal taxa following exchange may be due to either (1) patchy distribution of the organisms, hence a denser patch of organisms was sampled at T0 than had been sampled at T1, or (2) resuspension of the organisms in the water column by the turbulence induced during tank refill.

Zooplankton density changes in the control tanks were more variable than those in exchange tanks, but for the most part remained within ±30% of the initial density measured at T0. In a few instances, density increased or decreased by a large proportion for a target taxon in a control tank. Examples include, Mysidaceae in *Berge Nord*'s Tank 3 control (122%), *Eurytemora* sp. in *Federal Progress*' Tank 3 port (–99.9%), and *Limnoithona* sp. in *Kenai*'s Tank 2 port (-76%). Since the control tanks are held for the duration of the experiment, density changes must be due to the sampling of heterogeneously distributed populations (i.e. "patchy" distribution in the tank leads to unpredictable highs or lows) (Murphy et al. 2002) and/or natural attrition due to unfavorable conditions in the tank (waning food sources, temperature changes, predation).

In the case of the *Federal Progress,* both of the zooplankton target taxa (*Eurytemora* sp. and Rotifera) declined by 99.9% from their original abundance in the control tanks. This result could be due to a number of factors.

(1) After boarding the *Federal Progress* and discussing which tanks were available to us, we realized that the #3 tank pair was the only pair that could work as our control tanks. Later we were informed that this tank pair had been recently treated with an anti-rust treatment. The treatment was greasy to the touch and left a slippery film on equipment used in the tank. It also had a strong petroleum odor that was noticeable before and after the tanks were filled. This chemical treatment may have had a negative effect on the survivorship of organisms entrained in these tanks and accelerated any natural attrition that took place.

(2) All tanks on this voyage underwent an extreme temperature change between initial sampling at T0 and final sampling at T1. The control tanks experienced an approximate 15˚C increase in temperature in this timeframe. Temperature stress may have played a role in the attrition rate in these tanks (and possibly in the exchange tanks, as well).

(3) There was significant seiching (i.e. "sloshing") in all the tanks of this vessel. Organisms in these tanks may have been subjected to a heavy battering as a result.

In cases such as this, a very large decline in the control tank makes it difficult to accurately assess the effect of exchange. Since exchange efficacy is assessed as a change in concentration that occurs only as a result of the exchange process, one must account for what would happen "naturally" in the tank without the exchange (i.e. what occurs in the control tank). If a proportionally large decline occurs in the control tank, it is assumed that an equivalent decline will also occur on average in the paired exchange tank. Any additional decline beyond this is assumed to be a result of the exchange. If the density of a target taxon has already declined significantly at T1 without exchange, it becomes increasing difficult to assess the effectiveness of the exchange itself based on the very small proportion of organisms remaining. For this reason, we instated a threshold of $\Delta C \leq -85\%$ for the percent change in control tanks for any given target taxa. At or below this threshold, the control tank is no longer useful in calculating the exchange efficacy and so no calculation is performed. In the case of the *Federal Progress*, both targets (*Eurytemora* sp. and Rotifera) declined in density by approximately 100% in both the control tank and the exchange tank. As a result, no conclusions can be made about the effect of exchange on target taxa in the tanks of the *Federal Progress*, because there was nothing left in the controls for comparison. Other instances of target taxa that did not meet the -85% threshold in these experiments were barnacle nauplii, decapod zoea, and Cumacea, all occurring in the Tank 3 control on the *Berge Nord.*

The results for phytoplankton as biological tracers were much more variable than for zooplankton. In general, cell concentrations were highly variable for individual taxa from all voyages, as evidenced by the standard deviations given in Tables 5.7, 5.11, and 5.15**.** When phytoplankton are entrained in darkened ballast tanks, a large proportion die (i.e. exponential decline in chlorophyll-*a* concentration—see Task 2). Unlike zooplankton, which are not directly dependent on sunlight to stay alive, most phytoplankton species need light to photosynthesize and acquire energy. Phytoplankton may also experience significant mortality due to predation from zooplankton or infection by bacteria or viruses present in ballast tanks. Variability in abundance may also be due to resuspension of taxa from bottom sediments during exchange, or the potentially low likelihood of acquiring a representative sample from restricted tank access ports. (Note that little is known about spatial variability or patchiness of phytoplankton in ballast tanks).

A complicating issue in interpreting the phytoplankton results from the *Berge Nord* and *Federal Progress* experiments is that samples were stored for 12-17 months before analysis, and this likely compromised them. Many of the samples contained fungal contamination with evidence of cell deterioration (H. Marshall, Old Dominion University, pers. comm.).

As a result of these issues, there were significant challenges in choosing target taxa to calculate phytoplankton exchange efficacies. While an exchange effectiveness of 100% was achieved for the diatom *Skeletonema costatum* in the *Kenai*'s port-side exchange tank, all other targets experienced marked declines in the control tanks, suggesting that natural attrition plays a bigger role than exchange in the removal of these species. This is supported by the significant decline in chlorophyll-*a* concentration in control tanks on the *Berge Nord* (see Fig. 5.6). In any case, the low number of target taxa available for analysis makes it difficult to determine the effectiveness of ballast water exchange in removing coastal phytoplankton species.

The situation is different when considering the smallest components of the microbial population—bacteria and viruses. Our analyses showed significant changes in bacteria and virus density as a result of exchange, changes greater than the dynamics observed in control tanks. This statement is clearly demonstrated by Fig. 5.10b, which shows the unique nature of the virus to bacteria ratio in exchange tanks following open-ocean exchange. Further, in all experiments, bacteria abundance was significantly higher (*Berge Nord*) or lower (*Federal Progress and Kenai*) in exchange compared to control tanks (Table 5.21). While these results cannot provide a method for determining exchange efficacy (because we cannot identify the difference between "new" and original cells inside tanks), it is clear that relative changes in microbial abundance can provide an index of exchange effectiveness on a ship-by-ship basis.

It should be noted that our data only assess the presence or absence of organisms and do not make assumptions regarding their viability at the time of sampling. It is possible that the health of organisms counted as present following exchange was compromised in some fashion. The post-exchange presence of an organism does not imply its viability at the time of ballast release. Therefore, the analyses presented here are a minimum estimate of the efficacy of exchange.

5.4.2. Toxicity Effects of Saltwater Exposure

Though exchange experiments are useful in determining the efficacy of exchange in removing certain organisms from a ballast tank, they do not determine what effect exposure to high salinity has on any low-salinity organisms remaining in the tank after exchange. One method of exploring this question is through shipboard salinity tolerance experiments like the one carried out on the *Berge Nord*. However, in the context of the Task 3 exchange experiments, this salinity tolerance experiment provided very limited information. Only one organism large enough to "cage" (a mysid shrimp) was collected in sufficient densities on the *Berge Nord* to provide statistically valid results. There was no short-term effect of salinity increase for this organism, possibly due to the fact that the salinity only increased by 5 ppt during the exchange process. Most estuarine organisms have developed tolerance to a much broader degree of change due to the extremely variable conditions in their environment (Day et al. 1989). It is preferable to conduct these experiments on vessels with a larger difference between starting salinity and post-exchange salinity, but comparable experiments were not possible on the *Federal Progress* and the *Kenai* due to a lack of appropriately sized organisms in high abundance.

Given the limited availability of organisms suitable for shipboard experiments, it was necessary to expand the scope of this project to include laboratory-based salinity tolerance experiments. Experimental trials run on 14 different zooplankton taxa from low-salinity and freshwater habitats in the Upper Chesapeake Bay watershed demonstrated varying degrees of response to high salinity exposure across taxa (Fig. 5.24). Overall, the empty-refill treatment more consistently had a negative effect on survivorship than did the flow-through treatment. Four taxa experienced 100% mortality in both the flow-through and the exchange treatments (Rotifera, Cladocera, *Acartia* spp. and *Eurytemora* spp.); however, these 4 taxa also experienced higher mortality in the control dishes than the majority of taxa tested. It is possible these taxa are more sensitive to handling in the laboratory than others, as well as being sensitive to increased salinity.

Groups that exhibited high survivorship in both of the exchange treatments *and* the controls are the ones that warrant close scrutiny with regard to invasion potential. In this set of experiments, those were the amphipods *Gammarus* spp. and *Corophium* spp., zoea larvae of the crab *Rhithropanopeus harrisii*, and an unidentified species of juvenile harpacticoid copepod. These results are particularly interesting in light of the two amphipod invaders (*Gammarus fasciatus* and *Echinogammarus ischnus*) and four harpacticoid copepod species (*Nitocra hibernica, Nitocra incerta, Heteropsyllus* cf. *nunni*, and *Schizopera borutzkyi*) that have already become successfully established in the Great Lakes (Mills et al. 1993, Ricciardi 2001, Grigorovich *et al.* 2003), and the notable number of amphipod invasions that have occurred in the Baltic region in recent years (Jazdzewski 2002, Jazdzewski et al. 2004). It would be worthwhile to include known amphipod and harpactacoid invaders in future experiments to clarify if mid-ocean exchange is a useful management technique with regard to these taxa.

Chapter 6. Summary and Recommendations

The issue of NOBOB (no-ballast-on-board) vessel operations in the Great Lakes has risen from a position of relative obscurity to become a major concern in the Great Lakes basin today. NOBOB vessels escape scrutiny under existing U.S. and Canadian federal, state, and provincial laws designed to prevent the introduction of nonindigenous species (NIS) into the Great Lakes, yet their ballast tanks retain residual volumes of unpumpable ballast water and sediment that may contain live aquatic organisms and resting stages - eggs, spores, and cysts - accumulated over numerous previous ballasting operations. At the same time, open-ocean ballast water exchange (BWE) is the only presently accepted management practice for reducing the invasion risk associated with pumpable ballast water discharge. For the Great Lakes the use of BWE as a prevention tool has been based on the assumption of two processes: physical exchange whereby freshwater and coastal organisms – those representing the greatest threat to the Lakes' ecosystems – would be greatly reduced in abundance by physical flushing from the tanks, and "salinity shock", whereby organisms not adapted to the salinity of open ocean water would be killed. Yet the efficacy of BWE with respect to minimizing species introductions has been unclear, and experimental results to evaluate it have varied widely.

This multidisciplinary research program was designed to directly characterize and assess the NIS invasion risk from ballast water discharges associated with overseas vessels operating in the Great Lakes. It addresses both the NOBOB and the BWE concerns. For the NOBOB issue, it provides the beginnings of a scientific foundation for developing new policies and for identifying effective preventive measures and treatments. For BWE it expands our understanding of both exchange efficacy and "salinity shock."

This chapter provides a synopsis of our major observations, findings, and conclusions. Although we reproduce some of the details from the body of this report, the reader is encouraged to refer to the individual chapters for the complete presentation of data and discussions not presented below.

6.1. Water Ballast and Sediment Management in NOBOB Ships

In Chapter 2 we provided a summary of saltwater vessel traffic entering the Great Lakes and an analysis of the current ballast management practices being applied on a cross-section of Great Lakes saltwater vessels (103 on-board surveys). Imbedded within these topics was an extensive discussion of the nature of ballast tanks and related operational and structural constraints, as well as possible design improvements that would be beneficial in reducing sediment accumulation.

With very few exceptions there was awareness by ship staff of NIS issues, and whether for environmental or commercial reasons there were conscientious efforts being made to minimize the amounts of total residuals and especially sediment residuals being carried in ballast tanks.

We compared records of vessel entries into the Great Lakes over the period 1995 through 2000 and found discrepancies in the number of entries and their ballast condition, but general agreement that the total number of entries in recent years has generally been in the range of 500-

600 hundred ships annually. While the St. Lawrence Seaway authorities and the U.S. Coast Guard both keep fact-based vessel entry records, their methods of classifying the ballast condition of each vessel are for either regulatory or business purposes and do not necessarily agree, nor provide the direct details needed for accurate assessment of NOBOB vs. BOB status from the NIS risk perspective. In particular, ships that enter Canadian or U.S. waters as NOBOBs, but ballast at freshwater ports in the St. Lawrence River between Quebec City and Montreal are not subject to the deep-water ballast exchange requirements, yet are classified as ballasted vessels. For purposes of NIS risk assessment, these ships should be counted as NOBOBs.

The total amount of ballast residuals (water + sediment) carried per ship captured by our on-board survey ranged from negligible to 200 metric tonnes (t), averaging ~62 t. The amount of sediment residual ranged from negligible to 100 t, however, 60% of the ships carried less than 10 t of sediment, resulting in an overall average of 15 t. *Drainage restrictions resulting from inadequate design or construction considerations were identified as a significant contributory factor in sediment retention and accumulation.*

The sources of residual ballast being carried into the Great Lakes by the vessels we surveyed were global in nature, but Western Europe was the region from which the greatest number of ballasting operations had occurred. The Great Lakes were the second most predominant ballast source region among the vessels we surveyed.

Based on our analysis of the vessel traffic records we reviewed, we confirm the conclusion of Colautti et al. (2004) that over 90% of the ocean ships entering the Great Lakes do so as NOBOBs in terms of considering NIS risk assessment. One major difference between our results and those of Colautti et al. is the identification of the Great Lakes as a significant ballasting source contributor. However, given the multiple port cargo operations typical of the Great Lakes salties, this comes as no surprise, but does fill a gap in the otherwise excellent analysis by Colautti et al. (2004).

The most common management practices used to control sediment accumulation were to limit the total amount of ballast and/or the number of tanks utilized when ballasting in high turbidity locations, or to exchange such ballast in the first clean water location reached on passage. Flushing of ballast tanks to specifically loosen and remove sediment, either in conjunction with ballast water exchange or by introducing a small volume of seawater ballast during a loaded passage was the next most common practice. The latter practice, when possible, also subjects any freshwater biota to saline conditions, similar to open-ocean ballast water exchange.

We also found that numerous ships trading between North Europe and the Great Lakes repeatedly ballast in low-salinity or fresh water at both ends of their trade, but make their crossing as NOBOBs, i.e., fully loaded with cargo, and thus contain no declarable ballast water and are not subject to ballast water exchange requirements. Although some of the vessels surveyed that were operating under these conditions did flush or otherwise exchange their ballast tanks in mid-ocean, many did not. Specifically 31 of 49 vessels in our survey entered the Seaway with fresh or low-salinity water residuals from their last overseas ballasting operation. *From the perspective of ecosystem protection, NOBOBs entering with fresh or low-salinity water*

residuals represent the greatest threat to the Great Lakes for NIS introductions. Open-ocean flushing could provide an immediate method of potentially reducing that risk. We strongly recommend that this situation be more fully evaluated and appropriate procedures to mitigate this particular threat be sought as soon as possible.

6.2. Biological Assessment of Ballast Residuals in Tanks

In Chapter 3 we provided our findings related to direct sampling of 82 ballast tanks on 42 NOBOB vessels in the Great Lakes during the study period from December 2000 – December 2002.

Microbiology

We found no evidence that ballast tanks are serving as incubators of viral or bacterial growth. Concentrations of viruses (in the context of this report, "virus-like-particles" or VLPs) and bacteria in ballast residuals were highly variable, covering ranges both below and above those typically found in natural aquatic environments. No enteric bacteria (*E. coli* and enterococci, which would signify contamination by sewage) were detected in our ballast residual samples. Throughout freshwater and marine aquatic environments, including pristine ones, there are typically about 10^6 bacteria per ml. The overwhelming majority of these bacteria are natural, nonpathogenic forms, and their constancy of number is the result of a balance between nutrient supply and grazing by predators. Aquatic viruses are more variable and usually 10 to 100 times more abundant than bacteria in their natural concentrations, and seek naturally occurring bacteria and phytoplankton as hosts.

We also screened residual water and sediment samples for the presence of selected pathogens, and one or more of the microbial pathogens *Vibrio cholerae, Cryptosporidium parvum, Giardia lamblia, Encephalitozoon intestinalis, Pfiesteria piscicida, P. shumwayae,* and *Aureococcus anophagefferens* were detected in 26 of the 42 (62%) ships we sampled. However, we only tested for presence and did not determine absolute concentrations of these pathogens, and thus cannot ascribe a level of human health risk from their presence. There were few incidences of pathogen co-occurrence: 1 tank in 2001 and 2 tanks in 2002 had three pathogens. Four tanks in 2001 and 8 tanks in 2002 had two pathogens. Tanks with water from Antwerp (Belgium) had the greatest pathogen frequency, with other European ports, Matanzas (Cuba) and Maracaibo (Venezuela) having moderate pathogen frequency.

Our study also emphasized the importance of biological "resting stages" when assessing the risks associated with residual ballast water and sediments in particular. Several of the microbial pathogens, such as *Cryptosporidium* and *Giardia,* have encysted forms that allow them to lay dormant for long periods of time, yet remain viable even when exposed to harsh conditions. Similarly, dinoflagellates (including several harmful species) also produce cysts that remain viable for many years. Both the ODU and the NOAA labs investigated dinoflagellate abundances and viability. The ODU lab documented a range (80 to 850 per gram of sediment) of dinoflagellate and other unicellular algae cysts in residual sediments, 33-44% of which germinated in laboratory culture experiments even a year after they were collected. However,

since dinoflagellates are typically marine organisms, they do not likely pose a significant threat to the Great Lakes.

Some harmful algal bloom (HAB) species that produce resistant resting stages (e.g. *Pfiesteria piscicida* or *shumwayae*) and some that don't (e.g. brown tide organism, *Aureococcus anophagefferens*) were detected in 3-10% of residual water and sediment samples. These HAB species tolerate a wide range of salinities, but those found in our residual samples did not grow in freshwater cultures, and thus do not appear likely threats to the Great Lakes. On the other hand, as noted below, there are nonindigenous phytoplankton species that are identified as marine but which are established in the Great Lakes or that grew quite well in some freshwater cultures used by the NOAA lab. Therefore we cannot completely eliminate the potential for other marine algal forms to become established in the Great Lakes, although evidence suggests that the risk related to dinoflagellates and harmful algal bloom organisms is low.

While the risk from pathogens carried in ballast tank residuals is not zero, we found no evidence that the risk is, in general, particularly high. However, we suggest that due-diligence requires a more thorough assessment of the occurrences, and particularly, the range and frequency of concentrations of pathogens in ballast tank discharges and closer examination of their human-health implications.

In the case of cholera, there is little likelihood for infection of humans 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 we do not know the concentrations of cholera bacteria in the samples processed for this study (we know only that they were present), we make reference to the Chesapeake Bay study performed by Ruiz et al. (2000). Assuming that the concentrations of *V. cholerae* in residuals is about the same as found in ballast water in that study, a healthy adult would need to directly ingest between one and ten liters of undiluted ballast residual water to become ill.

With the exception of dinoflagellates there is no conclusive evidence linking ballasting operations to successful invasions by aquatic microorganisms. Nonetheless, it would be simplistic and possibly very wrong to consider that aquatic microbial invasions do not occur or could not be mediated by ballast water. Unlike many of their invertebrate counterparts, microbial invaders cannot be seen without a compound microscope and their presence might only be noticed in spectacular cases, e.g., red tides or outbreaks of illness. Thus, there is a bias inherent in the detection of nonindigenous microorganisms.

Phytoplankton

Every ballast residual sample tested at the NOAA lab for phytoplankton viability produced significant phytoplankton growth in at least one of five culture media (two common freshwater media, one standard seawater media, filtered Grand River (Michigan) water, and filtered Lake Michigan water) used in our laboratory growth experiments. This is a clear indication that phytoplankton can remain viable in spite of the complete darkness and highly variable water quality conditions inside ballast tanks.

Both of the common freshwater media produced germination and growth in approximately 80% of the samples. Diatoms were the dominant species that grew in all of the experiments, with lesser amounts of green algae, small flagellates and dinoflagellates. A total of 154 phytoplankton species were identified among all ballast residuals tested. Of these 154 species, 41 taxa (30 identified species) were nonindigenous diatoms which had originally been described from a marine environment. However, despite their marine origin, nine of these nonindigenous diatom species have been found in the Great Lakes.

Cysts of dinoflagellate species were also identified by the NOAA lab, including cysts belonging to potentially toxic species of the genus *Alexandrium*, which are known to cause paralytic shellfish poisoning. Five *Alexandrium* species were identified based on morphological descriptions in the literature. However, unlike the ODU lab (see above), the GLERL lab did not observe germination of any of the marine dinoflagellates in their culture experiments. We don't assign any significance to these different results because the two labs used different culture media and techniques.

Interestingly, the number of dinoflagellate species was negatively correlated with ship's age, salinity of the residual water, and whether or not the tank had been flushed. While a relationship with ship's age does not suggest anything significant, the negative relationship with salinity of the residual water and with flushing suggests a possible approach for reducing the invasion risk posed by phytoplankton in ballast residuals.

It must be noted that the actual abundance (i.e., number of cells) of nonindigenous phytoplankton species in the residual ballast samples was typically <5% of the total phytoplankton abundance in treatments where positive growth was noted. While 18% of our experimental treatments had more than 4 NIS present in the same sample, almost 30% did not have any NIS present at all.

We conclude that ballast residuals can be a significant vector for introducing nonindigenous phytoplankton to the Great Lakes. Regular flushing of tanks may help reduce the vector risk by reducing the number of species. Based on their abundance in the samples examined for this study and their survival and growth in freshwater cultures, we identified at least seven nonindigenous phytoplankton species that we believe pose a high potential invasion risk to the Great Lakes.

Live Invertebrates

In residual sediments, nematodes dominated the overall relative invertebrate abundance (91%) in sediment residuals and were the most species rich group, with 48 taxa recorded from a subset of only ten ships, including numerous taxa not reported from the Great Lakes, or North America. Harpacticoid (5%) and cyclopoid copepods (3%) were the next most abundant, and based on our samples, taken together, these taxa (nematodes and copepods) contribute almost 99% of all invertebrates entering the Great Lakes region in residual ballast sediment.

Twenty harpacticoid copepod species were identified, of which three are native to the Great Lakes and three are nonindigenous but already established in the Great Lakes. Two of the 20 harpacticoid species were freshwater taxa not known to have populations in the Great Lakes,

while four are brackish water fauna, and the remaining eight species are typically associated with more saline conditions.

Twelve cyclopoid copepod species were identified in residual sediments, six of which are known in the Great Lakes, and four are freshwater species that do not have established populations in the Great Lakes. Three marine copepods were also recorded. Two epibenthic cladoceran species were also found, both of which are cosmopolitan taxa presumably native to the Great Lakes. Another group with a reported invasion history in the Great Lakes, the oligochaetes, comprised only four species and 0.2% of the animals recorded and were present in 14.3% of ships.

Thus, of thirty-two copepod species identified in residual sediments, at least six (freshwater taxa) would pose a high risk to the Great Lakes as potential new invaders, while the risk associated with the remaining species (brackish and saline water taxa) will depend on their tolerance of freshwater environments.

The taxonomic composition organisms in residuals water differed greatly from that of sediments with copepods as the most abundant (97.3% of organism abundance per ship: 66.0% nauplii, 20.4% cyclopoids, 10.8% harpacticoids, plus others) and species-rich group. Rotifers were the next most abundant taxon at 1.2% of the total. Remaining taxa collectively comprised <1.5% of total abundance.

Five calanoid, twelve cyclopoid and ten harpacticoid copepod taxa were identified, including thirteen species already recorded from the Great Lakes. Ten of the remaining fourteen species were marine taxa, which presumably would not survive if introduced to the Great Lakes, leaving four freshwater or brackish-water species that could potentially tolerate conditions in the lakes.

At least eight cladoceran species were also found, of which three are not established in the Great Lakes (one North American and two European species). However, only one specimen each of the latter was found, so if this reflects actual inoculum pressure from these species, the associated invasion risk would be quite low, but not zero.

Seven rotifer species were identified, all of which are native to the Great Lakes, while three *Gammarus* species were identified that were all from European estuarine brackish waters. Small bivalves were recorded on several ships, including *Dreissena* veligers. However, these were typically present in low abundance and frequency overall.

A statistically significant negative relationship was found between pore water salinity and total invertebrate abundance in both water and sediment residuals. None of the other variables we assessed were important in determining animal abundances. This suggests the potential benefit of making sure that residual water in NOBOBs entering the Great Lakes is at least at open-ocean salinity.

Resting Stages

The density of invertebrate resting stages in residual sediments ranged from 4.0×10^4 to 9.1×10^7 per metric tonne. Taxonomic identity was made for 12 groups. Seventy-six distinct taxa were isolated from sediment and hatched under laboratory conditions. Of these, 23 NIS were

identified, consisting of 14 rotifers and seven cladocerans. However, both the total abundance and frequency of occurrence of NIS from resting stages was low compared to species considered native to the Great Lakes.

Higher salinity and lower temperature, and burial in sediment each suppressed total abundance and species richness of hatched taxa. Less than 43% of individuals that were successfully hatched when isolated from sediment did so when buried. All other ballast history variables were found to be insignificant in relation to resting stage density.

Relative Risks of Invertebrate Sources

Many of the active and dormant sediment taxa we identified are buried or have adaptations to ensure they remain in association with sediments, even during flow or turbulent conditions, and will thus have little chance for discharge from ballast tanks. Our analyses, combined with a propagule supply model, suggest that despite sediments containing higher densities of nonindigenous propagules overall, only the most frequently occurring epibenthic species appear likely to be able to invade the Great Lakes system from sediment residuals. Our in situ hatching studies suggest that less than 1% of invertebrate diapausing eggs will hatch in ballast tanks (see Mesocosm Experiments, below) and thus become available for introduction. *Therefore, fresh and brackish residual ballast water may pose the greatest risk for introduction of invertebrates due to the presence of live taxa readily available to move into the water column and be discharged with ballast water added during Great Lakes transits.*

6.3. Great Lakes NOBOB Ballast Tank Mesocosm Experiments

In Chapter 4 we described and summarized in-situ ballast tank experiments designed to examine the effects of adding Great Lakes water on germination and growth of NIS in NOBOB tanks ballast residuals and on their potential to be released from ballast tanks.

In general, VLP and bacterial abundances declined by about a factor of two during ballast transits within the Great Lakes. Pathogens were detected intermittently, but there was large variability between experiments. Phytoplankton species diversity declined during vessel transit and there tended to be a shift in species dominance, with the potentially harmful blue-green alga, Microcystis, being a favored competitor in ballast tanks.

Zooplankton densities for most taxa also declined during ballast transits. The amount of decline increased in proportion to voyage duration. On three occasions, however, some Rotifera species increased in abundance as the voyage progressed to the upper Lakes. Several NIS were detected in the Great Lakes ballast water loaded at the beginning of the experiments in the lower Lakes. Although these organisms are already present in and likely originated from the Great Lakes water, *the operational pattern of NOBOB vessels clearly presents a risk of spreading these invaders from the lower to the upper Lakes.*

Two NIS rotifers that are not presently reported in the Great Lakes were detected in ballast water samples collected from filled tanks. One was also detected in harbor samples collected during the same voyage and thus may constitute a new invasion by this species. The other was detected

in the tank 10 days after ballasting and may have hatched in tank, so we cannot assume that it was associated with the incoming ballast water or that it represents a new invasion.

Resting egg hatching experiments using Emergence Traps were conducted on four voyages. In total, 19 individuals hatched in 41 experimental replicates, producing an average hatching abundance of 0.5 individuals per 500g of sediment. *Both total abundance and species richness of organisms hatched was significantly lower in situ than in laboratory hatching experiments with the same sediments. In addition, the effect of burial appeared to have a significant impact on the number of eggs that hatched.*

Despite the fact that NOBOB vessels carried, on average, ~15 t of sediment, the probability that resting stages of NIS will be present and receive hatching cues is small, and the calculated inoculum size is estimated to be only 87-375 individuals per taxa per ship. Based on our analysis of vessel traffic and trade patterns over the course of a typical year, we estimate that approximately 250 NOBOB vessels provide conditions suitable for hatching and introduction of resting stages each year. From our combined in situ hatching and biological characterization data, we estimate that 5.7×10^3 to 3.0×10^4 nonindigenous individuals may be introduced to the Great Lakes basin via hatching from resting stages within residual sediments in NOBOB ballast tanks per year. However, there is some concern that low oxygen conditions may have been generated within the in situ hatching chambers, which could have suppressed hatching and thus explains the low hatching rate we observed. At the same time, indicator organisms added to a duplicate experimental chamber on each experiment did not exhibit any negative effects as would have been expected on exposure to sustained low oxygen conditions. Further experimentation will be required to resolve this concern.

These findings are consistent with our previously stated conclusion that *residual fresh and brackish ballast water likely poses the greatest risk for introduction of invertebrates compared to hatching from resting stages or the live organisms in the residual sediments.*

6.4. Low-Salinity Ballast Water Exchange

In Chapter 5 we detailed the outcomes of three ballast water exchange experiments conducted on board ships of opportunity. These experiments were designed to obtain quantitative measures of the efficacy of mid-ocean ballast water exchange (BWE) in reducing abundances of fresh- or brackish-water organisms in ballast tanks.

We examined the efficacy for volumetric water exchange of three-tank-volume exchange by measuring the concentration over time of two physical tracers, salinity and rhodamine dye. We examined the biological efficacy based on two approaches: (1) We measured the effect of BWE on the concentration of coastal organisms, comparing changes in abundance within exchanged ballast tanks to those in paired unexchanged (control) tanks on the same voyage. (2) We compared survivorship of a range of coastal zooplankton, which were exposed to water in simulated (exchange) conditions versus control (unexchanged) conditions, to measure effects of "salinity shock". These experiments consisted of both a shipboard trial and laboratory experiments.

For all three experimental voyages, we initially targeted ships making repeat voyages from low salinity ports in the Baltic/northern Europe region to the U.S. east coast or Great Lakes region. However, it proved exceedingly difficult to identify candidate vessels that met all our experimental design criteria. Therefore we found it necessary to shift our efforts to vessels originating from any low salinity port and engaging in a voyage of at least 5 days length, regardless of the destination port. We selected three ports where advance information indicated that the salinity would be suitably low. However, the salinity in each location was higher than anticipated for each experiment. While not in the preferred range of 5 ppt or less, the initial salinities for two of the three experiments were sufficiently below those typical of the mid-ocean to allow analyses based on changes in salinity. For the other experiment the salinity was much higher, but when combined with dye-tracer results, provided a measure of comparison between exchange efficacy for high- versus low- salinity ballast water source.

Exchange efficacy ranged from 80-100% based on salinity measurements and from 86-99% based on rhodamine dye measurements. There was no noticeable difference in calculated exchange efficacies when comparing the flow-through versus the empty-refill method of exchange. Similarly, there was no noticeable difference in efficacy based on the starting salinity of the initial ballast water over the range tested (11-33 ppt).

The efficacy of exchange in removing biological specimens was more variable, both among and within vessels. A total of 16 zooplankton taxa across all three voyages were used as target indicator organisms to measure biological exchange efficacy. The majority of these taxa experienced changes in density between -85% and -100% in the exchange tanks, although two taxa, *Polychaeta* and *Gastropoda*, actually increased in one tank following exchange. Changes in the control tanks were more variable than those in the exchange tanks, with the majority of targets changing in abundance between -30% to +15%. Comparison across target zooplankton taxa indicates that in most cases, ballast water biological exchange efficacy was >90%. *Overall, the empty-refill treatment more consistently had a negative effect on organism survivorship than did the flow-through treatment.*

For phytoplankton, there were significant challenges in choosing target taxa to calculate exchange efficacies due to high variability and high mortality. While on one occasion an exchange effectiveness of 100% was achieved for the diatom *Skeletonema costatum,* all other targets experienced marked declines in the control tanks, suggesting that natural attrition plays a bigger role than exchange in the removal of these species.

We conducted laboratory-based salinity tolerance experiments to address the question of whether "salinity shock" improves the effectiveness of ballast water exchange with respect to killing organisms from low-salinity environments. Experiments run on 14 different zooplankton taxa from low-salinity and freshwater habitats in the Upper Chesapeake Bay watershed showed a variable response to high salinity exposure across taxa, and indicated that empty-refill exchange would have the most significant negative effect on survival.

The combined results of our ballast water exchange and salinity tolerance experiments make it evident that *while exchange is highly effective for reducing the concentration of organisms*

entrained with coastal ballast water, the range of tolerance to high salinity exposure that exists across low-salinity taxa makes it difficult to generalize about the frequency with which species from low-salinity environments are killed by "salinity shock" via mid-ocean ballast water exchange. Organisms that exhibited high survivorship in both of the exchange treatments and the controls are the ones that warrant close scrutiny with regard to invasion potential. In this study, those were the amphipods *Gammarus* spp. and *Corophium* spp., zoea larvae of the crab *Rhithropanopeus harrisii*, and an unidentified species of juvenile harpacticoid copepods. These results are particularly interesting in light of recent invasion successes by amphipod and harpacticoid copepod species in the Great Lakes, and the notable number of amphipod invasions that have occurred in the Baltic region in recent years. It would be worthwhile to include known amphipod and harpacticoid invaders in future experiments to clarify if mid-ocean exchange is a useful management technique with regard to these taxa.

6.5. Final Comments

The microbial, phytoplankton, and invertebrate data and evaluations developed during this study confirm that NOBOB vessels are a vector for NIS introductions to the Great Lakes Basin, especially for algal and invertebrate biota.

We assign the greatest risk to NOBOB vessels that enter the Great Lakes containing fresh or low-salinity residual ballast water and urge that methods to eliminate this risk be developed as soon as possible.

The potential benefits of regular flushing of NOBOB tanks with open ocean water should be more fully tested and evaluated, both for reduction of accumulated sediment and for the potential benefits of "salinity shock.".

Ballast water exchange is an imperfect, but generally beneficial management practice in the absence of more effective and consistent management tools.

The assumption that "salinity shock" is an additional advantage for protecting the Great Lakes ecosystem from invasive species must be viewed with caution and regarded, like volumetric water exchange, as imperfect and subject to widely variable efficacy depending on taxa and the form in which they are represented in ballast tanks. This is not to say that it is ineffective, but to caution that nature in all its diversity had provided many organisms with various means to frustrate our attempts at prevention management.

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Appendix 1.

Ballast management and history survey form used in interviews with ship's master during the Great Lakes NOBOB study.

Philip T. Jenkins & Associates Ltd.
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Services

BALLAST WATER SURVEY

SHIP: ..... **FLAG:** .....

TYPE: ..... **DATE OF BUILDING:** .....

MANAGER: .....

OAL: ..... **BREADTH:** ..... **M DEPTH:** .....

TOTAL NO. BALLAST TANKS: .....

TOTAL BALLAST CAPACITY: .....

INCLUDES W.B. HOLD(S): **YES:** **NO:** **CAPACITY:** .....

LAST DRY DOCKING: **FULL:** ..... **INTERMEDIATE:** .....

WERE ANY TANKS CLEANED FOR SURVEY: **YES:** **NO:**

INDICATE TANKS CLEANED: .....

IS TANK CLEANING - FLUSHING PRACTICED: **YES:** **NO:**

.....

IS A MUD DISPERSENT USED: **YES:** **NO:**

PRODUCT NAME & MANUFACTURER: .....

CURRENT VOYAGE: **FROM:** ..... **TO:** .....

LAST BALLAST PASSAGE: **(1)** ....

BALLAST DENSITY: ..... **BALLAST EXCHANGE : YES NO**

PREVIOUS BALLAST PASSAGE: ....

BALLAST DENSITY: ..... **BALLAST EXCHANGE : YES NO**

PREVIOUS BALLAST PASSAGE: ....

BALLAST DENSITY: ..... **BALLAST EXCHANGE : YES NO**

PREVIOUS BALLAST PASSAGE: ....

BALLAST DENSITY: ..... **BALLAST EXCHANGE : YES NO**

PREVIOUS BALLAST PASSAGE: ....

BALLAST DENSITY: ..... **BALLAST EXCHANGE : YES NO**

NORMAL UNPUMPABLE BALLAST: .....

CURRENT DRAFT: **FORWARD:**..... **AFT:**.....

DIMENTIONS OF TANK(S) ENTERED: (USE MEASUREMENT UNITS DISPLAYED - IMPERIAL / METRIC)

TANK #	L	B	H	CAPACITY	RESIDUE MEASURED

ADDITIONAL OBSERVATIONS:

..... **.....**

SURVEY AT: **DATE:**

Appendix 2.

Ballast tank observations and ballasting history database.

SHIP #	TYPE	BUILT	LAST DRY DOCK	TANKS CLEANED AT DRY DOCK	SURVEY / SAMPLE DATE	CURRENT VOYAGE	TANKS FLUSHED	BALLAST HISTORY	EXCHANGE ON PASSAGE	TOTAL BALLAST CAPACITY M3	TOTAL RESIDUALS MT	OBSERVATIONS	SALINITY
1	BC	1990	10/00	All DB & Peak Tanks	Toronto 9/12/00	Itaji (Brazil) to Toronto	Yes Fore Peak exchanged	Gydnia FW: 10/00	Yes	6656	80. % Sediment not known	First cargo voyage following dry docking. Now using mud/sediment conditioner.	N/A
2 **1001** **ND 1/00**	BC	1999	New Build	New	Thorold 10/12/00 7-Dec-01	Kamsar (Guinea) to Thorold	No. Aft Peak exchanged 25/11/00 38° 16N 47° 05W	Skikda (Algeria) SW: 10/30/01 Stettin (Poland) FW: 10/3/01	No No	7651	< 20 20% Sediment	Tank flushing sporadic only. Fore Peak tank < 1.0 m^3 water with patches of congealed mud. No. 1 DB's < 2.0 m^3 water with only slight evidence of sediment deposits along bilge longitudinals.	ND ND
3	BC	2000	New Build	New	Hamilton 12/12/00	Buena-ventura (Colombia) to Montreal Hamilton	No	Guyaquil (Ecuador) FW: 11/00 Buenaventura (Colombia) FW : 11/00 Haiphong (Vietnam) BR: 10/00 Chandpur (Bangladesh) FW: 8/00	Yes Yes No No	5053	< 50. % Sediment not known	Management program requires annual inspection of all tanks - crew cleaning as necessary.	N/A
4	BC	1988	New Build	New	Hamilton 3/5/01	Puerto Cortes (Honduras) to Hamilton	No	Lazaro Gardenas (Mexico) BR: 4/01 Puerto Vinceo de Azur (Dom-inican Republic) SW: 4/01 Esmeraldis (Ecuador) SW: 3/01	No No No	2373	Negligible	New Building laid up at shipyard 1988-1998. Has efficient ballast stripping system. No specific management procedures for tank flushing/cleaning.	N/A

5	BC	1984	02/01	No	Thorold 5/5/01	Weiba (Australia) to Gaspe Thorold	No	Gaspe SW: 4/01 Kunsan (S. Korea) BR: 3/01 Jeddah (Saudi Arabia) SW: 1/01 Great Lakes FW: 11/00	No Yes No No	79170	< 200. % Sediment mud not known	No formal management procedures for flushing but for exchanging ballast fully whenever practical if taken in turbid locations.	N/A
6 **1002**	BC	1997	07/99	No	Hamilton 5/8/01	Beaumont, TX & Tampa. Florida to Montreal Lakes	Yes	Brownsville (Texas) SW: 4/01 Cork (Ireland) SW: 3/01 Vera Cruz (Mexico) SW: 3/01 Great Lakes FW: 11/00	No No Yes No	18682	< 40. 25% Sediment	Fore Peak < 2.0 m^{-3} water around bellmouth with only a light film of sediment on horizontal surfaces. 6 DB Port 5.0 m^{-3} water at aft end < 0.25 m^{-3} sediment along bilge stringer and outboard of bilge longitudinals. 6 DB Stbd. Negligible water, sediment as in port side.	SW SW ND
7	GC	1994	1999	Not known	Hamilton 8/5/01	Santos (Brazil) to Hamilton	No	La Plata (Argentina) to Santos (Brazil)	No	2287	N/A	The last ballast carried on board was chlorinated under the direction of Brazilian Authorities on arrival at Santos, Brazil. Ship has no formal ballast management procedures.	N/A
8	GC	1986	01/00	Not known	Toronto 9/5/01	Puerto Quetzal (Guatemala) to Toronto	No	Caldera (Costa Rica) SW: 4/15/01 New Orleans FW: 3/15/01	No Yes 3/31/2001 25^{o} 46 N 85^{o} 48 W	9000	< 180. % sediment not known	Only the most recent ballast history made available due to change of ownership. Formal ballast management plan yet to be developed.	N/A

9 **1003**	BC	1999	New Build	New	Cleveland 5/22//01	Ijmuiden (Netherlands) to Cleveland	No	Hamburg (Germany) FW: 5/01 Aviles (Spain) SW: 4/01 Police (Poland) FW: 4/01 Sfax (Tunisia) SW: 3/01 Swinovjscie (Poland) FW: 2/01	No No No No No	11088	< 25. 10% sediment	Fore Peak: Very clean - water and sediment localized around bellmouth < 1.0 m^3. 2 DB Port: Some evidence of sediment accumulation at the aft end of the tank and along centre girder. Monthly tank inspections performed to assess cleanliness and coating condition.	FW BR/SW
10 **ND 1/01**	BC	1986	1998	Yes all Double Bottoms	Toronto 5/24/2001	Campinas (Brazil) to Great Lakes	Yes	Skikda (Algeria) SW: 3/20/01 Wilmington FW: 2/01	Yes	17959	+/_ 50. 75% sediment	3 DB Port: Negligible water, mud concentrated along two bilge longitudinals to depths of 7.5 cm in pockets between drainage holes and over bottom shell total approx. 2.0 m^3. 3 DB Stbd: As port side.	ND ND
11	BC	1984	04/01	Yes all Double Bottoms	Hamilton 29/05/01	Constanza (Romania) to Sorel Hamilton	Yes	Varna (Bulgaria) FW: 4/24/01 Odessa (Ukraine) FW: 04/04/01	No No	10600	< 50. % sediment not known	Ship carries ISO 14000 certification. Has detailed ballast management plan with emphasis on keeping tanks clean, stripping ballast and exchanging ballast at every opportunity.	N/A
12	BC	1982	02/00	Not known	Toronto 5/29/2001	Puerto Quezil (Guatemala) to Toronto	No	Matarani (Peru) SW: 5/2/01 New York BR: 3/25/01 Tarragona (Spain) SW: 2/16/01 Huelva (Spain) BR: 1/27/01 Morocco SW: 1/18/01	No No No No No	8650	Not known	Record of residuals not made available only practice used to clean tanks is when ballast is exchanged where required by Port State.	ND

13	BC	1983	01/01	Yes 4 & 5 Double Bottoms only	Hamilton 6/18/2001	Immingham (UK) to Great Lakes	No	Rotterdam (Netherlands) FW:6/1/01 Montreal FW:5/8/01 Great Lakes FW: 5/01 Dubai (United Arab Emirates) SW: 1/01	No No No No	10497	Vary between 50/110 % sediment not known	Ship can be difficult to strip ballast at certain trims. Ballast management procedures basic, full exchange only.	N/A
14 **1004**	BC	1983	04/01	No	Hamilton 6/25/2001	Antwerp (Belgium) and Bremen (Germany) to Great Lakes	No	Current voyage Antwerp FW: #s 3,5,6, DB's Bremen FW: # 4 DB's Great Lakes FW: 5/01 Antwerp BR: 5/01 Brunsbuttel (Germany) FW: 4/01	Yes all 4 sets of tanks 59^{o} 30 N 21^{o} 30 W 6/13/2001 Yes Yes	18840	+/- 90. <15 sediment	2 DB Port: 5.4 m^3 water - thin layer of sediment on bottom shell but ridge concentrated along bilge stringer above slosh water line. 2 DB Stbd: Residual as port side, mud ridge varies slightly because of list. Tanks not flushed because of difficulty in evacuating water at even keel trim. Management procedures require regular tank inspections and cleaning as required.	BR FW
15 **1005**	BC	1984	11/99	Yes 5 Double Bottoms only	Thorold 6/27/2001	Fangchen (China) to Providence RI & Thorold	Yes	Inchon (S. Korea) BR: 5/1/01 Inchon BR: 3/01 Inchon BR: 2/01 Hong Kong SW: 1/01 Ghent FW: 12/00	Yes East China Sea Yes North Pacific Yes Indian Ocean Yes Indian Ocean No	10878	+/- 50. 90% mud	4 DB Port: < 1.0 m^3 water at aft end. Mud collecting along bilge longitudinals throughout and in patches on shell + 5.0 m^3. 4 DB Stbd: Slightly more water in this side bud mud sediment pattern identical.	SW SW

16 **1006**	BC	2000	New Build	New	Windsor 6/30/2001	Ghent (Belgium) & Brunsbuttel (Germany) to Contrecoeur & Windsor	Yes	Lake Maracaibo (Venezuela) FW: 5/01 Becancoeur FW: 5/01 Vera Cruz (Mexico) SW: 4/01 Ghent (Belgium) FW: 4/01	Yes Offshore Venezuela No No No	19730	< 40 < 10% Sediment	6 DB Port: 3.8 m^3 water residual with negligible sediment. 6 DB Stbd: 2.7 m^3 water residual with negligible sediment. Indications in both tanks of sediment starting to accumulate along bilge longitudinals despite constant flushing. Management procedures call for regular tank inspections and exchange/flushing whenever possible.	SW SW
17 **ND 2/01**	GC	2000	New Build	New	Hamilton 19/07/01	Stettin (Poland) to Hamilton	No Aft Peak exchanged 52° 28w 33° 17w 5/7/2001	Immingham (UK) BR: 6/19/01 Baltimore (MD) BR: 5/1/01 Galveston (TX) SW: 4/12/01 Rivena (Italy) SW: 3/01	No No No No	2630	Vary between 10/40 depending on trim % sediment not known	After peak sampled but not entered. Management procedures only require exchange where mandated for tank cleaning.	SW
18	CT	1982	01/99	Not necessary tanks clean	Hamilton 22/07/01	New York (NY) to Hamilton	No	Saint John (NB) SW: 7/10/01 Houston (TX) SW: 6/30/01 Santos (Brazil) SW: 5/15/01 St. Croix (Virgin Isle) SW: 4/01 St. Croix SW: 4/01	No No No No No	4441	Negligible	Management procedures call for close attention to tank condition. Inspections every 2 months - crew hand cleaning as necessary.	N/A
19 **ND 3/01**	BC	1981	02/00	Not known	Hamilton 22/07/01	Bremen (Germany) & Rotterdam (Netherlands) to Montreal Hamilton	No Fore-peak exchanged 56° 06n 43° 53w 12/7/2001	Rotterdam FW: 6/25/01 Great Lakes FW: 05/01	No No	16294	Not indicated	Exchanged ballast in forepeak sampled only. Tank not entered. Ballast management program still being formulated. Tanks flushed during exchange only.	SW

20 **1007** **ND 4/01**	BC	1987	01/01	Yes Double Bottoms 4,5, & 6	Hamilton 7/26/2001	Ghent & Antwerp (Belgium) to Great Lakes	Yes	Great Lakes FW: 6/01 Rotterdam (Netherlands) FW: 6/01 Great Lakes FW: 5/01 Bremen (Germany) BR: 5/01 Setubal (Portugal) SW: 3/01	No No No No Yes	25533	+/- 150. Varies with trim % sediment not known	Fore Peak: Relatively clean 0.5 m^3 water around bellmouth. Mud deposits concentrated in breast hooks where drainage poor due to construction. Total accumulation less than 1.0 m^3. 4 Top Wing Tank Stbd: Dry & clean. 6 Top Wing Tank Stbd: Clean but with 3.0 m^3 water entrapped by temporary repair.	FW N/A BR
21 **ND 5/01**	RoRo	1992	1990	Not known	Hamilton 7/26/2001	Cape Town (South Africa) to Philadelphia Pa Montreal & Hamilton	No	Continual ballasting for stability & trim	Yes See Observations	4344	N/A	Tanks exchanged on passage 1: 2/07/01: Fore Peak - #1: 12° 07S 10° 42 W DB 2: 11° 47S 11° 04W DB 3/4: 9° 53S 13° 02W DB 5/6: 5° 38s 17° 22W Tanks 1 & 3 sampled only without opening - tanks 2 & 5 sounding pipes not condusive to sampling.	SW SW
22 **1008** **ND 6/01**	BC	2000	New Build	New	Hamilton 7/28/2001	Antwerp (Belgium) to Contrecoeur & Great Lakes	Yes	Hamburg & Brunsbuttel (Germany) FW: 7/07/01 Osaka SW: 5/7/01	No No	19730	< 30 < 25% sediment	4 DB Port: 2.0 m^3 water residual at aft end with sediment starting to accumulate outboard of bilge longitudinals, light patches on bilge stringer and bottom shell. 4 DB Stbd: 2.5 m^3 water residual - sediment pattern as port side. Management program calls for regular flushing - salinity readings of water samples indicates not all tanks flushed or program sporadic.	FW
23 **1009** **ND 7/01**	CT	1998	08/00	Yes P & S 6 DB side tanks blasted & recoated all others clean	Hamilton 12/10/00 and 8/4/01	San Juan (Puerto Rico) Hamburg (Germany) Newcastle (UK) & Malaga (Spain) to Hamilton	No	Nassau (Bahamas) SW: 7/01 Malaga (Spain) SW: 6/01 Nassau (Bahamas) SW: 5/01	No Yes Yes	2232	Negligible	Ship is on regular Europe, Caribbean, North America rotation that can include Baltimore & Trinidad. Ballast is only carried if insufficient cargo on board for directional stability. Eductor system is very efficient leaving no water residual. Tanks are kept clean by regular exchange & hand washing. Dry sediment samples removed from 4 P & S tanks - total accumulation measured in region of 25 kg each. Retained ballast sampled in 2c & 4c DB tanks.	N/A SW

24 **ND 8/01**	RoRo	1990	1999	Not known	Hamilton 5/22/2001	Cape Town (South Africa) to Savannah Ga Philadelphia Pa Montreal & Hamilton	No (See Obser vations)	Continuous ballasting de-ballasting for trim & stability	Yes 7/30/2001 28° 00N 63° 07W	4404	Records indicate when "Dry" <100 unpumpable % sediment not known	Tanks # 31 & 32 retained ballast sampled only - no entry. Ballast is carried in loaded condition for trim & stability purposes. Flushing is virtually impossible - cleaning through full exchange only. Sediment collected in water samples indicates measurable accumulation.	ND
25 **1010** **ND 9/01**	BC	1980	3/2000	Not known	Hamilton 8/15/2001	Constanta (Romania) to Hamilton, Montreal Sorel	Yes	Last ballast taken on board entering the Black Sea from Bosporus	Yes	10189	See observations	Ship changed ownership this voyage - previous records not available. Fore Peak: Relatively clean but light scale overall. Sediment collected on lower horizontal members & between deep floors 14.0 m3 water retained with <3.0 m^3 mud. 3 Top Wing Tank - Dry & clean. Unable to access double bottom tanks due to port time.	FW N/A
26 **1011** **ND 10/01**	BC	2001	New build	New	Hamilton 8/15/2001	Bremen (Germany) Antwerp (Belgium) to Sorel & Hamilton	Yes	Liverpool (UK) FW/BR: 7/29/01 Hamilton & Sault Ste. Marie FW: 7/01 Annawaba (Algeria) SW: 4/01 Three Rivers FW: 3/01 Toyohashi (Japan) SW: 1/01	No No Yes No No	18246	Not indicated	Fore Peak: 1.0 m^3 water around bellmouth. All flat surfaces coated with a thin layer of sediment - deeper concentrations along shell frames where canted. Total accumulation < 0.25 m^3.	FW
27 **ND 11/01**	HL	1999	New build & emerg-ency 05/01	4 DB Local cleaning for repair	Hamilton 8/22/2001	Yokohama (Japan) to Hamilton & Detroit	No	Continual ballast & de-ballast for trim & stability. Last full Singapore SW: 6/01	Yes	2838	Negligible when dry	Management plan calls for full exchange at all times & 2 year tank inspection. Master has 6 month tank inspection program. Samples exchange ballast in 4 tanks: 2 DB Port: 33° 50N 68° 06W 14/08/01 2 DB Stbd: 28° 00N 71° 50W 13/08/01 1 TWT Stbd: 34° 35N 67° 32W 14/08/01 3 TWT Stbd: 34° 04N 67° 58W 14/08/01	SW SW SW SW

28	BC	1980	02/00	Not known	Hamilton 8/27/2001	Antwerp (Belgium) Dunkirk (France) to Hamilton Cleveland	Yes	Antwerp (Belgium) FW: 8/1/01 Becancour FW: 7/14/01 Mississippi River FW: 6/28/01 Tilbury (UK) FW/BR: 5/26/2001	No No No No	16294	< 80 % sediment not known	Planned sampling of sediment & water residuals in #'s 3 & 6 double bottoms aborted due to change in stevedore activity. Effective ballast management plan & program appears to be in place.	N/A
29	GC	1997	05/00	Not known	Hamilton 9/7/01	St. Petersburg (Russia) & Klaipeda (Lithuania) to Great Lakes	No	Ghent (Belgium) FW: 8/9/01 Mantylueto (Finland) FW: 7/30/01 Dunkirk (France) SW: 7/24/01 Dordrecht (Netherlands) FW: 7/10/01 Almeira (Spain) SW: 6/28/01 Kemi (Finland) FW: 6/20/01	No No No No No No		Negligible	No formal ballast management plan - trades normally European Coastal Trade - Black Sea to Baltic. Trims well & has educator system. Records indicate no measurable residuals.	N/A
30 **ND 12/01**	BC	1980	01/00	No found not necessary for enhanced survey	Toronto 09/06/01	Santos (Brazil) to Montreal & Toronto	Yes Fore Peak and Aft Peak Exchanged	Casablanca (Morocco) SW: 7/18/01 Burns - Harbor, In FW: 6/20/01 Amsterdam FW: 5/15/01 Hamilton FW: 4/15/01 Eregli (Turkey) FW: 3/01	Yes No No No Yes	16294	< 50 sediment % said to be minimal	All tanks cleaned when ship changed ownership & reported to have stayed clean through flushing & regular inspection. Samples of exchanged ballast taken from fore & aft peak tanks - forepeak already mixed with lakes water & discarded.	SW

31 **1012**	BC	2000	New build	New	Cleveland 9/18/2001	Ijmuiden (Netherlands) to Cleveland, Oh & Burns-Harbor, In	No	Police (Poland) FW: 8/01 JiJel (Algeria) SW: 8/01 Burns Harbor, In FW: 7/01 Police (Poland) FW: 7/01	Yes Yes No No	11088	< 30 < 25% sediment	1 DB Stbd: <1.0 m^3 water in proximity of the bellmouth. Thin layer of sediment on bottom shell. 4 DB Stbd: < 1.5 m^3 water concentrated at aft end of tank. Some sediment accumulation along turn of bilge at aft end.	FW FW
1007	BC	1986	08/00	Yes #'s 4 & 5 Double bottoms	Hamilton 10/01/01	Antwerp (Belgium) Dunkirk (France) to Montreal & Great Lakes	Yes Aft Peak Exchanged	Amsterdam (Netherlands) FW: 9/16/01 Ghent (Belgium) FW: 8/2/01 Takoradi (Ghana) SW: 4/15/01 Pohang (S. Korea) SW: 1/11/01 Maracaibo (Venezuela) FW: 11/21/00	No Yes Yes South Atlantic Yes Indian Ocean	24907	< 50. % sediment not known	Ballast management plan calls for regular flushing & tank inspections for cleanliness & coatings every 6 months. Fore Peak: Generally clean on horizontal surfaces - some sediment along shell stringers where canted. Large pockets of mud retained in breast hook area of bulb & between deep floors. 1.0 m^3 water retained around bellmouth, estimate total sediment 5.0 m^3. Aft Peak: Exchanged water ballast sampled only.	FW SW
33 **1013**	BC	1999	New build	New	Cleveland 10/5/01	Ijmuiden (Netherlands) to Cleveland	No	Antwerp (Belgium) FW: 9/01 Cleveland, Oh FW: 9/01 Amsterdam (Netherlands) FW: 8/01 Cleveland, Oh FW: 8/01 Antwerp FW: 7/01	No No No No No	11087	< 50 < 20% sediment	3 DB Stbd: 1.5 m^3 water residual at aft end. Local areas of mud accumulating along longitudinals and turn of bilge. 6 DB Port: 4.0 m^3 water residual at aft end - mud pattern as in # 3 - shell plating mostly clean.	FW FW

34 **1007** **ND 14/01**	BC	1987	01/01	Not known	Windsor 10/7/2001	Ventspils (Latvia) Stettin (Poland) Bremen (Germany) to Sorel & Great Lakes	Yes	Tanala (Finland) FW: 9/01 Great Lakes FW: 9/01	No	25533	Varies with trim +/- 150. < 25 % sediment	2 DB Port: 10.0 m^3 water residual mud concentrated in piles along bilge longitudinals, floors and intercoastals. Estimate total 2.0 m^3. 2 DB Stbd: 8.0 m^3 water residual - mud pattern and quantity similar to port side.	SW SW
35 **ND 15/01**	BC	1984	04/99	Not known	Hamilton 10/11/01	St. Petersburg (Russia) to Hamilton & Detroit	Yes	Aarhus (Denmark) SW: 9/19/01 Quebec FW: 8/22/01 Ghazaouet (Algeria) SW:7/25/01 Brownsville, TX BR/SW: 4/1/01	No No No Yes	7783	+/- 100. % sediment not known	Management procedures call for annual tank inspections. Last inspection 03/01. Fore Peak: 1.5 m^3 water residual around bellmouth. Lower horizontal surfaces have light mud accumulation. Heavy concentrations of mud at forward end up to lightening holes in floors - estimated up to 7.0 m^3.	SW
36	BC	1984	02/00	Not known	Hamilton 10/14/2001	Foz (Spain) to Montreal & Great Lakes	No	Torre Annunziate (Italy) SW: 10/1/01 Alexandria (Egypt) SW: 9/2/01	No No	17426	< 89 % sediment not known	Ship had a ten day port stay in Alexandria & Hull heavily encrusted with marine growth through wind & water area. Growth had been exposed to air for a prolonged period - dry - & samples not viable. Ballast management program calls for flushing as part of any exchange process.	N/A
37	BC	1980	06/01	Yes all 5 DB tanks & Fore Peak	Hamilton 10/14/2001	Mylaki (Greece) to Montreal & Hamilton	Yes	Sfax (Tunisia) SW: 9/01 Houston, TX BR: 7/01 Piraus (Greece) SW: 6/01	No No No	20120	< 75 sediment said to be negligible	Ship has comprehensive ballast management plan with emphasis of maintaining clean tanks & procedures for different exchange sequences. Cargo distribution and ballast from Montreal in fore peak negated sampling.	N/A

38	BC	1981	1999	Yes all tanks	Hamilton 10/14/2001	Antwerp (Belgium) to Montreal & Great Lakes	Yes	Antwerp (Belgium) FW: 9/01 Casablanca (Morocco) SW: 9/01 Liverpool (UK) FW: 8/01 Great Lakes FW: 8/01 Christensand (Denmark) SW: 7/01	No Yes No No No	16294	Between 70-110 MT depending on trim % sediment said to be slight	All ballast tanks cleaned by new owners during last dry docking. Ballast management plan calls for regular tank inspection with cleaning as required. Latest inspection indicates very little sediment accumulation and mainly at forward end. Already has taken Lakes ballast.	
39 **ND 16/01**	BC	1978	09/01	Yes all DB tanks	Thorold 10/19/2001	Aalborg (Denmark) to Three Rivers & Thorold	No	Tallinn (Estonia) SW: 9/16/01 Aalborg BR: 10/5/01	No Yes No. 1 DB only 53° 28 N 44° 54 W	5149	< 25. sediment said to be negligible	Ship is undergoing major refurbishment. No formal ballast management procedures but ballast exchanged by complete pump out and replacement. Cleaning by permitting sloshing during the process. Exchanged ballast in No. 1 DB sampled only.	SW
40 **1014**	BC	1999	New Build	New	Cleveland 10/22/2001	Ijmuiden (Netherlands) to Cleveland	No	Rotterdam (Netherlands) FW: 10/01 Liverpool (UK) BR: 9/01 Rio Grande FW: 8/01 Parangua (Brazil) FW/BR: 8/01	No No No No	11089	< 40 10% sediment	3 DB Port: 1.6 m^3 water residual across bottom of tank with mud deposits along turn of bilge. 3 DB Stbd: 3.3 m^3 water residual across bottom of tank, mud deposits along turn of bilge.	BR/SW BR/SW
41	BC	1981	08/01	Yes All Top Wing & DB tanks	Hamilton 10/22/2001	Fukijama (Japan) to Hamilton & Sault Ste. Marie	Yes See Obser- vations	Jinjiiang (China) FW: 8/01	Yes East China Sea 32° 03 W 134° 06E	10443	< 30 % sediment not known	Ship has comprehensive ballast management plan with emphasis on tank cleanliness and regular inspection. In view of turbidity of ballast taken leaving the dry dock a full ballast exchange & flushing was carried out en route to Japan. After loading cargo tanks were again flushed on Pacific Passage. Ballast was taken again in Caribbean Sea to improve motion during hurricane season dumped prior to entering Canadian waters. Limited port stay did not permit tank entry.	N/A

42 **ND 17/01**	BC	1981	On dry dock	No	Port Weller Dry Dock 10/20-24/01	Callao (Peru) to Belledune & Duluth	No	Belledune, WS SW: 10/01 Saint Vincente (Chile) SW: 8/01 Algiers (Algeria) SW: 7/15/01	No No No	8308	Varies between 60/100 depending on trim % sediment not known	Hull below ballast waterline has considerable marine growth which sampled as soon as dock dewatered. Indications that crustacean deposits occurred during a prolonged port stay in Algiers, Algae/weed growth occurring during a prolonged stay on the coast of Chile in late August and early September.	N/A
43 **1015**	BC	1996	02/01	No	Chicago, Ill 10/25/2001	Itea (Greece) to Chicago	No	Naples (Italy) SW: 10/01 Burns Harbor,In FW: 9/01 Valetta (Malta) SW: 8/01 Chicago FW: 7/01 Philadelphia, Pa FW: 6/01 Santos (Brazil) SW: 5/01	Yes No No No Yes No	18682	Not ascertained	1 Top Wing Tank Port: Clean & dry with small pocket of sediment against one frame collected as sample. 2 Top Wing Tank Port: Dry with patches of dried mud along longitudinals. Quantities in both tanks minimal.	ND
44 **ND 18/01**	BC	1985	01/00	Yes Fore Peak and Top Wing Tanks	Thorold 8/31/2001 Hamilton 11/1/01	Mantanzas Cuba to Great Lakes	Yes Fore Peak and 1DB Tanks exchanged	San Juan (Puerto Rico) SW: 10/01 Great Lakes FW: 9/01 Cape Town (S. Africa) SW: 8/01 Taiwan SW: 6/01	No No Yes Yes	15633	< 40 %sediment not known	Ballast management plan calls for full exchange whenever practical & for emphasis to be placed on cleaning tanks by flushing & sloshing. Exchanged ballast samples taken: Fore Peak: 23 ^{o} 59N 59^{o} 36W I DB Port: 29^{o} 46N 59^{o} 34W 1 DB Stbd: 29^{o} 46N 59^{o} 34W Water temperature at time of exchange 25^{o}C - samples 10.2^{o}C.	SW SW SW
45	BC	1983	02/99	Not known	Toronto 11/8/01	Constanza (Romania) to Montreal & Toronto	Yes	Oristano (Italy) SW: 10/01 Rotterdam (Netherlands) FW/BR: 8/01 Murmansk (Russia) SW/BR: 8/01 Spitzbergen SW: 7/01	Yes at entrance to Black Sea No No No	6818	< 40 10% sediment	Tanks already contain FW ballast from Montreal - no sampling possible. Management procedures call for full exchange & flushing whenever practical. Tank inspections made every 6 months. Last inspection indicates mud accumulation between frames in bilge areas.	N/A

46 **1016**	BC	1999	New build	New	Burns Harbor 11/7/01	Ijmuiden (Netherlands) to Burns Harbor	No	Hull (UK) SW/BR: 10/01 Barry (UK) SW: 10/3/01 Liverpool (UK) FW: 9/27/01 Parangua (Brazil) SW: 8/24/01 Vlissengen (Netherlands) FW: 8/5/01	No No No No	11088	< 20 5-10% sediment	2 DB Port: <1.0 m^3 water retained at the aft end, patches of mud along bilge longitudinals. 2 DB Stbd: Both water and sediment residuals as port side. Sediment residual appears to be restricted by frequency of ballasting.	BR/SW BR/SW
47 **ND 19/01**	RoRo	1992	06/98	Yes all tanks	Hamilton 11/16/2001	Cape Town (S. Africa) to Philadelphia, Pa Montreal Hamilton & Baltimore, Md	Yes see observa-tions	Continuous ballast movement for stability & trim considerations	Yes 15 tanks with different parcels of ballast	4456	See observations	Because of nature of operation and trade vessel is seldom without ballast. Newly purchased, unpumpables still being assessed. All retained ballast is exchanged, or retained under USCG direction. Samples attempted from 4 tanks but sounding pipe arrangement did not permit in tanks 11 & 12. DBW Tank 9: 04° 10N 27° 00W DBW Tank 10: 04° 52N 27° 25W	 SW SW
48 **1017**	BC	1998	1999	No	Burns Harbor 11/19/2001	Ghent (Belgium) & Teurneuzen (Netherlands) to Contrecoeur & Burns Harbor	Yes	Decertion Bay (NWT) SW: 11/11/01 Antwerp (Belgium) FW: 10/01 Cheng Xi (China) FW: 8/01 Kaoshung (Taiwan) SW: 7/01	No Yes Yes No	18682	< 50 < 10% sediment & mud	2 Port DB: 3.0 m^3 water residual over bottom shell, slight accumulation of sediment along turn of bilge. 2 Stbd. DB: Both water and sediment residuals as port side. Ballast management plan calls for regular flushing of tanks both during exchange and on loaded passages.	SW SW
49 **1018** **ND 20/01**	BC	1982	09/00	No	Hamilton 11/21/2001	Oita (Japan) & Pusan (Korea) to Hamilton	Yes	Shanghai (China) FW: 10/4/01 Baltimore, Md SW: 8/3/01 Puerto Cabello (Venezuela) SW: 7/17/01 Burns Harbor, W FW: 6/13/01 Porto Vesme (Italy) SW: 5/10/01	Yes (Due to turbidity) Yes No No No	11149	+/- 100. Varies with trim < 40% sediment and mud	Fore Peak: Very little water residual but concentrations of mud between forward deep floors up to lightening hole level - drainage at shell blocked estimate 10.0 m^3. 3 DB Stbd: Sediment and mud accumulating heavily along out board side of bilge longitudinals. Drainage in tank still good - accumulation between limber holes. Ballast was exchanged and tanks flushed in East China Sea 30° 55N 130° 05E 6/10/01 due to silt in river at Shanghai. Vessel has good ballast management program.	ND SW

50 **1019** **ND 21/01**	BC	1993	03/01	Yes Fore Peak & 1 DB's	Hamilton 11/21-23/01	Brake (Germany) to Montreal & Hamilton	Yes	Antwerp (Belgium) FW: 11/1/01 Sept Isles, PQ SW/BR: 10/8/01 Augusta (Sicily) SW: 9/14/01 Montreal, PQ FW: 8/08/01 Ravena (Italy) SW: 6/16/01	No No No No Yes	14067m^3	< 50 < 10% sediment	Ship has good ballast management and tank inspection program, although samples may indicate flushing restricted to double bottoms only. Had taken ballast in peaks and double bottoms in Montreal. No 8 trim tanks P & S entered and sampled for water and sediment residuals. Sediment samples may be corrupted by soft coating used on some tank members Water residual in each # 8 tank 2.0 m^3 with small localized sediment deposits	FW
51 **1020** **ND 22/01**	BC	1984	03/99	No	Hamilton 11/29/2001	Ventspils (Latvia) to Hamilton	No Aft Peak exchanged	Stettin (Poland) FW: 11/6/01 Cartagena & Valencia (Spain) SW: 10/01 Bremen (Germany) FW: 9/20/01 Ghent (Belgium) FW: 9/11/01 Livorno (Italy) SW: 8/16/01	No No No No No	13276	< 130 50% sediment	Ship does not flush tanks on loaded passage due to problems with evacuation. Tanks flushed as part of exchange program. Fore Peak: 3.0 m3 water retained around bellmouth - tank generally has light coating of sediment on all horizontal surfaces. Heavy concentration of mud between deep floors estimated +/- 20.0 m^3. Aft Peak: Very confined - no evidence of sediment collection but heavy rust scale. Tank generally filled in deep water. Tank inspections performed every 6 months.	ND SW
52 **ND 23/01**	BC	1982	03/99	Not known	Hamilton 11/30/2001	Bremen (Germany) to Great Lakes	Yes	Santander (Spain) SW/BR: 4/8/01 Montreal & Les Escoumins Combined FW/SW: 10/15/01 Great Lakes FW: 9/01 Rio Grande (Argentina) FW: 8/13/01 Jen Jen (Algeria) SW: 7/01	Yes No No No No	15947	< 90 Varies with trim	Excellent example of older ship with good tank cleaning program. Managers have comprehensive management program with tank flushing in loaded condition and whenever exchanging ballast. Fore Peak: Light scale but hand cleaned by crew and virtually sediment free, <1.5 m^3 water retained around bellmouth.	ND

53 **ND 24/01**	BC	1985	22/00	No See Obser vations	Hamilton 11/30/01	Morehead City, NC to Hamilton	Yes	San Juan (Puerto Rico) SW: 4/15/01 Burns Harbor, Ind FW: 10/01 Stravanger (Norway) SW: 9/5/01	Yes No Yes	17228	< 30 25% sediment	Managers have comprehensive ballast management plan which requires regular tank inspection and crew cleaning. No's 1,2 & 3 double bottoms cleaned during September passage from Stavenger to Brazil - all other tanks exchanged and flushed. 2 DB Port: 1.7 m3 of water retained at aft end with about 25% sediment content. No significant accumulation anywhere in tank. 2 DB Stbd: As port side	 ND ND
54	BC	2001	New Build	New	Thorold 12/3/01	Fanshan (China) to Thorold	Yes See Obser-vations	Shanghai (China) FW: 9/14/01 and East China Sea SW: 9/15/01	Yes	8670	NIL	This ship was delivered from a shipyard on the Yagtzee River above Shanghai in September. To minimize the amount of silt ingested only sufficient ballast to permit steerage was taken until clear of the river outflow - then exchanged & flushed and/or topped off. Comprehensive ballast management plan calls for regular flushing. Stripping system permitted all ballast to be evacuated at first loading.	NA
55	BC	1987	03/01	Not known	Hamilton 12/3/01	Pyongyang (N. Korea) to Maracaibo & Porto Caballo (Venezuela) Sorel & Hamilton	No	Samarinda (Indonesia) SW/BR: 9/17/2001 Chon Buri (Thailand) SW: 7/30/01 Dalian (China) SW : 3/01	No Yes Yes	13249	90-130. Varies with trim % sediment not known	This ship has to continually adjust ballast for seaway passages due to air draft restrictions. Ballasting begun in Sorel and tank entry and sampling not possible. Managers are in the process of obtaining Flag State and Class approval of the ballast management plan - not available for reference.	N/A
56	BC	1983	10/01	Yes all DB tanks	Hamilton 12/3/01	Bartin (Turkey) & Ceuta (Spain) to Sorel & Great Lakes	No	Piraeus (Greece) SW: 10/01	Yes at entrance to Black Sea	7978	< 70 Nil sediment	First voyage after change of ownership and extensive dry docking at which double bottom tanks were cleaned. Only rudimentary ballast management and note no flushing after discharge of Black Sea ballast.	ND
57 **ND 25/01**	HL/ RoRo	1983	07/01	no not necessary	Port Weller 12/06/01	Kingston (Jamaica) to Montreal & Port Weller	No but # 4 DB's P & S and # 9 WT P & S & exchanged	Multiple movements in Far East & discharged & re ballasted Kingston (Jamaica) SW: 11/21/01	Yes	6000	Negligible	This ship continuously carries and/or handles ballast for loading/ discharging and transit trim & stability. System is capable of stripping out ballast completely. Exchanged ballast tanks sampled only. 4 DB Port & Stbd: 29^{o} 39N 65^{0} 41W 9 Wing Tank Port & Stbd: 34^{o} 46N 66^{o} 01W 28/11/01	

58	CT	1993	11/98	No not necessary	Hamilton 12/07/01	Porvo (Finland) to Quebec Hamilton & Sarnia	Yes	Rotterdam (Netherlands) BR/FW: 11/12/01 Great Lakes FW: 10/23/01 Rotterdam BR/FW: 10/5/01 Great Lakes FW: 9/18/01 Faieringe Haven (Greenland) SW: 8/17/01	No No No No No		Nil water &/or sediment	This ship constantly trades Trans Atlantic but seldom in ballast - flushing of tanks with seawater is performed to both clean and sanitize. Segregated ballast tanks are all accessible from deck hand washed by crew every six months using pressure hoses. Short port stay does not permit sufficient time to ventilate and survey/ sample tanks.	N/A
59	BC	1986	09/01	Yes all tanks	Hamilton 12/10/01	St. Petersburg (Russia) to Montreal & Great Lakes	No	Bilbao (Spain) BR/SW: 10/21/01 Hull (UK) BR/FW: 10/1/01 St. Petersburg (Shipyard) to load berth FW: 9/01	Yes Yes No	13124	< 30 nil sediment	Ballast management plan calls for minimum uptake of water in port. Ship to proceed and complete ballasting on reaching cleaner water. Port water to be exchanged during process. Ship also dry-docks 12-18 month intervals where tanks cleaned. Short port stay precluded tank entry.	N/A
60	CT	1981	05/01	Yes all tanks	Hamilton 12/13/2001	Rotterdam (Netherlands) to Montreal, PQ Chicago, Ill Green Bay, Wis Hamilton, Ont	Yes	See observations	See observations	2409	NIL	Ship trades constantly Trans Atlantic & seasonally Great Lakes. Ballast movements are generally very short and tanks are flushed in North Atlantic to clean and sanitize. Access to double bottom tanks is difficult, consequently tank cleaning is performed with dry docking. Ship was boarded to evaluate suitability for scientific experiments.	N/A
61 **ND 26/01**	GC	1982	09/01	Not known	Hamilton 12/8/01	(S. Korea) and Bilbao (Spain) to Hamilton	No	Tientsin (China) FW: 9/01	Yes	1984	Not known but see observations	Appears to have only ballasted on leaving the shipyard in China in September. Fore Peak: Exchanged 11/28/01 Fwd. Deep Tank: Between 47° 31N 38° 44W 3 DB Stbd: and 47° 22N 42° 24W 4 DB Stbd: Samples taken through sounding pipes indicated significant sediment accumulation.	SW

62 **ND 1/02**	BC	1982	11/01	Not known	Hamilton 29/5/02	Borgas (Bulgaria) and Gemlik (Turkey) to Hamilton	No Fore Peak exchanged 5/5/02 37^{o} 03N 17^{o} 10W	Monfalcone (Italy) SW: 5/02 Philadelphia FW: 4/02 Leixios (Portugal) SW: 3/02 Wilmington(NC) FW: 3/02	Yes Yes No No	15952	+/- 150 % Sediment not known	Ballasting in Hamilton had already commenced. Fore Peak only sampled after mid ocean exchange. Ballast valve arrangement not conducive to mid ocean flushing, and results in unpredictable residuals when deballasting depending on loading circumstances.	N/A SW
63 **ND 2/02**	HL	1983	7/01	Not necessary tanks clean	Port Weller 1/6/02	Corpus Christie to Montreal & Port Weller	See obser-vations	Multiple movements in Far East, Persian Gulf & Red Sea, partial ballast in Corpus Christie	Yes (See obser-vations)	4945	See obser-vations	Compensate for trim and stability requirements as a result of project cargo, thus tanks frequently flushed. The tanks sampled as follows: 4 ST Stbd: Ballasted at Jebel Ali (SW) 19/4/02 - exchanged at 26^{o}19N, 163^{o} 11W, 10/4/02. 5 DB Port: Ballasted at Ulsan (SW) 15/3/02 - exchanged at 25^{o} 00N, 89^{o} 56W 30/4/02. 8 ST Port: Ballasted at Ulsan (SW) 15/3/02 - exchanged 30^{o} 00N, 174^{o} 52W 8/4/02. 9 ST Stbd: Ballasted at Ulsan (SW) 15/3/02 - exchanged 29^{o}58N 170^{o} 02W 7/4/02 Sampling indicated there may be significant sediment accumulation in tanks ballasted in Ulsan.	SW SW SW SW
64 **ND 3/02**	BC	1983	7/01	Believed all cleaned	Toronto 5/6/02	Klaipeda (Lithuania) & Szczecin (Poland) to Toronto & Windsor	Yes, tanks with ballast fully exchanged	Valleyfield FW: 4/02 Antwerp (Bel) FW: 4/02	No Yes	9610	+/- 80 % Sediment not known	1 CDB: exchanged 59^{o} N 21^{o} 00W. Unable to access Fore Peak due to bend in sounding pipe.	SW

1021 **65** **ND 4/02**	BC	1997	3/02	All tanks drained	Toronto 6/6/02	Lian Yun Gang & Shanghai (China) Pohang & Kwong Yung (S. Korea) to Vancouver, Porto Cabello, Sorel and Toronto	Yes	Shanghai FW: 3/02	Yes	19149	< 50 25% Sediment	Only one ballasting following dry-docking, tanks both exchanged and flushed due to turbidity in river. Fore Peak tank exchanged 5/02 28° 07N 64° 27W and No. 6 Double Bottoms flushed in same position. Fore Peak tank < 10 m^3 mostly around bellmouth. 6 DB Port: 1.0 m^3 residuals water/slurry with sediment deposits on bilge and side longitudinals. 6 DB Stbd: 2.0 m^3 residuals water/slurry with sediment deposits on bilge and side longitudinals.	SW SW SW SW
1022 **66** **ND 5/02**	BC	1981	4/02	All DB and TW tanks	Toronto 6/6/02	Nemrut/ Istanbul (Turkey) to Sorel and Hamilton	Not necessary this trip	Nemrut BR/FW: 4/02	No	15952	+/- 80 10% Sediment	Fore Peak tank only accessed, sediment samples taken will contain scale as repairs were performed to chain locker during recent dry-docking. Estimated I.0 m^3 water residual with pockets of sediment in restricted areas.	FR/BR
67 **ND 6/02**	BC	1983	4/01	Not known	Toronto 5/6/02	Inchon & Pyong Ye (S. Korea) to Acajutla (El Salvador) Christobal (Panama), Rio Haina (Dom Republic) San Juan (Puerto Rico) Newark & Toronto	Only during ballast exchange	Inchon (S. Korea) SW: 4/02 Fan Cheng (China) SW: 3/02 Nantung (China) FW: 2/02 Onsan (S. Korea) SW: 1/02	Yes Yes Yes Yes	7783	+/- 100 % Sediment not known	Management program calls for ballast exchange on all passages irrespective of regulatory requirements to keep tanks clean. Fore Peak ballasted Balboa (SW) exchanged 32° 31.6N, 70° 50.2W 1DB Port: Ballasted Cristobal (SW) exchanged as above. 1 DB Stbd: Ballasted Cristobal (SW) exchanged as above.	SW SW SW

1023 **68** **ND 7/02**	BC	2000	New building	N/A	Hamilton 13/6/02	Matanzas (Venezuela) to Hamilton	No See comments	Maracaibo (Venezuela) FW: 5/02 Burns Harbour/ Thunder Bay FW: 5/02 Puerto Cabello (Venezuela) SW: 3/02 Contrecoeur FW: 2/02	No Yes No No	19731	35-50 > 25% Sediment	Ship has tank flushing program but was unable to utilize on this voyage due to draft concerns. Last ballasting in Venezuela carried out in extremely turbid conditions. Fore Peak tank washed down with hoses but residual mud still contained in panting area and other restricted locations. Estimated < 1.0 m^3 water and 2.0 m^3 mud residual. With two meter trim by stern residuals in after end of double bottom tanks primarily water with mud residuals concentrated in the forward end and outboard of bilge intercostal and lower bilge longitudinal stringer. 5 DB Port: < 3.0 m^3 water and 1.5 m^3 mud/sediment. 5 DB Stbd: < 3.0 m^3 water and 1.5 m^3 mud/sediment. Tank coating in extremely good condition.	FR/BR FW FW
69	BC	1986	3/01	Not known but probable	Hamilton 22/6/02	Tubarao (Brazil) to Sorel & Hamilton	Yes	Sfax (Tunisia) SW: 5/02 Trieste (Italy) SW: 4/02 Richards Bay (S. Africa) SW: 2/02 Maputo/Beira (Mozambique) SW: 1/02 Abidjan (Cote D'Ivoire)/ Douala (Cameroon) SW: 1.02	Yes Yes Yes Yes Yes Yes	10837	+/- 60 % Sediment not determined	Management plan calls for ballast exchange and flushing at every opportunity even if not required by regulation. Six month ballast tank inspection last inspection indicated tanks relatively clean.	N/A
1024 **70**	BC	1986	3/02	No	Windsor 24/6/02	Tampico (Mexico) to Toledo, Windsor & Detroit	Yes	Port Everglades SW: 5/02	No	9000	+/- 50 < 25% Sediment		SW

71 **ND 8/02**	HL	1991	8/01	Yes all Top Wing Trim Peak & DB Tanks	Port Weller 3/7/02	Beaumont to Erie	N/A	Beaumont SW: 6/02	Yes	4494	Not determined	This ship carries ballast for trim and stability on a continuous basis. On this full ballast passage departed Beaumont with 1400 m^3 ballast to fill and/or exchange all tanks enroute. Only two tanks available for sampling due to head pressure. USCG dictated retention of this ballast water due to location taken. 4 TWT Port: Taken 39^{O} 35N 69^{o} 06W. 6 TWT Port: Taken 41^{o} 50N 65^{o} 35W.	SW SW
72	BC	2000	New building	N/A	Hamilton 8/7/02	Praia Mole (Brazil) to Hamilton & Detroit	Yes	Porto Caballo (Venezuela) SW: 6/02 Sault Ste. Marie FW: 5/02 Porto Caballo (Venezuela) SW: 5/02 New Orleans FW: 4/02	Yes No Yes No	19313	+/- 40 % Sediment not ascertained but said to be minimal	Managers have comprehensive tank inspection and tank flushing program. Regular reports indicate little or no sediment accumulation.	N/A
73 **ND 9/02**	GC/HL	1999	5/02	No	Hamilton 10/7/02	Tampico (Mexico) to Hamilton & Contrecoeur	Yes	Beaumont,(TX) FW: 6/02 Genoa (Italy) SW: 5/02 Beaumont,(TX) FW: 4/02 Gibraltar SW: 3/02	No No No No	2947	See observations	This ship carries ballast on most passages due to the nature of it's trade, and exchanges ballast, flushing tanks whenever the opportunity presents. This inbound voyage included ballast taken in the Gulf of Mexico between latitudes 25^{o} 41N and 25^{o} 50N, and longitudes 86^{o} 50W and 87^{o} 17W. This ballast to be retained on board in the Great Lakes at USCG direction. These two tanks, No. 1 side tank stbd and No. 3 side tank stbd both sampled.	SW
74 **ND 10/02**	BC	1998	6/01	Not known	Hamilton 17/7/02	Teesport (UK) & Avilla (Spain) to Hamilton & Cleveland	No	Emshaven (Holland) SW: 6/02 Alexandria (Egypt) SW: 6/02 Civitavecchia (Italy) SW: 5/02 Milwaukee FW: 4/02 Istanbul (Turkey) BR/SW: 3/02	Yes No No No No	1485	Normally Nil See observations	This ship was carrying partial ballast in the fore peak and No. 1 Port & Stbd Double Bottoms for trim and stability purposes in addition to a break bulk cargo. Fore Peak: Exchanged 43^{o} 05N 33^{o} 43W. 1 DB Port: Exchanged 43^{o} 04N 34^{o} 09W. 1 DB Stbd: Exchanged 43^{o} 04 N 34 24 W.	SW SW SW

1025 **75** **ND 11/02**	BC	1984	2/01	Yes all DB's and Top Wing Tanks	Hamilton 19/7/02	Praia Mole & Tubarao (Brazil) to New Haven, Halifax and Hamilton	No	Paranagua (Brazil) BR/SW: 5/02 Tuapse (Russia Black Sea) BR/FW: 4/02 Klaipeda (Lithuania) FW: 2/02	No Yes No	10600	+/- 70 43% Sediment	See also ship No. 11, this ship changed name in 2002. Samples taken from No. 1 Double Bottoms Port and Stbd. 1 DB Port: 7.0 m^3 water/mud with deposits primarily in bilge area. 1 DB Stbd: 7.0 m^3 water/mud with deposits primarily in bilge area.	 BR/FW BR/FW
1026 **76** **ND 12/02**	BC	1981	12/00	All Ballast Tanks	Hamilton 22/7/02 Cleveland 25/7/02	Antwerp (Belgium) to Hamilton, Cleveland & Toledo	Not this voyage	Ghent (Belgium) FW: 7/02 Great Lakes FW: 6/02 Ghent (Belgium) FW: 5/02 Great Lakes FW: 4/02 Antwerp (Belgium) FW: 3/02	No No No No No	16294	70 - 110 <10% Sediment	Ballast water management plan calls for flushing/exchange at every opportunity. No flushing this voyage due to draft considerations. Samples taken from No. 1 Port DB and Fore Peak tanks, No. 1 Stbd DB not entered due to poor atmosphere. Fore peak: < 1.0 m^3 each water and mud. 1 DB Port: 12.0 m^3 water with < 10% sediment evenly coating bottom shell in forward end of tank.	 FW BR/FW
77	BC	1982	1/01	All Ballast Tanks	Hamilton 1/8/02	Archangel (Russia) to Becancoeur & Hamilton	No	Huelva (Spain) SW: 7/02 Huelva SW: 6/02 Huelva SW: 6/02 Le Havre (France) BR/FW: 5/02	No No No No	2524	+/- 100 % Sediment not known	Ship purchased in 2001 as one of four for newly formed ship manager. Has double bottom and side tanks for water ballast but side tanks unusable due to perforations in tank/hold bulk heads. Ballast management plan and procedures still under development.	N/A
1006 **78** **ND 13/02**	BC	2000	New building	N/A	Hamilton 6/8/02	Matanzas (Venezuela) to Hamilton	Not this voyage	Cienfuegos (Cuba) SW: 7/02 Veracruz (Mexico) SW: 6/02 Great Lakes FW: 5/02 Rauma (Finland) BR/FW: 4/02 Montreal FW: 3/02	Yes Yes No No No	19730	+/- 20 > 50% Sediment	Ship previously sampled in 2001(No. 16) and revisited to examine sediment buildup since delivery. Managers have comprehensive ballast management program which includes frequent flushing and exchange. 5 DB Port & Stbd: Tanks examined, sediment buildup apparent despite this program between outer intercostal and longitudinal frames extending both tanks upturn of bilge. Bottom shell remains clean full length of tanks between intercostal and fuel tank longitudinal bulkhead. < than 2.0m^3 unpumpable ballast, but an estimated 1.3 MT sediment in each tank.	SW

1027 **79** **ND 14/02**	BC	1980	1/00	Yes	Hamilton 7/8/02	Antwerp (Belgium) to Montreal, Hamilton and Burns Harbor	Not this voyage	Liverpool (UK) SW: 7/02 and Antwerp (Belgium) FW: 7/02 Great Lakes FW: 6/02 Antwerp FW: 5/02 Great Lakes FW: 4/02 New Orleans FW: 3/02	No No No No No	16294	+/- 70 > 50% Sediment	Fore Peak and No. 7 Port & Stbd DB's entered for sampling. Fore Peak: <5.0 m^3 water residual with heavy local concentrations of sediment/mud as a result of ship yard debris blocking drainage through floors. 7 DB Port: < 2.0 m^3 water with heavy localized concentration of mud and sediment between longitudinals directly below ballast main - accumulation appears to be a result of scale from pipe restricting drainage. Bottom shell generally coated with sediment through out. 7 DB Stbd: Conditions as Port side.	FW FW FW
1028 **80**	BC	1983	3/02	Not known	Detroit 8/8/02	Antwerp (Belgium) Rotterdam (Netherlands) to Sorel, Cleveland & Detroit	Yes	Antwerp (Belgium) FW: 7/02 Sparrows Point SW: 6/02	No Yes	18150	Not ascertained	Fore Peak tank only available for access, DB tanks already containing Great Lakes ballast. Tank found dry with less than 0.5 MT sediment.	N/A
1029 **81** **ND 15/02**	BC	1995	5/02	Fore Peak only for damage repairs	Windsor 13/8/02	Szczecin (Poland) to Montreal & Windsor	Yes but not this voyage	Policie (Poland) FW: 7/02 Monfalcone (Italy) SW: 6/02 Algeciras (Spain) SW: 6/02 Falmouth (UK) SW: 5/02	No No No No	7658	Unpumpable ballast +/- 30 Sediment/ Mud +/- 100	This ship returned to owners/managers in May after Bare Boat charter since building. Current managers are in the process of refurbishing which includes ballast tank cleaning and rehabilitation. Comprehensive flushing program will be undertaken but impossible on this passage due to load line limitations. Two adjacent combined side/DB tanks opened for examination and sampling. In both No. 5 tank and No. 6 tank < 1.0 m3 of water found, but extensive mud/sediment deposits found on side tank stringer plates and in heavy patches on bottom shell and in bilge area, depths ranging from 1-10 cm.	FW
1030 **82**	BC	1983	11/01	Yes all Double Bottoms	Hamilton 15/7/02	Antwerp (Belgium) & Brunsbuttel (Germany) to Contrecoeur, Hamilton & Detroit	Yes but not this voyage	Ijmuiden (Holland) SW: 7/02 Great Lakes FW: 6/02 Antwerp (Bel) FW: 5/02 Great Lakes FW: 4/02 Amsterdam (Holland) FW: 3/02	No No No No No	18842	+/- 170 < 10% Sediment	Ship Management plan calls for regular tank inspections and flushing to control sediment buildup, but pumping arrangement combined with restricted draft means flushing can not be done on all Seaway bound passages. Accessed # 2 DB Port and Stbd. In both tanks sediment/mud accumulation restricted mainly to upper turn of bilge above unpumpable water level. Tanks had 10.9 m^3 and 8.2 m^3 unpumpable ballast respectively.	SW
83	BC	1984	4/02	Not known	Hamilton 22/8/02	Salvador (Brazil) to Sorel and Hamilton	No	Porto Cabella (Venezuela) SW: 7/02 Great Lakes FW: 6/02 Tampa SW: 5/02	Yes No No	15200	+/- 50 % Sediment not known	Vessel changed ownership following April dry docking, all previous records removed. Ballast Management Plan calls for flushing during exchange only.	N/A

84	BC	1993	1/01	Not known	Hamilton 23/8/02	Immingham (UK) to Montreal, Hamilton, Cleveland and Detroit	No	Bilbao (Spain) SW: 7/02 Great Lakes FW: 7/02 Sete (France) SW: 6/02 Mumbai (India) BR/SW: 4/02 Zhanjiang (China) SW: 3/02	No No No No No	5955	+/- 70 < 25% Sediment	Vessel changed ownership in April. Tank inspection reports indicate tanks in good condition but some mud accumulation in double bottom hopper tanks.	N/A
1031 **85**	BC	2002	New building	N/A	Hamilton 13/9/02	Matanzas (Venezuela) to Hamilton	Yes but not this voyage	Aguide (Venezuela) SW: 8/02 Great Lakes FW: 7/02 Liverpool (UK) BR/SW: 5/02 Montreal FW: 4/02 Shanghai (China) FW: 2/02	No No No No Yes	18830	< 10 Negligible Sediment	4 DB Port: Tank extends outboard to bilge girder only. <0.5 m^3 water with only partial light film of sediment. 4 DB Stbd: As port side but some small clumps of sediment in local areas. Ship has completely separate stripping system with independent bellmouths, eductors. Separate pumping system exists for side tanks which extend down to lower turn of bilge.	SW SW
86	BC	1984	8/02	Yes All tanks cleaned by crew	Hamilton 20/9/02	Izmir (Turkey), Foz and Santander (Spain) to Hamilton	Yes	Istanbul (Turkey) SW: 8/02	No	7987	< 100	First voyage following dry docking, ballast taken at anchorage following dry docking to avoid turbidity. No attempt made to enter tanks.	N/A
87	BC	1983	3/00	Not known	Hamilton 22/9/02	Antwerp (Belgium) to Hamilton, Cleveland, Detroit and Ludington	Only during exchange procedures	Antwerp (Belgium) FW: 8/02 Great Lakes FW: 8/02 Maracaibo (Venezuela) BR/FW: 7/02 Great Lakes FW: 6/02 Casablanca (Morocco) SW: 4/02	No No Yes No Yes	17471	Not ascertained	Sampling of Double Bottom tanks arranged for Ludington but subsequently aborted due to security at discharge terminal.	N/A

88	BC	1985	2/02	Partial cleaning only to survey require-ments	Hamilton 29/2/02	Praia Mole (Brazil) to Hamilton	Yes	Altamira (Mexico) SW: 8/02 Dunkirk (France) SW: 7/02 Djenet (Algeria) SW: 6/02 Great Lakes FW: 5/02 Lake Charles FW: 5/02	Yes Yes Yes Yes Yes	18081	< 30 % Sediment not known	Records indicate ship exchanges ballast on every voyage regardless of regulations, tanks flushed as part of exchange procedures.	N/A
1027 **89**	BC	1980	1/00	Yes	Windsor 5/10/02	Antwerp (Belgium) to Windsor, Detroit, Milwaukee & Burns Harbor	No	Santander (Spain) SW: 9/02 Great Lakes FW: 8/02 See also # 79	No No	16294	+/- 70 < 50 % Sediment	Fore Peak tank and # 7 DB's cleaned following earlier inspection in August, debris causing sediment accumulation removed. Samples taken and Task 2 experiment set up in forepeak.	FW
90	BC	1982	7/02	Yes	Windsor 09/10/02	Korea to Great Lakes	No	Shipyard China		9336		3 DB Port tank and 3 DB Stbd tanks sampled.	SW
1033 **91**	BC	2002	New building	N/A	Windsor 20/10/02	Ghent (Belgium), Stettin (Poland) and Bremen (Germany) to Montreal, Windsor, Detroit, Milwaukee & Burns Harbor	No	Ghent (Belgium) FW: 10/02 Great Lakes FW: 9/02 Jing Jiang (China) FW: 6/02	No No Yes	18068	< 10 < 10 % Sediment	Fore Peak and No 6 Wing Tank Stbd. entered and sampled. In both tanks a film of sediment is apparent on all horizontal surfaces with some minor accumulation in patches. Also accumulation on bottom shell of Fore Peak locally in way of floors.	FW
1007 **92**	BC	1987	1/01	No	Burns Harbor 24/10/02	Antwerp (Belgium) to Burns Harbour	No	Rotterdam (Netherlands) FW: 10/02 Great Lakes FW: 9/02	No No	24911	< 55	6 DB Port & Stbd tanks sampled.	FW

93	CT	1998	8/00	Yes includes # 6 Port & Stbd blasted & recoated	Hamilton 3/11/02	San Juan (Puerto Rico) and Nassau (Bahamas) to Hamilton	Yes	Malaga (Spain) SW: 10/02 Southampton (UK) SW: 10/02 Hamburg (Germany) FW: 10/02 Trinidad BR/SW: 9/02 Southampton (UK) SW: 9/02 Hamburg (Germany) FW: 8/02	No No No No No No	2232	< 1.0	Ship is on continual mixed passages of cargo and ballast, ballast tanks are flushed when ever possible, has comprehensive cleaning program.	N/A
94	BC	1980	7/02	Yes all ballast tanks	Hamilton 6/11/02	Gefle (Sweden), Sauda (Norway) to Sorel, Hamilton, Cleveland & Detroit	No	Ghent (Belgium) FW: 9/02 Great Lakes FW: 8/02 Tallin FW: 7/02	No No No	5324	+/- 20	Ballast Management Plan calls for flushing/cleaning in conjunction with ballast exchange, and caution related to the unnecessary intake of sediment or other turbidity when ballasting	N/A
95	BC	1995	3/00	Not known	Hamilton 11/11/02	Mizushima (Japan) to Hamilton	Yes	Ulsan (S. Korea) SW: 9/02 Kuantan (Malaysia) SW:: 8/02 Shimizu (Japan) SW: 5/02 Pasir Gudang (Malaysia) BR/SW: 4/02	No Yes Yes No	5053	< 30 % Sediment not known	Port time did not permit sampling, tank inspections performed every six months and cleaning and flushing if ballasting to US/Canadian or Australian ports.	N/A

1014 **96**	BC	1999	4/02	Yes	Cleveland 12/11/02	Gdansk (Poland) to Cleveland & Duluth	Yes	Bremen (Germany) FW: 10/02 Nordenham (Germany) FW: 9/02 Bremen (Germany) FW: 9/02 Bremen (Germany) FW: 9/02 Casablanca (Morocco) SW: 8/02	No No No No No	19060	+/- 45 15% Sediment	4 DB Stbd: Has 8-10cm (+/- 15 m^3) water throughout with sediment accumulation throughout turn of bilge area and light film covering bottom shell. 6 DB Stbd: <1 m^3 water but heavy sediment accumulation throughout turn of bilge area. Salinity and water accumulation tend to indicate No. 4 Double Bottom flushed during ocean passage but not No. 6 Double Bottom.	SW FW
97	BC	1984	12/01	All tanks to meet survey requirements	Hamilton 17/11/02	Tubarao (Brazil) to Hamilton	Not on this voyage	Santos & Paranagua (Brazil) BR/SW: 10/02 Oran (Algeria) SW: 8/02 Great Lakes FW: 7/02 Tubarao/ Paranagua (Brazil) BR/SW: 6/02 La Goulette (Tunisia) SW: 4/02	No No No No No	7782	+/- 80 % Sediment not known	Tank flushing is practiced in conjunction with ballast exchange. No Double Bottom or PeakTank inspections carried out since last crew change, condition related to sediment accumulation not known.	N/A
98	BC	1987	1/02	All DB tanks to survey requirements	Hamilton 19/11/02	Praia Mole (Brazil) to Hamilton	Not on this voyage	Barcelona (Spain) SW: 10/02 Great Lakes FW: 9/02 Skikda (Algeria) SW: 8/02 Great Lakes FW: 7/02 Ghent (Belgium) FW: 6/02	Yes No No No No	14137	+/- 30 Said to be negligible Sediment	Ballast Management Plan calls for tank flushing when draft or other conditions permit. Regular tank inspections every six months, July report indicates tanks in good and clean condition.	N/A

99	BC	1999	New building	N/A	Cleveland 19/11/02	Rotterdam (Netherlands) to Cleveland	No	Rotterdam (Netherlands) BR/SW: 10/02 Montreal FW: 10/02 Bremen (Germany) FW: 9/02 Police (Poland) FW: 8/02 Limassol (Cyprus) SW: 7/02	No No No No	19061	+/- 45 40 % Sediment	4 DB Port: < 10 m^3 water distributed through tank with significant sediment accumulation throughout. 4 DB Stbd: < $2m^3$ water with significant sediment/mud accumulation throughout.	BR/SW BR/SW
100	BC	2001	New building	N/A	Hamilton 25/11/02	Kaohsung (Taiwan) Pohang (S. Korea) to Sorel & Hamilton	Yes	Shanghai (China) FW: 10/02 Kaohsung (Taiwan) SW: 10/02 Valparaiso (Chili) SW: 8/02 Djen Djen (Algeria) SW: 7/02 Sorel FW: 5/02 Savannah/ Philadelphia BR/FW: 4/02 Tobata (Japan) SW: 3/02	Yes No No Yes No No Yes	9718	< 20 % Residual not known	Ship has separate stripping system for all tanks with eductors, good ballast management plan and records indicates cleaned and flushed whenever circumstances permit. Potential for very little sediment accumulation.	N/A
101	BC	1983	10/01	Yes all ballast tanks	Hamilton 25/11/02	Diliskales (Turkey) to Three Rivers, Hamilton and Chicago	Not this voyage	Gabes/Sfax (Tunisia) SW: 10/02 Santos (Brazil) BR/FW: 6/02 Paranagua (Brazil) BR/FW: 6/02 Bilbao (Spain) SW: 05/02	No See remarks See remarks Yes	4627	< 10 mt percentage sediment negligible	On both ballastings in Brazil tanks where chlorinated prior to discharge in Argentina. Ship has excellent ballast management plan and ballast records. Drydock report and subsequent Masters reports indicate excellent tank conditions with negligible sediment accumulation. Independent striping system fitted.	

1034 **102**	BC	1977	03/01	Partially, Top Side tanks scraped & coated, DB's flushed & drained.	Hamilton 26/11/02	Morehead City to Hamilton	Yes	Tampa SW: 11/02 Maracaibo (Venezuela) BR/FW: 10/02 Abidjan (Cote D' Ivoire) SW: 9/02	Yes Yes Yes	11109	< 60	Company policy is to exchange ballast on every voyage, and ballast management plan calls for flushing at every opportunity. 3 DB Port & Stbd. Tanks accecces for sampling. 3 DB Port: +/- 25 m^3 water residual with approximately 1% sediment in light film across bottom. 3 DB Stbd: < 2 m^3 water residual with approximately 10% sediment covering < 20% of exposed bottom and bilge shell plating. Sediment is accumulating primarily in areas where loose scale has been piled during tank maintenance for future disposal.	BR/FW SW
1035 **103**	BC	1984	01/02	Yes all ballast tanks	Windsor 6/12/2002	Tampico (Mexico) to Windsor	No	Veracruz (Mexico) SW: 11/02 Windsor FW: 11/02 Tampico (Mexico) SW: 10/02 New Orleans FW: 09/02	No No No No	7783	+/- 30		

Appendix 3.

NOBOB ballast tank sample summary database.

Task	Date	Year	Jday	Port	Ship Code	Ship #	Tank Type	Tank ID	Tank #	Sample ID	Medium	Temp	Salinity
1	7-Dec-00	2000	341	Hamilton	1001	1	FPK	Forepeak	1	1-00341-01-wa	wa	ND	48
1	7-Dec-00	2000	341	Hamilton	1001	1	FPK	Forepeak	1	1-00341-01-ws	ws		
1	7-Dec-00	2000	341	Hamilton	1001	1	DBT	DB ## -	2	1-00341-02-wa	wa	ND	36
1	7-Dec-00	2000	341	Hamilton	1001	1	DBT	DB ## -	2	1-00341-02-ws	ws		
1	7-Dec-00	2000	341	Hamilton	1001	1	AFT	Aft	3	1-00341-03-wa	wa	ND	ND
1	8-May-01	2001	128	Hamilton	1002	2	DBT	DB ## - port	4	1-01128-01-wa	wa	8	24
1	8-May-01	2001	128	Hamilton	1002	2	DBT	DB ## - port	4	1-01128-01-ws	ws		
1	8-May-01	2001	128	Hamilton	1002	2	DBT	DB ## - starboard	5	1-01128-02-ws	ws		
1	8-May-01	2001	128	Hamilton	1002	2	FPK	Forepeak	6	1-01128-03-wa	wa	ND	44
1	22-May-01	2001	142	Cleveland	1003	3	DBT	DB #2 - port	7	1-01142-01-wa	wa	12	22
1	22-May-01	2001	142	Cleveland	1003	3	DBT	DB #2 - port	7	1-01142-01-ws	ws		
1	22-May-01	2001	142	Cleveland	1003	3	FPK	Forepeak	8	1-01142-02-wa	wa	12	2
1	22-May-01	2001	142	Cleveland	1003	3	FPK	Forepeak	8	1-01142-02-ws	ws		
1	25-Jun-01	2001	176	Hamilton	1004	4	DBT	DB #2 - port	9	1-01176-01-wa	wa	20.2	10
1	25-Jun-01	2001	176	Hamilton	1004	4	DBT	DB #2 - port	9	1-01176-01-ws	ws		
1	25-Jun-01	2001	176	Hamilton	1004	4	DBT	DB #2 - starboard	10	1-01176-02-wa	wa	19.8	2
1	25-Jun-01	2001	176	Hamilton	1004	4	DBT	DB #2 - starboard	10	1-01176-02-ws	ws		
1	27-Jun-01	2001	178	Thorold	1005	5	DBT	DB #4 - port	11	1-01178-01-wa	wa	20	34
1	27-Jun-01	2001	178	Thorold	1005	5	DBT	DB #4 - port	11	1-01178-01-ws	ws		
1	27-Jun-01	2001	178	Thorold	1005	5	DBT	DB #4 - starboard	12	1-01178-02-wa	wa	20.1	32
1	27-Jun-01	2001	178	Thorold	1005	5	DBT	DB #4 - starboard	12	1-01178-02-ws	ws		
1	30-Jun-01	2001	181	Windsor	1006	6	DBT	DB #6 - starboard	13	1-01181-01-wa	wa	22.5	34
1	30-Jun-01	2001	181	Windsor	1006	6	DBT	DB #6 - starboard	13	1-01181-01-ws	ws		
1	30-Jun-01	2001	181	Windsor	1006	6	DBT	DB #6 - starboard	13	1-01181-01-sl	ws		
1	30-Jun-01	2001	181	Windsor	1006	6	DBT	DB #6 - port	14	1-01181-02-wa	wa	22.7	29
1	30-Jun-01	2001	181	Windsor	1006	6	DBT	DB #6 - port	14	1-01181-02-ws	ws		
1	30-Jun-01	2001	181	Windsor	1006	6	DBT	DB #6 - port	14	1-01181-02-sl	ws		
1	26-Jul-01	2001	207	Hamilton	1007	7	UWT	Upper wing #6 - starboard	15	1-01207-01-wa	wa	21.4	8
1	26-Jul-01	2001	207	Hamilton	1007	7	FPK	Forepeak	16	1-01207-02-wa	wa	20.1	9
1	26-Jul-01	2001	207	Hamilton	1007	7	FPK	Forepeak	16	1-01207-02-ws	ws		
1	28-Jul-01	2001	209	Hamilton	1008	8	DBT	DB #4 - starboard	17	1-01209-01-wa	wa	21.2	2
1	28-Jul-01	2001	209	Hamilton	1008	8	DBT	DB #4 - starboard	17	1-01209-01-ws	ws		
1	28-Jul-01	2001	209	Hamilton	1008	8	DBT	DB #4 - port	18	1-01209-02-wa	wa	21	5
1	4-Aug-01	2001	216	Hamilton	1009	9	DBT	DB #4 - port	19	1-01216-01-ds	ds		
1	4-Aug-01	2001	216	Hamilton	1009	9	DBT	DB #4 - starboard	20	1-01216-02-ws	ws		
1	15-Aug-01	2001	227	Hamilton	1010	10	FPK	Forepeak	21	1-01227-01-wa	wa	23.9	1
1	15-Aug-01	2001	227	Hamilton	1010	10	FPK	Forepeak	21	1-01227-01-ws	ws		
1	15-Aug-01	2001	227	Hamilton	1011	11	FPK	Forepeak	22	1-01227-02-wa	wa	22.9	0
1	15-Aug-01	2001	227	Hamilton	1011	11	FPK	Forepeak	22	1-01227-02-ws	ws		
1	18-Sep-01	2001	261	Cleveland	1012	12	DBT	DB #4 - starboard	23	1-01261-01-wa	wa	20.7	0
1	18-Sep-01	2001	261	Cleveland	1012	12	DBT	DB #4 - starboard	23	1-01261-01-ws	ws		
1	18-Sep-01	2001	261	Cleveland	1012	12	DBT	DB #1 - starboard	24	1-01261-02-wa	wa	20.7	0
1	18-Sep-01	2001	261	Cleveland	1012	12	DBT	DB #1 - starboard	24	1-01261-02-ws	ws		
1	1-Oct-01	2001	274	Hamilton	1007	13	FPK	Forepeak	25	1-01274-01-wa	wa	17.1	5
1	1-Oct-01	2001	274	Hamilton	1007	13	FPK	Forepeak	25	1-01274-01-ws	ws		
2	1-Oct-01	2001	274	Hamilton	1007	2.13.1	FPK	Forepeak	25	2-01274-01-wa	wa	17.6	
1	5-Oct-01	2001	278	Cleveland	1013	14	DBT	DB #6 -port	26	1-01278-01-ws	ws		
1	5-Oct-01	2001	278	Cleveland	1013	14	DBT	DB #3 - starboard	27	1-01278-02-wa	wa	18.7	7
1	5-Oct-01	2001	278	Cleveland	1013	14	DBT	DB #3 - starboard	27	1-01278-02-ws	ws		

2	7-Oct-01	2001	280	Windsor	1007	2.13.2	FPK	Forepeak	25	2-01280-01-wa	wa		
1	7-Oct-01	2001	280	Windsor	1007	15	DBT	DB #2 - starboard	28	1-01280-01-wa	wa	11.6	23
1	7-Oct-01	2001	280	Windsor	1007	15	DBT	DB #2 - starboard	28	1-01280-01-ws	ws		
1	7-Oct-01	2001	280	Windsor	1007	15	DBT	DB #2 - port	29	1-01280-02-wa	wa	11.9	3
1	7-Oct-01	2001	280	Windsor	1007	15	DBT	DB #2 - port	29	1-01280-02-ws	ws		
2	11-Oct-01	2001	284	Chicago	1007	2.13.3	FPK	Forepeak	25	2-01284-01-wa	wa	15.3	2
1	22-Oct-01	2001	295	Cleveland	1014	16	DBT	DB #3 -port	30	1-01295-01-wa	wa	14	20
1	22-Oct-01	2001	295	Cleveland	1014	16	DBT	DB #3 -port	30	1-01295-01-ws	ws		
1	22-Oct-01	2001	295	Cleveland	1014	16	DBT	DB #3 - starboard	31	1-01295-02-wa	wa	13.5	22
1	22-Oct-01	2001	295	Cleveland	1014	16	DBT	DB #3 - starboard	31	1-01295-02-ws	ws		
1	25-Oct-01	2001	298	E. Chicago	1015	17	UWT	Upper Wing #1 - port	32	1-01298-01-wa	wa	10	7
1	25-Oct-01	2001	298	E. Chicago	1015	17	UWT	Upper Wing #1 - port	32	1-01298-01-ws	ws		
1	25-Oct-01	2001	298	E. Chicago	1015	17	UWT	Upper Wing #1 - port	32	1-01298-01-ds	ds		
1	25-Oct-01	2001	298	E. Chicago	1015	17	UWT	Upper Wing #2 - port	33	1-01298-02-ds	ds		
1	8-Nov-01	2001	312	Burns Harbor	1016	18	DBT	DB #2 - starboard	34	1-01312-01-wa	wa	11.8	22
1	8-Nov-01	2001	312	Burns Harbor	1016	18	DBT	DB #2 - starboard	34	1-01312-01-ws	ws		
1	8-Nov-01	2001	312	Burns Harbor	1016	18	DBT	DB #2 - port	35	1-01312-02-wa	wa	12.2	22
1	8-Nov-01	2001	312	Burns Harbor	1016	18	DBT	DB #2 - port	35	1-01312-02-ws	ws		
1	19-Nov-01	2001	323	Burns Harbor	1017	19	DBT	DB # - starboard	36	1-01323-01-wa	wa	11.7	35
1	19-Nov-01	2001	323	Burns Harbor	1017	19	DBT	DB # - starboard	36	1-01323-01-ws	ws		
1	19-Nov-01	2001	323	Burns Harbor	1017	19	DBT	DB # - port	37	1-01323-02-wa	wa	11.7	35
1	19-Nov-01	2001	323	Burns Harbor	1017	19	DBT	DB # - port	37	1-01323-02-ws	ws		
1	21-Nov-01	2001	325	Hamilton	1018	20	FPK	Forepeak	38	1-01325-01-ws	ws		
1	21-Nov-01	2001	325	Hamilton	1018	20	DBT	DB #3 - starboard	39	1-01325-02-wa	wa	10.3	37
1	21-Nov-01	2001	325	Hamilton	1018	20	DBT	DB #3 - starboard	39	1-01325-02-ws	ws		
1	23-Nov-01	2001	327	Hamilton	1019	21	DBT	DB #8 - port	40	1-01327-01-wa	wa	10.6	7
1	23-Nov-01	2001	327	Hamilton	1019	21	DBT	DB #8 - port	40	1-01327-01-ws	ws		
1	23-Nov-01	2001	327	Hamilton	1019	21	DBT	DB #8 - starboard	41	1-01327-02-wa	wa	9.4	1
1	23-Nov-01	2001	327	Hamilton	1019	21	DBT	DB #8 - starboard	41	1-01327-02-ws	ws		
1	29-Nov-01	2001	333	Hamilton	1020	22	DBT	DB #3 - port	42	1-01333-01-wa	wa	8.4	2
1	29-Nov-01	2001	333	Hamilton	1020	22	DBT	DB #3 - port	42	1-01333-01-ws	ws		
1	29-Nov-01	2001	333	Hamilton	1020	22	FPK	Forepeak	43	1-01333-02-wa	wa	8.3	7
1	29-Nov-01	2001	333	Hamilton	1020	22	FPK	Forepeak	43	1-01333-02-ws	ws		
1	6-Jun-02	2002	157	Toronto	1021	23	FPK	Forepeak	44	1-02157-01-wa	wa	9.7	35
1	6-Jun-02	2002	157	Toronto	1021	23	FPK	Forepeak	44	1-02157-01-ws	ws		
1	6-Jun-02	2002	157	Toronto	1021	23	DBT	DB #6 Starboard	45	1-02157-02-wa	wa	9.4	32
1	6-Jun-02	2002	157	Toronto	1021	23	DBT	DB #6 Starboard	45	1-02157-02-ws	ws		
1	6-Jun-02	2002	157	Toronto	1021	23	DBT	DB #6 Port	46	1-02157-03-wa	wa	9.4	30
1	6-Jun-02	2002	157	Toronto	1021	23	DBT	DB #6 Port	46	1-02157-03-ws	ws		
1	6-Jun-02	2002	157	Toronto	1022	24	FPK	Forepeak	47	1-02157-04-wa	wa	8.6	15
1	6-Jun-02	2002	157	Toronto	1022	24	FPK	Forepeak	47	1-02157-04-ws	ws		
1	13-Jun-02	2002	164	Hamilton	1023	25	FPK	Forepeak	48	1-02164-01-wa	wa	18.5	8
1	13-Jun-02	2002	164	Hamilton	1023	25	FPK	Forepeak	48	1-02164-01-ws	ws		
1	13-Jun-02	2002	164	Hamilton	1023	25	DBT	DB #5 Starboard	49	1-02164-02-wa	wa	18.1	2
1	13-Jun-02	2002	164	Hamilton	1023	25	DBT	DB #5 Starboard	49	1-02164-02-ws	ws		
1	13-Jun-02	2002	164	Hamilton	1023	25	DBT	DB #5 Port	50	1-02164-03-wa	wa	18.2	6
1	13-Jun-02	2002	164	Hamilton	1023	25	DBT	DB #5 Port	50	1-02164-03-ws	ws		

1	24-Jun-02	2002	175	Windsor	1024	26	DBT	DB #2 Starboard	51	1-02175-01-wa	wa	21	16
1	24-Jun-02	2002	175	Windsor	1024	26	DBT	DB #2 Starboard	51	1-02175-01-ws	ws		
1	24-Jun-02	2002	175	Windsor	1024	26	DBT	DB #2 Port	52	1-02175-02-wa	wa	21	26
1	24-Jun-02	2002	175	Windsor	1024	26	DBT	DB #2 Port	52	1-02175-02-ws	ws		
1	19-Jul-02	2002	200	Hamilton	1025	27	DBT	DB #1 Port	53	1-02200-01-wa	wa	21.3	19
1	19-Jul-02	2002	200	Hamilton	1025	27	DBT	DB #1 Port	53	1-02200-01-ws	ws		
1	19-Jul-02	2002	200	Hamilton	1025	27	DBT	DB #1 Starboard	54	1-02200-02-wa	wa	21.1	26
1	19-Jul-02	2002	200	Hamilton	1025	27	DBT	DB #1 Starboard	54	1-02200-02-ws	ws		
1	25-Jul-02	2002	206	Cleveland	1026	28	DBT	DB #5 Port	55	1-02206-01-wa	wa	22.4	18
1	25-Jul-02	2002	206	Cleveland	1026	28	FPK	Forepeak	56	1-02206-02-wa	wa	22	4
1	25-Jul-02	2002	206	Cleveland	1026	28	FPK	Forepeak	56	1-02206-02-ws	ws		
1	6-Aug-02	2002	218	Hamilton	1006	29	DBT	DB #5 Starboard	57	1-02218-01-wa	wa	20.3	37
1	6-Aug-02	2002	218	Hamilton	1006	29	DBT	DB #5 Starboard	57	1-02218-01-ws	ws		
1	6-Aug-02	2002	218	Hamilton	1006	29	DBT	DB #5 Port	58	1-02218-02-wa	wa	19	37
1	6-Aug-02	2002	218	Hamilton	1006	29	DBT	DB #5 Port	58	1-02218-02-ws	ws		
1	6-Aug-02	2002	219	Hamilton	1027	30	DBT	DB #7 Starboard	59	1-02219-01-wa	wa	22.2	5
1	6-Aug-02	2002	219	Hamilton	1027	30	DBT	DB #7 Starboard	59	1-02219-01-ws	ws		
1	6-Aug-02	2002	219	Hamilton	1027	30	DBT	DB #7 Port	60	1-02219-02-wa	wa	21.9	8
1	6-Aug-02	2002	219	Hamilton	1027	30	DBT	DB #7 Port	60	1-02219-02-ws	ws		
1	6-Aug-02	2002	219	Hamilton	1027	30	FPK	Forepeak	61	1-02219-03-wa	wa	23.3	2
1	6-Aug-02	2002	219	Hamilton	1027	30	FPK	Forepeak	61	1-02219-03-ws	ws		
1	6-Aug-02	2002	221	Detroit	1028	31	FPK	Forepeak	62	1-02221-01-ws	ws		
1	13-Aug-02	2002	225	Windsor	1029	32	DBT	DB #6 Port	63	1-02225-01-wa	wa	24.5	8
1	13-Aug-02	2002	225	Windsor	1029	32	DBT	DB #6 Port	63	1-02225-01-ws	ws		
1	13-Aug-02	2002	225	Windsor	1029	32	DBT	DB #5 Starboard	64	1-02225-02-wa	wa	24.5	3
1	13-Aug-02	2002	225	Windsor	1029	32	DBT	DB #5 Starboard	64	1-02225-02-ws	ws		
1	15-Aug-02	2002	227	Hamilton	1030	33	DBT	DB #2 Starboard	65	1-02227-01-wa	wa	20.7	22
1	15-Aug-02	2002	227	Hamilton	1030	33	DBT	DB #2 Starboard	65	1-02227-01-ws	ws		
1	15-Aug-02	2002	227	Hamilton	1030	33	DBT	DB #2 Port	66	1-02227-02-wa	wa	N/A	31
1	15-Aug-02	2002	227	Hamilton	1030	33	DBT	DB #2 Port	66	1-02227-02-ws	ws		
1	13-Sep-02	2002	256	Hamilton	1031	34	DBT	DB #4 Starboard	67	1-02256-01-wa	wa	22.6	57
1	14-Sep-02	2002	256	Hamilton	1031	34	DBT	DB #4 Starboard	67	1-02256-01-ws	ws		
1	15-Sep-02	2002	256	Hamilton	1031	34	DBT	DB #4 Port	68	1-02256-02-wa	wa	22.6	75
1	5-Oct-02	2002	278	Windsor	1027	35	FPK	Forepeak	69	1-02278-01-wa	wa	20.2	36
1	5-Oct-02	2002	278	Windsor	1027	35	FPK	Forepeak	69	1-02278-01-ws	ws		
2	5-Oct-02	2002	278	Windsor	1027	2.35.1	FPK	Forepeak	69	2-02278-01T-wa	wa	19.19	0.93
2	5-Oct-02	2002	278	Windsor	1027	2.35.1	FPK	Forepeak	69	2-02278-01B-wa	wa	19.36	3.37
2	7-Oct-02	2002	280	Detroit	1027	2.35.2	FPK	Forepeak	69	2-02280-01T-wa	wa	18.27	2.06
2	7-Oct-02	2002	280	Detroit	1027	2.35.2	FPK	Forepeak	69	2-02280-01B-wa	wa	18.21	2.08
2	10-Oct-02	2002	283	Burns Harbor	1027	2.35.3	FPK	Forepeak	69	2-02283-01T-wa	wa	16.24	0.74
2	10-Oct-02	2002	283	Burns Harbor	1027	2.35.3	FPK	Forepeak	69	2-02283-01B-wa	wa	16.18	0.74
2	11-Oct-02	2002	284	Milwaukee	1027	2.35.4	FPK	Forepeak	69	2-02284-01T-wa	wa	17.3	0.6
2	11-Oct-02	2002	284	Milwaukee	1027	2.35.4	FPK	Forepeak	69	2-02284-01B-wa	wa	15.9	0.56
1	11-Oct-02	2002	284	Windsor	1032	36	DBT	DB #3 Port	70	1-02284-01-wa	wa	N/A	32
1	11-Oct-02	2002	284	Windsor	1032	36	DBT	DB #3 Port	70	1-02284-01-ws	ws		
1	11-Oct-02	2002	284	Windsor	1032	36	DBT	DB #3 Starboard	71	1-02284-02-wa	wa	N/A	20
1	11-Oct-02	2002	284	Windsor	1032	36	DBT	DB #3 Starboard	71	1-02284-02-ws	ws		

1	20-Oct-02	2002	293	Windsor	1033	37	FPK	Forepeak	72	1-02293-01-wa	wa	11.5	2
1	20-Oct-02	2002	293	Windsor	1033	37	FPK	Forepeak	72	1-02293-01-ws	ws		
1	20-Oct-02	2002	293	Windsor	1033	37	ST	Side Tank #6 Starboard	73	1-02293-02-wa	wa	10.9	1
1	20-Oct-02	2002	293	Windsor	1033	37	ST	Side Tank #6 Starboard	73	1-02293-02-ds	ds		
1	23-Oct-02	2002	297	Burns Harbor	1007	38	DBT	DB #6 Starboard	74	1-02297-01-wa	wa	13.7	1.9
1	23-Oct-02	2002	297	Burns Harbor	1007	38	DBT	DB #6 Starboard	74	1-02297-01-ws	ws		
1	23-Oct-02	2002	297	Burns Harbor	1007	38	DBT	DB #6 Port	75	1-02297-02-wa	wa	13.6	1.1
1	23-Oct-02	2002	297	Burns Harbor	1007	38	DBT	DB #6 Port	75	1-02297-02-ws	ws		
1	12-Nov-02	2002	316	Cleveland	1014	39	DBT	DB#4 Starboard	76	1-02316-01-wa	wa	12.3	26
1	12-Nov-02	2002	316	Cleveland	1014	39	DBT	DB#4 Starboard	76	1-02316-01-ws	ws		
1	12-Nov-02	2002	316	Cleveland	1014	39	DBT	DB#6 Starboard	77	1-02316-02-wa	wa	12.3	2
1	12-Nov-02	2002	316	Cleveland	1014	39	DBT	DB#6 Starboard	77	1-02316-02-ws	ws		
1	19-Nov-02	2002	323	Cleveland	1013	40	DBT	DB #4 Starboard	78	1-02323-01-wa	wa	9.8	21
1	19-Nov-02	2002	323	Cleveland	1013	40	DBT	DB #4 Starboard	78	1-02323-01-ws	ws		
1	19-Nov-02	2002	323	Cleveland	1013	40	DBT	DB #4 Port	79	1-02323-02-wa	wa	9.2	20.6
1	19-Nov-02	2002	323	Cleveland	1013	40	DBT	DB #4 Port	79	1-02323-02-ws	ws		
1	26-Nov-02	2002	330	Hamilton	1034	41	DBT	DB #3 Starboard	80	1-02330-01-wa	wa	7.4	28
1	26-Nov-02	2002	330	Hamilton	1034	41	DBT	DB #3 Starboard	80	1-02330-01-ws	ws		
1	26-Nov-02	2002	330	Hamilton	1034	41	DBT	DB #3 Port	81	1-02330-02-wa	wa	7.6	12
1	6-Dec-02	2002	340	Windsor	1035	42	FPK	Forepeak	82	1-02340-01-wa	wa	-0.7	34
1	6-Dec-02	2002	340	Windsor	1035	42	FPK	Forepeak	82	1-02340-01-ws	ws		
2**	7-May-03	2003	127	Windsor	1036	43	DBT	DB #5 Port		2**-03127	ws		
2**	7-May-03	2003	127	Windsor	1036	43	DBT	DB #5 Starboard		2**-03127	ws		
2**	7-May-03	2003	127	Windsor	1036	43	DBT	DB #1 Port		2**-03127	ws		
2**	7-May-03	2003	127	Windsor	1036	43	DBT	DB #1 Starboard		2**-03127	ws		
2**	2-Jul-03	2003	183	Hamilton	1037	44	FPK	Forepeak		2**-03183	ws		
2	2-Jul-03	2003	183	Hamilton	1037	2.44.1	UWT	Hamilton Harbor		2-03183-Harbor	wa	19.6	
2	2-Jul-03	2003	183	Hamilton	1037	2.44.1	UWT	UWT #5 Port	83	2-03183-T(o)	wa	19.6	
2	6-Jul-03	2003	187	Windsor	1037	2.44.2	UWT	UWT #5 Port	83	2-03187-T(1)	wa	23.2	0.4
2	10-Jul-03	2003	191	Burns Harbor	1037	2.44.3	UWT	UWT #5 Port	83	2-03191-T(2)	wa	22.6	0.4
2	12-Jul-03	2003	193	Thunder Bay	1037	2.44.4	UWT	UWT #5 Port	83	2-03193-T(3)	wa	18.49	0.4
2	14-Jul-03	2003	195	Hamilton	1038	2.45.1	UWT	Hamilton Harbor	84	2-03195-Harbor	wa	20.8	0.4
2	14-Jul-03	2003	195	Hamilton	1038	2.45.1	UWT	UWT #3 Starboard	84	2-03195-T(o)	wa	18.8	0.4
2	17-Jul-03	2003	198	Detroit	1038	2.45.2	UWT	UWT #3 Starboard	84	2-03198-T(1)	wa	24.6	0.4
2	24-Jul-03	2003	205	Milwaukee	1038	2.45.3	UWT	UWT #3 Starboard	84	2-03205-T(2)	wa	22.6	0.4
1	15-Sep-03	2003	258	Cleveland	1033	46	ST	Side Tank #5 Starboard	85	1-03258-01-ws	ws		
2	15-Sep-03	2003	258	Cleveland	1033	2.46.1	ST	Cleveland Harbor	85	2-03258-Harbor	wa	22.7	0.27
2	15-Sep-03	2003	258	Cleveland	1033	2.46.1	ST	Side Tank #5 Starboard	85	2-03258-T(o)	wa	23.4	0.27
2	19-Sep-03	2003	262	Windsor	1033	2.46.2	ST	Side Tank #5 Starboard	85	2-03262-T(1)	wa	21.9	0.27
2	22-Sep-03	2003	265	Burns Harbor	1033	2.46.3	ST	Side Tank #5 Starboard	85	2-03265-T(2)	wa	19.2	0.34
2	26-Sep-03	2003	269	Duluth	1033	2.46.4	ST	Side Tank #5 Starboard	85	2-03269-T(3)	wa	12.6	0.37
2	26-Sep-03	2003	269	Duluth	1033	2.46.4	ST	Duluth Harbor	85	2-03269-Harbor	wa	10.7	0.24

Appendix 4.

Live Invertebrates recorded in residual sediment and water from NOBOB ships entering the Great Lakes during the project period December 2001 – December 2003.

	Sediment	Water
ROTIFERA		
Brachionus angularis		X
Keratella crassa		X
Kelicottia longispina		X
Keratella cochlearis		X
Lecane closterocerca	X	
Lecane hamata	X	
Polyarthra dolichoptera		X
Proales decipiens		X
Synchaeta oblonga		X
Bdelloids	X	X
COPEPODA		
Harpacticoida		
Ameira parvula	X	X
Bryocamptus pygmaeus	X	
Bryocamptus zschokkei	X	
Canthocamptus staphylinoides	X	
Canthocamptus staphylinus	X	
Ectinosoma californicum	X	
Halectinosoma curticorne	X	
Harpacticus uniremis	X	
Longipedia minor	X	X
Mesochra pygmaea	X	
Microarthridian littorale	X	
Microsetella norvegica	X	X
Nitocra affinis affinis	X	
Nitocra hibernica	X	
Nitocra lacustris	X	X
Nitocra spinipes	X	X
Nitocra sp.	X	
Onychocamptus mohammed	X	
Schizopera baltica	X	
Schizopera borutzkyi	X	X
Schizopera knabeni	X	X
Tachidius littoralis		X
Tisbe furcata		X
Tisbe gracilis		X
Indet. harpacticoida	X	X
Cyclopoida		
Acanthocyclops robustus	X	X
Acanthocyclops venustus		X
Acanthocyclops vernalis		X

Acanthocyclops sp.	X	
Cyclopina litoralis		X
Cyclops abyssorum		X
Cyclops strenuus	X	X
Diacyclops nanus		X
Diacyclops thomasi	X	X
Eucyclops serrulatus		X
Halicyclops sp.	X	X
Mesocyclops americanus	X	
Mesocyclops edax	X	X
Mesocyclops leukarti	X	
Mesocyclops sp.	X	
Paracyclops chiltoni	X	X
Paracyclops fimbriatus	X	
Thermocyclops crassus	X	
Thermocyclops oithonoides	X	
indet. cyclopoids	X	X
Calanoida		
Calanus sp.		X
Epischura lacustris		X
Eurytemora affinis		X
Leptodiaptomus minutus		X
Senecella calanoides		X
Temora longicornis	X	
Poecilostomatoida		
Oncaea media	X	
Oncaea borealis	X	
Saphirella sp.		X
CLADOCERA		
Alona quadrangularis	X	X
Bosmina liederi		X
Bosmina maritima		X
Bosmina sp.		X
Chydoris sphaericus		X
Daphnia atkinsoni		X
Daphnia cristata		X
Daphnia magna		X
Daphnia retrocurva		X
Ilyocryptus sordidus	X	
AMPHIPODA		
Gammarus chevreuxi		X
Gammarus salinus		X

Gammarus zaddachi		X
Indet. *Gammarus* spp.	X	X
OTHER ARTHROPODA		
Decapoda juveniles	X	
Crangon crangon		X
Cirriperdia juveniles	X	X
Mysidacea		
Neomysis integer		X
Ostracoda	X	X
Chironomids	X	X
Other Insecta	X	
Acarina	X	X
ANNELIDA		
Oligochaeta		
Amphichaeta americana	X	
Pristina sp.	X	X
Vejdovskyella intermedia	X	
Indet. Tuberficidae	X	
Aphanoneura		
Aelosoma sp.	X	
Polychaeta	X	X
TARDIGRADA	X	X
MOLLUSCA		
Gastropoda	X	X
Fissurellidae		X
Bivalvia (incl. *Driessena*)	X	X
HYDROZOA	X	X
BRYOZOA	X	
ECHINODERMATA - Class Crinoidea		X
NEMATODES		
Acrobeles sp.	X	
?*Anonchus* sp.	X	
Anoplostoma sp.	X	
Aphanolaimus sp.	X	

Ascolaimus sp.	X
Axonolaimus sp.	X
Bathylaimus sp.	X
Campylaimus sp.	X
Choriorhabditis sp.	X
Chromadoridae	X
Cobbia sp.	X
?*Criconema* sp.	X
Cyatholaimidae	X
Daptonema sp.	X
Dichromadora sp.	X
Diplogasteridae	X
Diplogasterioides sp.	X
Diplolaimella sp.	X
Diploscapter sp.	X
Dorylaiminae	X
Dorylaimus sp.	X
Ironus sp.	X
Leptolaimidae	X
Leptolaimoides sp.	X
Leptolaimus sp.	X
Mesorhabditis sp.	X
Microlaimus sp.	X
Molgolaimus sp.	X
Monhystera sp.	X
Monhysteridae	X
Mononchoides sp.	X
Oncholaimus oxyuris	X
cf. *Paracyatholaimus* sp.	X
Paraphanolaimus sp.	X
Plectidae	X
Plectus sp.	X
Rhabditis sp.	X
Sabatieria sp.	X
Sphaerolaimus sp.	X
Teratocephalus sp.	X
Thalassomonhystera cf. *parva*	X
Thalassomonhystera sp.	X
Theristus flevensis	X
Tobrilus sp.	X
Tripyla sp.	X
Tripyloides sp.	X
cf. *Viscosia* sp.	X
?Xiphinema sp.	X

Appendix 5.

List of invertebrate taxa hatched from resting stages during this study, arranged taxonomically. Occurrence lists number of ships that the species was collected on, from a possible 35. Abundance lists the range (median) number of individuals emerging from 40 g sediment for all ships. Experiment type lists presence of species in maximum diversity (isolated from sediment) and whole sediment (buried in sediment) trials. All species able to hatch in 0‰ medium unless otherwise indicated: †denotes exclusively in 8‰; ‡denotes exclusively in 32‰.

Taxon	Occurrence	Abundance	Experiment type	
			Maximum Diversity	Whole Sediment
Gastrotricha				
Chaetonotidae, unidentified	1	3	X	
Rotifera				
Ascomorpha ecaudis	1	0.25	X	
Ascomorpha saltans	1	0.25	X	
Ascomorpha sp.	1	1	X	
Asplanchna brightwelli	4	0.25-1 (0.75)	X	X
Asplanchna girodi	2	0.5 (0.5)	X	X
Asplanchna priodonta	3	1-11.5 (3)	X	X
Brachionus angularis	21	0.25-21.8 (4)	X	X
Brachionus bennini	1	0.25	X	
Brachionus budapestinensis	15	0.75-341.5 (3)	X	X
Brachionus calyciflorus	25	0.63-77.77 (3)	X	X
Brachionus caudatus	2	0.5-2 (1.25)	X	
Brachionus diversicornis	1	0.25	X	
Brachionus forficula	1	1	X	
Brachionus havanaënsis	2	1 (1)	X	
Brachionus leydigi	4	0.25-1 (0.78)	X	X
Brachionus nilsoni	1	1	X	
Brachionus quadridentatus	4	0.5-12.25 (1.25)	X	X
Brachionus urceolaris	9	0.25-78 (1)	X	X
Cephalodella catellina	2	0.25 (0.25)	X	
Cephalodella forficula	1	0.25	X	
Cephalodella ?*stenroosi*	1	0.3		X
Cephalodella sterea	1	4.75	X	
Cephalodella cf. *theodora*	1	0.25	X	
Cephalodella sp.	1	1	X	
Conochilus coenobasis	1	0.5	X	
Conochilus dossuarius	1	1	X	
Conochilus hippocrepis	2	1 (1)	X	
Conochilus cf. *natans*	1	0.25	X	
Conochilus unicornis	1	0.8		X
Dicranophoridae, unidentified	1	83	X	
Euchlanis cf. *dilatata*	2	0.25-1 (0.63)	X	
Filinia brachiata	1	0.25	X	
Filinia cornuta	3	0.5-1 (0.5)	X	
Filinia longiseta	6	0.25-4 (1)	X	
Filinia passa	4	0.25-1 (0.75)	X	
Filinia terminalis	5	0.38-2.5 (1)	X	
Floscularidae, unidentified	1	0.25	X	
Hexarthra intermedia	1	0.25	X	
Hexarthra mira	3	0.25-1 (1)	X	

Keratella cochlearis	3	0.25-1 (1)	X	
Keratella quadrata	5	0.25-4 (0.5)	X	X
Keratella tropica	1	2	X	
Keratella sp.	1	1	X	
Lacinularia sp.	1	0.25	X	
Lecane closterocerca	2	0.3-0.5 (0.4)	X	X
Lecane flexilis	1	0.25	X	
Lindia truncata	1	0.5	X	
Ploesoma truncatum	3	0.25-2 (2)	X	
Polyarthra dolichoptera	9	0.5-5 (1)	X	
Polyarthra vulgaris	6	0.25-21 (2)	X	
Polyarthra spp.	2	0.25-1 (0.63)	X	
Pompholyx sulcata	4	0.25-7 (3.5)	X	
Synchaeta bacillifera	1	2.25	X†	
Synchaeta baltica	1	2.75		X‡
Synchaeta kitina	1	0.25	X	
Synchaeta oblonga	1	0.25	X	
Synchaeta stylata	4	0.25-1 (0.28)	X	X
Synchaeta tremula	2	1-3.5 (1)	X	X
Synchaeta sp.	1	0.25		X†
Trichocerca multicrinis	1	39	X	
Trichocerca pusilla	7	1-17.63 (1.25)	X	
Trichocerca rattus	1	1	X	
Trichocerca similis	1	0.25	X	
Monogonont, unidentified	2	1 (1)	X	
Bryozoa				
Plumatella casmiana	2	0.25-1 (0.63)	X	
Plumatella sp.	1	0.25	X	
Anomopoda				
Alona rectangula	1	0.5	X	
Alona rustica	1	0.25	X	
Bosmina liederi	3	1-6 (1)	X	
Bosmina maritima	1	2	X	
Bosmina spp.	2	1 (1)	X	
Ceriodaphnia quadrangula	1	0.25	X	
Ceriodaphnia sp.	2	1 (1)	X	
Daphnia longiremis	2	1 (1)	X	
Daphnia magna	4	0.5-2 (1)	X	X
Daphnia retrocurva	1	2	X	
Disparalona leei	1	0.25	X	
Moina micrura	2	1-47.88 (24.44)	X	
Moina sp.	1	1	X	
Ctenopoda				
Diaphanosoma birgei	2	0.75-6 (3.38)	X	
Diaphanosoma brachyurum	1	0.25	X	
Diaphanosoma mongolianum	1	0.5	X	

Diaphanosoma orghidani	1	1.25	X	
Diaphanosoma sarsi	1	0.25	X	
Diaphanosoma spp.	6	1 (1)	X	
Onychopoda				
Evadne nordmanni	1	0.5	X[†]	
Copepoda				
Acanthocyclops robustus	1	0.8		X
Cyclopoida, unidentified	3	0.25-1.25 (0.25)		X
Nitocra lacustris	1	1		X
Copepod nauplii, unidentified	14	0.25-20 (3)	X	X

Appendix 6.

Products and Presentations

Scientific Presentations

Baier, R.E., R.L. Forsberg, A.E. Meyer, J.J. Zambon, B. Galil, N. Hulsmann, L.A. Drake, F.C. Dobbs, M. Doblin. 2003. Ballast water biofilms as "benchmarks" for bioinvasion risk management. American Chemical Society National Meeting, New York

Bailey, S.A., I. C. Duggan, C.D.A. van Overdijk and H.J. MacIsaac. 2003. Transoceanic Invasion Time Bombs? Viability of Resting Eggs Collected from Residual Sediments in NOBOB Vessels. Canadian Conference for Fisheries Research, Ottawa, ON (January).

Bailey, S.A., I. C. Duggan, C.D.A. van Overdijk and H.J. MacIsaac. 2003. Sleeping Stow-aways! Do resting eggs on transoceanic vessels pose an invasion risk? American Society of Limnology and Oceanography, Salt Lake City, Utah (February).

Bailey, S.A., I.C. Duggan, C.D.A. van Overdijk. and H.J. MacIsaac. 2003. Do resting eggs in ballast tanks pose an invasion risk? 12th International Conference on Aquatic Invasive Species, Windsor, Ontario (June).

Bailey, S.A., H.J. MacIsaac, C.D.A. van Overdijk, I.C. Duggan, K. Nandakumar, D.F. Reid, and T.H. Johengen. 2004. In situ assessment of NOBOB ships as a vector for invasive species in the Great Lakes. Baltic Sea - Great Lakes Workshop on Aquatic Invasive Species, Ann Arbor, Michigan (April).

Bailey, S.A. and H.J. MacIsaac. 2004. Sediments and ships: natural biota as biological contaminants. 6th International Symposium on Sediment Quality Assessment, Antwerp, Belgium (August).

Bailey, S.A., K. Nandakumar, I.C. Duggan, C.D.A. van Overdijk, H.J. MacIsaac, T.H. Johengen, and D.F. Reid. 2004. Modelling the risk of invasion by diapausing eggs in residual ballast sediments. 13th International Conference on Aquatic Invasive Species, Ennis, County Clare, Ireland (September).

Bandoni, S.A. and H.J. MacIsaac. 2002. Assesment of invasion risk posed by invertebrate resting eggs in residual ballast sediments in NOBOB vessels entering the Great Lakes. 11th International Conference on Aquatic Invasive Species. Alexandria, Virginia (February).

Bandoni, S.A. and H.J. MacIsaac. 2002. The Great Lakes NOBOB project. United Nations University, Managing Shared Waters International Conference, Hamilton, ON (June). Invited.

Dobbs, F.C. 2001. Invasion potential of microorganisms in ships' ballast waters. Second International Conference on Marine Bioinvasions, New Orleans, LA (April).

Dobbs, F.C., A.A. Diallo, M.A. Doblin, L.A. Drake, T.K. Graczyk, X. Jiang, T.H. Johengen, L.G. Kampschmidt, P.A. Rublee, D.F. Reid, and G.M. Ruiz. 2003. Pathogens in ships'

ballast water and sediment residuals. Third International Conference on Marine Bioinvasions, San Diego (March).

Dobbs, F.C., M.A. Doblin, L.G. Kampschmidt, A.A. Diallo, T.K. Graczyk, X. Jiang, and P.A. Rublee. 2003. Microorganisms in NOBOB Ships Arriving in the Great Lakes. Joint International Association for Great Lakes Research/ International Lake Environment Committee conference, Chicago, Illinois (June).

Dobbs, F.C. and A. Rogerson. 2004. Can we ever remove, kill, or inactivate all the microorganisms from ships' ballast water, and should we try? 2nd International Conference and Exhibition on Ballast Water Management, Singapore.

Doblin, M.A., D.F. Reid, F.C. Dobbs, D. Donahue, G.L. Fahnensteil, P.T. Jenkins, T.H. Johengen, H.J. MacIsaac, and G. M. Ruiz. 2001. Assessment of transoceanic NOBOB vessels and low-salinity ballast water as vectors for nonindigenous species introductions to the Great Lakes. Second International Conference on Marine Bioinvasions, New Orleans, LA (April).

Doblin, M.A., L.A. Drake, K.J. Coyne, P.A. Rublee, and F.C. Dobbs. 2002. *Pfiesteria* species identified in ballast water and residuals: A possible vector for introductions to coastal areas Xth International Conference On Harmful Algae, St. Pete Beach, Florida. http://www.xhab2002.com/PDF/D65_81.pdf (see page 74)

Doblin, M.A., L.C. Popels, S.C. Cary, K.J. Coyne, D.A. Hutchins, and F.C. Dobbs. 2003. Long-distance and local-scale transport of *Aureococcus* by ships and small boats in fresh and marine waters. Second Symposium on Harmful Marine Algae in the U.S., Woods Hole, MA. Subsequently published in Harmful Algae 3:200-201 (2004).

Doblin, M.A., F.C. Dobbs, L.A. Drake, M. Bednarski, K. Coyne, S. Heinemann, G. DiTullio, L.G. Kampschmidt, T. Mullady, K. Murphy, G.M. Ruiz. 2003. Effects of open-ocean exchange on microbial communities in ships' ballast tanks. Third International Conference on Marine Bioinvasions, San Diego (March).

Doblin, M.A., L.G. Kampschmidt, S. Heinemann, P.T. Jenkins, and F.C. Dobbs. 2003. Microbial dynamics in ships' ballast tanks during vessel transit in the Great Lakes. Joint International Association for Great Lakes Research/ International Lake Environment Committee conference, Chicago, Illinois (June).

Doblin, M.A., K.J. Coyne, L.C. Popels, D.A. Hutchins, S.C. Cary, P.A. Rublee, and F.C. Dobbs. 2004. Global transport and dispersal of HABs in ships' ballast water and recreational vessels. 11th International Conference on Harmful Algae, Capetown, South *Africa.*

Duggan, I.C., S.A. Bailey, C.D.A.van Overdijk, D.F. Reid, T.H. Johengen, and H.J. MacIsaac. 2003. Just Add Water: Evidence of in situ emergence of invertebrates from Resting Stages in NOBOB Ballast Tanks. Joint International Association for Great Lakes

Research/ International Lake Environment Committee conference, Chicago, Illinois (June).

Duggan, I.C., C.D.A. van Overdijk, S.A. Bailey, and H.J. MacIsaac. 2003. Nasties in NOBOBs?: Potential for introduction of invertebrates to the Great Lakes from residual ballast water. American Society of Limnology and Oceanography, Salt Lake City, Utah (February).

Fahnenstiel, G.L., Y. Hong, D.F. Reid, T.H. Johengen, H.J. MacIsaac, F.C. Dobbs, and G.M. Ruiz. 2002. Non-indigenous phytoplankton in the Great Lakes: NOBOB ships as potential vectors. Eos. Trans. AGU 83(4), Ocean Sciences Meet. Suppl, Abstract OS42H-177 (February).

Forsberg, R., R.E. Baier, A. Meyer, F.C. Dobbs, M.A. Doblin, L. Drake, and P.T. Jenkins. 2002. Internal hull fouling: Effects of surface properties and resuspension of NOBOB sediments. 11th International Congress on Marine Corrosion and Biofouling, San Diego.

Forsberg, R., D. Friedmann, L. Drake, B. Galil, N. Hηlsmann, A. Meyer, F.C. Dobbs, and R.E. Baier. 2002. Material surface-control of ballast biofilm complexes introduced to Great Lakes. International Association for Great Lakes Research, 45th Conference on Great Lakes Research, Winnipeg.
http://www.iaglr.org/conference/2002/abstracts/pub_abstract_view.php?abstract_id=1008856166

Gray, D.K., I.C. Duggan, S.A. Bailey, and H.J. MacIsaac. 2003. Can Partial Ballast Exchange Reduce the Viability of Resting Stages in the Ballast Tanks of NOBOB Ships? Joint International Association for Great Lakes Research/ International Lake Environment Committee conference, Chicago, Illinois (June).

Gray, D.K., S.A. Bailey, I.C. Duggan, and H.J. MacIsaac. 2004. Viability of invertebrate diapausing eggs exposed to saltwater: implications for Great Lakes' ship ballast management. 13th International Conference on Aquatic Invasive Species, Ennis, County Clare, Ireland (September).

Heinemann, S., F.K. Thomson, III, W.L. Hynes, F.C. Dobbs. 2003. Plasmid-borne antibiotic resistance in Vibrio cholerae isolated from ships' ballast water. Third International Conference on Marine Bioinvasions, San Diego (March).

Hong, Y., G.L. Fahnenstiel, and R. Stone. 2003. Dinoflagellate Cysts in NOBOB Ballast Tank Sediment: Potential Risk of Invasion to the Great Lakes. Joint International Association for Great Lakes Research/ International Lake Environment Committee conference, Chicago, Illinois (June).

Jenkins, P.T., S. Bailey, C.D.A. van Overdijk, T.H. Johengen, S.A. Constant, D.F. Reid, and D. Donahue. 2003. The Role of Ship Management in Mitigating or Preventing ANS Invasions. Joint International Association for Great Lakes Research/ International Lake Environment Committee conference, Chicago, Illinois (June).

Johengen, T.H., D.F. Reid, H.J. MacIsaac, F.C. Dobbs, M.A. Doblin, G.L. Fahnensteil, G.M. Ruiz, and P.T. Jenkins. 2001. Assessment of Transoceanic NOBOB Vessels and Low-salinity Ballast Water as Vectors for Nonindigenous Species Introductions to the Great Lakes. IAGLR Conference. June 10-14, 2001. Green Bay, WI.

Johengen, T.H., D.F. Reid, P.T. Jenkins, F.C. Dobbs, M.A. Doblin, H.J. MacIsaac, C.D.A. van Overdijk, S.A. Bandoni, G.L. Fahnenstiel, and H.Ying. 2003. The Great Lakes NOBOB Project: 38 ships and 82 tanks later. 12th International Conference on Aquatic Invasive Species, Windsor, Ontario.

Johengen, T.H., D.F. Reid, P.T. Jenkins, S.A. Bailey, C.D.A. van Overdijk, and S.A. Constant. 2003. Assessment of NOBOB ballast tank residual as a potential vector of ANS: A summary of field activities and chemical conditions within the tanks. Joint International Association for Great Lakes Research/ International Lake Environment Committee conference, Chicago, Illinois (June).

McGann, M., T.H. Johengen, D.F. Reid, G.M. Ruiz, and A.H. Hines. 2002. Transoceanic Ballasted and No-Ballast-On-Board Vessels as Vectors of Nonindigenous Foraminiferal Introductions. GSA Abstracts With Programs Vol. 34, No. 6, September 2002. (Presented at The Geological Society of America, 2002 Annual Meeting, October 27-30, Denver, Colorado.)

McGann, M., T.H. Johengen, and D.F. Reid. 2003. No-Ballast-On-Board Vessels: Potential Vector For Alien Invasive Foraminiferal Introductions In The Great Lakes. Joint International Association for Great Lakes Research/ International Lake Environment Committee conference, Chicago, Illinois (June).

Meyer, A.E, R.L. Forsberg, L.A. Drake, R.E. Baier, L.K. Scott, F.C. Dobbs, M.A. Doblin, M. Koch, W.P. Johnson. 2003. Ballast tank biofilms as "seed banks": Physicochemical and microbiological characterization. 12th International Conference on Aquatic Invasive Species, Windsor, Ontario.

Reid, D.F., S.A. Bailey, T.H. Johengen, S.A. Constant, P.T. Jenkins, C.D.A. van Overdijk, I.C. Duggan, and H.J. MacIsaac. 2003. Use Of Incubator-Emergence Traps For Hatching Studies In Ballast Tanks. Third International Conference on Marine Bioinvasions, San Diego (March).

Reid, D.F., S.A. Bailey, T.H. Johengen, S.A. Constant, P.T. Jenkins, C.D.A. van Overdijk, I.C. Duggan, and H.J. MacIsaac. 2003. NOBOB Project Task 2 – Ballast Tank Mesocosm Experiments and Use of Incubator-Emergence Traps. Joint International Association for Great Lakes Research/ International Lake Environment Committee conference, Chicago, Illinois (June).

Reid, D.F., T.H. Johengen, S.A. Constant, S.A. Bailey and H.J. MacIsaac. 2004. Design and testing of incubator-emergence traps (IETraps) for use in hatching studies in ballast tanks. Modelling the risk of invasion by diapausing eggs in residual ballast sediments.

13th International Conference on Aquatic Invasive Species, Ennis, County Clare, Ireland (September).

Ruiz, G.M., K.R. Murphy, G. Smith, T. Mullady, S. Chaves-Beam, M.A. Doblin, and F.C. Dobbs. 2003. Toward Understanding Transfer Dynamics of Organisms in Ships' Ballast Water: Measuring Survivorship and Efficacy of Ballast Water Exchange. Joint International Association for Great Lakes Research/ International Lake Environment Committee conference, Chicago, Illinois (June).

Therriault, T., H.J. MacIsaac and H. Limen. 2002. Overview and taxonomic assessment of live invertebrates in residual ballast sediment of NOBOB vessels entering the Great Lakes. 11thInternational Conference on Aquatic Invasive Species. Alexandria, Virginia (February).

Thomson, F.K., III, K.H. Choi, and F.C. Dobbs. 2001. Can bacterial data be used to verify ballast-water exchange? Second International Conference on Marine Bioinvasions, New Orleans. http://www.sgnis.org/publicat/thomsonf.htm

Thomson, F.K., III, S. Heinemann, and F.C. Dobbs. Patterns of antibiotic resistance in cholera bacteria isolated from ships' ballast water. 2003. Third International Conference on Marine Bioinvasions, San Diego.
http://www.sgmeet.com/mb/viewabstract2.asp?AbstractID=80&SessionID=23

van Overdijk, C.D.A, S.A. Bandoni, and H.J. MacIsaac. 2002. Identification of live invertebrates in residual ballast water of NOBOB vessels entering the Great Lakes. 11th International Conference on Aquatic Invasive Species. Alexandria, Virginia (February).

van Overdijk, C.D.A., S.A. Bailey, I.C. Duggan, and H.J. MacIsaac. 2003. Assessment of live invertebrates and resting stages from NOBOB vessels entering the Great Lakes: Potential vectors for new invasions. Third International Conference on Marine Bioinvasions, La Jolla, California (March).

van Overdijk, C.D.A, S.A. Bailey, I.C. Duggan, and H.J. MacIsaac. 2003. Transoceanic NOBOB vessels entering the Great Lakes: Vectors for new invasions. Joint International Association for Great Lakes Research/ International Lake Environment Committee conference, Chicago, Illinois (June).

van Overdijk, C.D.A., S.A. Bailey, I.C. Duggan, and H.J. MacIsaac. 2004. Transfer of nonindigenous species to the Laurentian Great Lakes in residual ballast water from no-ballast-on-board (NOBOB) vessels. 13th International Conference on Aquatic Invasive Species, Ennis, County Clare, Ireland (September).

Stakeholder Presentations

Dobbs, F.C. presented a talk on the NOBOB program at Old Dominion University's annual Environmental Forum. March 12, 2002.

Dobbs, F.C. presented a talk on the NOBOB program at the Department of Biology, University of North Carolina at Greensboro. April 3, 2002.

Dobbs, F.C. presented a talk on the NOBOB program at the Department of Biological Science, Louisiana State University. April 8, 2002.

Doblin, M.A. presented a seminar at the University of Technology, Sydney, Australia, providing an overview of the NOBOB program and ODU's microbial results. February 27, 2002.

Jenkins, P.T. Project overview presentation. International Joint Commission - Water Quality Group. November 2, 2000, Quebec City

Jenkins, P.T. Voyage of a NOBOB Vessel. Great Lakes Commission, ANS Symposium. Ann Arbor, 16-18 May 2001.

Jenkins, P.T. Voyage of a NOBOB Vessel. Great Lakes Protection Fund Board, Chicago, June 15, 2001.

Johengen, T.H. Presented a briefing and update on the NOBOB program to representatives from shipping Federation Canada and U.S. Great Lakes Shipping Association at their annual meeting held in conjunction with the U.S. Coast Guard's Marine Community Day. January 30, 2002.

Johengen, T.H. Presented a talk on the NOBOB program at the Michigan State University Water Resources Institute's Annual Symposium on Current Trends and Issues in the Great Lakes. March 7, 2002.

Johengen, T.H. Gave invited lecture on the NOBOB project to a Great Lakes policy class at Michigan State University, East Lansing, MI. October 16, 2002.

MacIsaac, H.J. and D.F. Reid. Progress Report and Briefing for Great Lakes Protection Fund - Assessment of Transoceanic NOBOB Vessels and Low-Salinity Ballast Water as Vectors for Nonindigenous Species Introductions to the Great Lakes. January 22, 2002.

Reid, D.F. Project Briefing - Assessment of Transoceanic NOBOB Vessels and Low-Salinity Ballast Water as Vectors for Nonindigenous Species Introductions to the Great Lakes. Meeting of the U.S. Great Lakes Shipping Association, Cleveland, January 31, 2001.

Reid, D.F. Project Briefing - Assessment of Transoceanic NOBOB Vessels and Low-Salinity Ballast Water as Vectors for Nonindigenous Species Introductions to the Great Lakes. U.S. Coast Guard Marine Community Day, Cleveland, January 31, 2001

Reid, D.F. Project Briefing - Assessment of Transoceanic NOBOB Vessels and Low-Salinity Ballast Water as Vectors for Nonindigenous Species Introductions to the Great Lakes. St. Lawrence Seaway Customer Relations Meeting, Montreal, April 5, 2001.

Reid, D.F. Project Briefing - Assessment of Transoceanic NOBOB Vessels and Low-Salinity Ballast Water as Vectors for Nonindigenous Species Introductions to the Great Lakes. Great Lakes Commission Symposium - Looking Forward, Looking Back: Assessing Aquatic Nuisance Species Prevention and Control, Ann Arbor, May 17, 2001.

Reid, D.F. Early Status Report and Picture Gallery. Great Lakes Protection Fund Board. Chicago, June 15, 2001.

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Reid, D.F. Participated in two Environmental Forums for the U.S. Coast Guard's Marine Community Day, presenting a briefing and update on the NOBOB program at both sessions. January 31, 2002.

Reid, D.F. presented NOBOB Program overview and progress-to-date at the ICES/IOC/IMO Study Group on Ballast and other Ship Vectors meeting in Sweden. This provided international exposure for the issue and our research program. March 18, 2002.

Reid, D.F. NOBOB Program Progress Update. National ANS Task Force Meeting, Honolulu, HI. November, 2002.

Reid, D.F. Ballast Tanks - "Transoceanic Highways" For Aquatic Invasive Species Movement To The Great Lakes (And Elsewhere). Science Research Club, University of Michigan, Ann Arbor, Michigan. December 2002.

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Pandey, Capt. A.K, K. Murphy, M. Bednarski, S. Heinemann, and M.A. Doblin. 2002. "Testing Ballast Water Exchange Efficiency". *Horizon*, Bergesen d.y. ASA, 4: 20-21. This article is the second of a two-part overview article on the *Berge Nord* experience—from both research and shipping industry perspectives—appeared in the Winter 2002 issue of *Horizon*, the company magazine of Bergesen (the owner/operator of the *Berge Nord*). This article focuses on the experiment conducted on the *Berge Nord* and was printed as a follow-up to Part 1, Fall 2002, which provided background on the subject of marine invasions and the reasons for conducting ballast water exchange. Both articles were co-authored by Capt. A.K. Pandey, Kate Murphy, Melinda Bednarski, Stefan Heinemann, and Martina Doblin.

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