NOBOB-A
EXECUTIVE SUMMARY

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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%) <u>ships</u> sampled tested "positive" for one or more pathogens.
- Overall, 40 of 82 (49%) <u>ballast tanks</u> 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 <u>frequency</u>, 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: <u>Filled Ballast Tank Experiments</u>

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 <u>can</u> 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: <u>Ballast Water Exchange Experiments</u>

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 <u>in the control tanks</u>. 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.